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AUTHOR LEGEND

Asterisk (*) Presenting author

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A1

HUMAN CORONAVIRUS OC43: POTENTIAL FOR SEVERE ILLNESS IN THE ELDERLY AND SEROLOGIC CROSS-REACTIVITY WITH THE SARS CORONAVIRUS

DM PATRICK*[1], M PETRIC[1], DM SKOWRONSKI[1], R GUASPARINI[2], TF BOOTH[3], M KRAJDEN[1], P MCGEER[4]
[1]British Columbia Centre for Disease Control, Vancouver; [2]Fraser Health Authority, Surrey, British Columbia; [3]Public Health Agency of Canada, Winnipeg, Manitoba; [4]Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, British Columbia

OBJECTIVES: In summer 2003, a respiratory outbreak occurred in a seniors care facility within a region that had two SARS cases earlier in the spring. SARS coronavirus (SARS-CoV) was initially identified by nucleic acid tests and serology. The purpose of these studies was to clarify the etiology of the outbreak and explain the unexpected laboratory findings.
METHODS: Cases at the facility were epidemiologically characterized and investigated for conventional agents of respiratory infection, SARS-CoV and other human coronaviruses. Possible serological cross-reactivity between SARS-CoV and human coronavirus OC43 (HCoV-OC43) was investigated by peptide spot assay.
RESULTS: 95/142 residents (67%) and 53/160 staff (33%) experienced symptoms of respiratory infection. Symptomatic residents experienced cough (66%), fever (21%), and pneumonia (12%). Eight died, six with pneumonia. Staff experienced similar symptoms without pneumonia. In specimens from 9 of 40 patients submitted to a national reference laboratory, SARS-CoV was diagnosed by RT-PCR and 9 specimens had antibody to SARS-CoV. Subsequent molecular testing by RT-PCR at a provincial facility did not corroborate these findings and on further investigation found evidence of HCoV-OC43 infection. Neutralizing antibody tests on convalescent sera ruled out SARS. Sera demonstrated cross-reactivity against peptide sequences common to HCoV-OC43 and SARS-CoV.
DISCUSSION: These findings underscore the virulence of human coronavirus OC43 in elderly populations and indicate that cross reactivity to antibody against such viruses must be considered in interpreting some serological tests for SARS-CoV. The possibility of false-positive RT-PCR results should be considered in the laboratory diagnosis of SARS.

A2

SEROTYPING OF IMPORTED DENGUE VIRUS CASES USING A MICRONEUTRALIZATION FORMAT: EXPOSURE OF CANADIAN TRAVELLERS TO DIVERSE SEROTYPES FROM 1996 TO 2004

KA HOLLOWAY*, H ARTSOB, MA DREBOT
Public Health Agency of Canada, Winnipeg, Manitoba

OBJECTIVES: Dengue virus is one of the most medically important flaviviruses and cases total over 50 million per year worldwide. Canadian travellers to Asia and the Caribbean are frequently exposed to dengue virus and account for the majority of imported flavivirus cases. The objective of this study was to determine the prevalence of dengue serotypes among Canadians returning from endemic areas and equate this to viral epidemiology in these regions.
METHODS: Over 300 sera were collected from Canadian travellers with clinical symptoms consistent with dengue fever or dengue hemorrhagic fever. Probable cases of dengue infection were identified using the hemaglutination inhibition assay (HAI) and/or the Dengue ELISA. Determination of dengue serotype was performed using a neutralization assay in a 96 well plate format with colorimetric detection of neutralizing activity.
RESULTS: Microneutralization tests confirmed dengue virus exposures in 85% of the samples tested. Dengue serotype 1 was the most commonly identified serotype and accounted for 40% of the cases in which serotypes could be determined. The next most prevalent serotypes were types 2 and 3. In approximately 20% of the cases, secondary infections appears to have precluded serotype identification due to enhanced serological cross-reactivity. Travel history of dengue cases were consistent with visits to endemic countries in SouthEast Asia and the Caribbean, in which various serotypes of dengue virus circulate. Higher rates of Canadian cases coincide with travel to countries with documented outbreaks of dengue virus associated illness.
CONCLUSION: Canadian travellers can act as sentinels for emerging infectious disease in other countries. The identification of imported dengue virus infections provides useful epidemiological information for assessing risk for exposure in countries where this virus circulates.

A3

NUCLEIC ACID AMPLIFICATION TESTS ENHANCE DETECTION OF RESPIRATORY VIRUSES IN A RANGE OF SPECIMEN TYPES

JD FOX*[1], B LEE[2], K FONSECA[1], JK PREIKSAITIS[2]
[1]Provincial Laboratory for Public Health, and University of Calgary, Calgary; [2]Provincial Laboratory for Public Health, and University of Alberta, Edmonton, Alberta

BACKGROUND AND OBJECTIVES: Nucleic acid amplification tests (NATs) are more sensitive than culture-based procedures for identification of respiratory viruses and can be used on a wider range of specimen types than direct fluorescent antigen (DFA) methods. We aimed to introduce respiratory virus NATs into routine diagnostics and assess the impact on laboratory work-flow, costs and results.
METHODS: Polymerase chain reaction (PCR) and nucleic acid sequence based amplification (NASBA) were utilized for identification of influenza (IFV) A and B, parainfluenza (PIV) 1-4, respiratory syncytial virus (RSV), adenovirus (ADV) and human metapneumovirus (hMPV). Nucleic acid extraction was automated (bioMérieux, Canada). In a two-phase process, NATs were first used to replace DFA and culture for lower respiratory specimens and then to replace culture for DFA-negative nasopharyngeal samples. The impact of these changes was assessed over a period of one year with more than 2000 specimens analyzed.
RESULTS: NATs identified a significant proportion of mixed infections and detected IFV, PIV and RSV positives missed by DFA. NATs identified hMPV and ADV as a probable cause of respiratory symptoms in a wider range of patients (from multiple specimen types) than previously appreciated. PCR

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provided a significantly shorter turn-around time for ADV diagnosis than the previous culture procedures. The positive rate for lower respiratory specimens was increased by more than 5-fold by the addition of NATs to the testing algorithm.

DISCUSSION AND CONCLUSION: Providing automated nucleic acid extraction is available, NATs for respiratory viruses can be incorporated into a routine diagnostic laboratory. There are significant cost implications if NATs and "conventional" methods are run alongside. As a DFA-positive result has immediate clinical impact and the test can be undertaken more rapidly than NATs, this procedure is still part of our algorithm for NP samples. In our laboratory, the DFA/NAT testing combination is optimal for respiratory virus diagnosis.

A4
DEVELOPMENT OF A NEW RESPIRATORY VIRUS PANEL (RVP) TEST FOR THE DETECTION OF HUMAN RESPIRATORY VIRUSES.

J MAHONY*, S CHONG, F MERANTE, K LUINSTRA, T SINHA, A PETRICH, R JANECZKO
McMaster University and St Joseph's Healthcare, Hamilton, Ontario

INTRODUCTION: With the emergence of five new respiratory viruses over the past four years, including SARS and avian influenza, there is a need for improved diagnostic tests to detect viral respiratory tract infections (RTI). Virology laboratories have historically used traditional methods to detect only six or seven clinically important respiratory virus infections. With the advent of new molecular diagnostic methodology over the past 15 years, it has become apparent that traditional methods of diagnosis viz. culture and DFA are suboptimal in sensitivity, have a slow turn-around time and do not detect newly described viruses. We have developed a multiplex nucleic amplification test that can detect 17 different respiratory viruses in a single test.

METHODS: The test employs a multiplex PCR step using 14 pairs of primers followed by a multiplexed Target Specific Primer Extension (TSPE) reaction (21 primer pairs) to detect and identify different viruses. Following completion of the TSPE step, the reaction products are hybridized to a fluid (microsphere)-based array and analyzed on a bench-top flowcell (Luminex Corporation). The entire assay from sample input to result takes 5 hours for 96 specimens.

RESULTS: Using cloned amplicons and in vitro generated transcripts, the test had an analytical sensitivity of 100-1000 viral genomes for all 17 viruses as assessed by quantitative real-time PCR using LightCycler assays. The 17 viruses detected included Influenza A and B, parainfluenza types 1-4, RSV A and B, adenovirus, rhinovirus, enterovirus, metapneumovirus, SARS-CoV, and coronviruses OC43, 229E, NL63 and HKU1. The test also identified influenza H1, H3 and H5 genes. In an evaluation of 119 NP specimens that were positive by either culture or DFA, the RVP test had a sensitivity of 94% (112/119).

DISCUSSION: This new multiplex RVP test should improve the diagnostic capabilities of hospital and public health laboratories in the detection of respiratory viruses, increase the detection rate for identifying etiologic agents in local outbreaks of RTI, and assist with global surveillance for potential pandemic influenza strains such as avian H5N1.

A5
COMPREHENSIVE LONG RT-PCR FOR THE AMPLIFICATION OF THE NEAR FULL-LENGTH GENOME OF NOROVIRUSES

J KOSTELA[1], M AYERS[1], J NISHIKAWA[1], L MCINTYRE[2], M PETRIC[2], R TELLIER*[1]
[1]Division of Microbiology, The Hospital for Sick Children, Toronto, Ontario; [2]British Columbia Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: Norovirus-induced diseases manifest typically as outbreaks of acute gastroenteritis. These viruses can periodically behave as "emerging viruses" with a sudden increase in incidence, associated with genetic changes. We sought to develop a long RT-PCR able to amplify most of the genome of noroviruses, with primers able to amplify any strain. Such a method would allow for a complete genetic characterization of these viruses.

METHODS: Primers targeting regions well conserved among prototype strains of genotype I and II noroviruses were designed and a long RT-PCR assay optimized. We also developed the parameters for nested long PCR to facilitate sequencing.

RESULTS: RNA extracted from samples positive for noroviruses by electron microscopy (EM) served as templates in the long RT-PCR to generate amplicons of approximately 7.6 kb (full length of norovirus genome: 7.654 kb). The long RT-PCR assay has the same sensitivity as EM but is considerably less sensitive than standard PCR assays. We completely sequenced the amplicons from two Toronto specimens obtained in 2002 and 2005. The 2002 amplicon sequence is very similar to that of the Oxford 2002 strain. The 2005 amplicon sequence is of the same lineage but has accumulated mutations.

CONCLUSION: We showed that the near complete genome of noroviruses can be amplified in a single RT-PCR assay, provided that the samples contain adequate amounts of viruses. The strain circulating in Toronto in 2002 was of the same lineage as the new variant that caused worldwide increases in norovirus activity in the winter of 2002. The strain from 2005 appears to have drifted from that strain. This method will facilitate the complete genetic characterization of noroviruses.

B1
NECROTIZING PNEUMONIA SECONDARY TO COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *S AUREUS* (CA-MRSA) USA300 STRAIN WITHOUT EVIDENCE OF ANTECEDENT VIRAL RESPIRATORY TRACT INFECTION

J JANVIER*[1], S ELSAYED[2], D GREGSON[2], K ZHANG[2], M LOUIE[5], K LAUPLAND[2], D CHURCH[2], T LOUIE[3], H RABIN[3], J CONLY[3]
[1]University of Calgary; [2]Calgary Laboratory Services; [3]Foothills Medical Centre; [4]Peter Lougheed Centre; [5]Provincial Laboratory of Alberta, Calgary, Alberta

OBJECTIVE: Necrotizing pneumonia (NP) secondary to CA-MRSA USA300 strain has been reported in the US to be related to antecedent viral respiratory tract infection (VRTI). We present a case series of NP secondary to CA-MRSA USA300 strain without clinical or laboratory evidence of predisposing VRTI.

METHODS: Patients with NP secondary to CA-MRSA USA300 strain were identified through clinical and laboratory review. Clinical and radiographic data was collected by chart review. MRSA was identified using standard laboratory procedures and confirmed by an in-house PCR assay for *nuc*, *fem*A, and *mec*A genes. After DNA extraction and digestion with *Sma*1, typing by pulsed-field gel electrophoresis (PFGE) was done using a standardized protocol from the National Microbiology Laboratory (NML). Genes for Panton-Valentine leukocidin (PVL) and SCC*mec* were identified using multiplex PCR assays. Characterization of isolates matching the USA300 strain was based on presence of PVL and SCC*mec* type IVa, PFGE patterns, staphylococcal protein A (spa) typing and multilocus sequence typing (MLST). Serological testing for influenza and parainfluenza viruses, adenovirus, and RSV were performed at the National Microbiology Laboratory.

RESULTS: Five patients with NP secondary to CA-MRSA USA300 were identified. All had radiological evidence of lung cavitation or abscess, and 4 of 5 patients required chest tube drainage. One death occurred. Mean hospital stay was 24 days (range 5 to 42 days). All sputa, BAL, pleural fluid and blood CA-MRSA isolates from the patients were found to be PVL+, contained SCC*mec* Type IVa, and had a PFGE pattern, spa type (t008), and MLST (ST8) type consistent with the USA300 pattern. No patients had a clinical history or fourfold change or a single static high titre to suggest serologic evidence of VRTI.

CONCLUSION: Our findings suggest a significant morbidity and mortality secondary to CA-MRSA NP and that it can occur spontaneously without a predisposing VRTI. Clinicians need to be aware of this clinical entity.

B2

POSSIBLE ROLE OF SEXUAL ACTIVITY IN PROPAGATION OF AN OUTBREAK OF CMRSA10 COMMUNITY-ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (CAMRSA) IN A MARGINALIZED URBAN POPULATION (MUP)

M GILBERT*[5], J CONLY[2], J MACDONALD[2], J SIUSHANSIAN[1], D GREGSON[3], S ELSAYED[3], K ZHANG[3], K LAUPLAND[2], M LOUIE[7], M MULVEY[1], T LOUIE[2], D NIELSEN[2], V WHEELER[2], T LYE[2], G KEYES[6], J GILLESPIE[2]

[1]Public Health Agency of Canada, Ottawa, Ontario; [2]Calgary Health Region; [3]Calgary Laboratory Services; [4]University of Calgary, Calgary, Alberta; [5]Vancouver Island Health Authority, Victoria, British Columbia; [6]Alberta Health and Wellness, Edmonton; [7]Provincial Laboratory for Public Health, Calgary, Alberta; [8]National Microbiology Laboratory, Winnipeg, Manitoba

OBJECTIVE: An outbreak of the CMRSA10 strain of MRSA has been ongoing in an MUP with histories of drug use, homelessness, and incarceration (DU/H/I) since January 2004. We conducted a prevalence survey and assessed demographic and behavioural factors associated with colonization and/or infection with the outbreak strain.

METHODS: Trained study personnel administered PDA-based questionnaires to participants recruited at 5 study sites (correctional facility, shelter, outreach van, detox centre, medical clinic) to assess demographics, medical history, substance use behaviours, hygiene, skin infections, and contacts. Swabs of nares, axillae, and any skin infections were collected. Isolates of SA were identified with standard techniques. MRSA isolates were subjected to pulsed-field gel electrophoresis (PFGE), SCC*mec* typing and PVL testing by PCR assay. Cases were defined as being colonized and/or infected with CMRSA10 based on PFGE. SPSS V12.0.1 was used for analysis.

RESULTS: We identified CMRSA10 in 5.5% (15/271), another strain of CMRSA in 1.8% (5/271), and MSSA in 35.4% (96/271). All CMRSA10 isolates were PVL-positive and SCC*mec* type IVa. Variables related to our original hypotheses were not associated with case status. However, CMRSA10 cases were more likely to report involvement with the sex trade (using drugs as/with a sex trade worker or income from the sex trade; odds ratio 3.16 [95% CI 1.02–9.79]), any use of drugs with casual sex partners (OR 5.29 [95% CI 1.61–17.41]), more frequent binge drug use (P=0.002) and binges of longer duration (P=0.01). The findings were unchanged if all CMRSA cases were included in the analysis.

CONCLUSION: The prevalence of CMRSA10 in this MUP was 5.5% [3.1% to 9.0%] and cases were more likely to report involvement with the sex trade, drug use with casual sex partners, and binge drug use (which may be associated with increased sexual activity). We postulate that sexual transmission is a potential route by which CMRSA10 is spreading in this population. Further studies to test this hypothesis are warranted.

B3

CHARACTERIZATION OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AMONG CLINICAL ISOLATES AT PROVIDENCE HEALTH CARE, VANCOUVER, BRITISH COLUMBIA

MW HULL*[1], S CHAMPAGNE[2], SH GOH[3], A MCNABB[3], MG ROMNEY[2]

[1]University of British Columbia; [2]Providence HealthCare; [3]British Columbia Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: Community-associated MRSA (CA-MRSA) has been reported as an emerging pathogen in North America; however, little is known regarding its role as an etiologic agent at our institution. We undertook a review of all MRSA isolates to determine whether CA-MRSA was present, and to correlate CA-MRSA with reported antibiotic susceptibility profiles and clinical presentation.

METHODS: MRSA isolates from clinical specimens were tracked for a 30-day period. Only one isolate per patient was included in the review. Clinical specimens from sterile sites were given preference over non-sterile sites if multiple specimens were available. Antibiotic susceptibility profiles, patient location at time of specimen collection and demographic data were collected. Molecular analysis was performed on a subset of isolates. The presence of CA-MRSA markers, namely SCC*Mec*IV, PVL and PFGE type, was evaluated.

RESULTS: One hundred fifty isolates were identified: 52% were from wound specimens, and 33% were sent from the ER. 57 isolates (86% wound specimens) had molecular analysis performed. Overall, 68% of these isolates were found to be SCC*Mec*IV- and PVL gene-positive (CA-MRSA). All CA-MRSA isolates belonged to the PFGE clonal group CMRSA10 (USA 300). 85% of ER wounds were CA-MRSA vs 40% non-ER specimens (P=0.006 by Fisher's exact test). Isolates were uniformly resistant to erythromycin (99%) and ciprofloxacin (98%) but retained sensitivity to rifampin (97%). Clindamycin susceptibility was used to predict the presence of CA-MRSA, with a positive predictive value of 86%. TMP-SMX resistance had a negative predictive value of 100%.

CONCLUSIONS: CA-MRSA is present among MRSA specimens isolated from clinical specimens at our institution. CA-MRSA is found predominantly in skin and soft tissue isolates collected in the ER setting suggestive of community spread and disease burden. The CA-MRSA isolates are clonal in nature and belong to the CMRSA10 (USA300) clonal type. Susceptibility to clindamycin may serve to predict CA-MRSA, while TMP-SMX resistance rules out CA-MRSA.

B4

NEW LABORATORY APPROACHES TO THE SELECTIVE DETECTION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM SURVEILLANCE SPECIMENS

BM WILLEY*, N KREISWIRTH, P AKHAVAN, A TYLER, S MALEK, V PONG-PORTER, G SMALL, N NELSON, A MCGEER, S POUTANEN, T MAZZULLI, DE LOW, M SKULNICK

Toronto Medical Laboratories and Mount Sinai Hospital, Toronto, Ontario

OBJECTIVES: This study compared MRSA yield, turn-around time (TAT), workload and cost for four cefoxitin-based selective media that use mannitol salt or chromogens to distinguish *S aureus* from other bacteria.

METHODS: 2500 screens from 1271 patients in 13 facilities were planted as per routine to Oxoid Mannitol Salt Cefoxitin [MSF] agar, and to Oxoid Modified-MSF [MOD], Bio-Rad MRSA*Select* [BR] and Becton-Dickinson CHROMagar MRSA [BD] study agars. Specimens included 178 wound, 777 rectal, 823 nasal and 713 nasal-axilla-groin-perineum swabs and 9 sputa. Incubation was at 37°C. Reading of MSF was at 18h+48h and MOD, BR and BD was at 24h+48h. MRSA were confirmed using Pastorex Staph Plus (BR), tube coagulase, PBP2a (Denka Seiken) and oxacillin screen plate. When possible, tests were done directly from study media except for MSF, where routine protocol required prior sub-culture. MRSA from any medium was the gold standard.

RESULTS: A total of 147 (5.8%) MRSA were detected. 24/48h results for BR, MOD, BD, and MSF were (respectively): % sensitivity – 91/91, 69/86, 67/81 and 52/82; % specificity – 98/48, 90/77, 97/81 and 91/68; % post-predictive value (PV) – 73/10, 30/19, 60/21 and 27/14. Negative PV for all were over 97%. Direct testing enabled MRSA reporting ≤24h of specimen receipt in 74%, 58% and 31% of cases from BR, MOD and BD, respectively, versus 0% from MSF (direct testing not done). Costs, expressed as % increase or decrease compared to MSF protocol, are shown below.

24 h/48 h	BR	MOD	BD
Materials	0/+59	–33/–14	+12/+17
Labour	–64/+24	–51/–36	–68/–52

CONCLUSIONS: BR, incubated for 24 h at 37°C, performed better than all other media (P<0.00001) and reduced laboratory costs compared to MSF. Incubation of BR beyond 24 h significantly reduced its specificity. The MOD rated second overall, and was more specific than MSF (P<0.00001). Direct testing from BR and MOD led to earlier

MRSA TAT without reducing accuracy. The BD TAT was limited by mixed growth.

B5
COMPARISON OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS (*MRSA)-SELECT AND MANNITOL SALT AGAR WITH CEFOXITIN FOR THE DETECTION OF MRSA FROM SCREENING AND SURVEILLANCE SPECIMENS

L LOUIE*[1], D SOARES[1], H GARDNER[1], H MEANEY[1], M VEARNCOMBE[1,2], AE SIMOR[1,2]
[1]Sunnybrook and Women's College Health Sciences Centre; [2]University of Toronto, Toronto, Ontario

OBJECTIVE: Detection of MRSA from screening and surveillance specimens is a tedious and time-consuming challenge. Newer chromogenic media such as MRSA-Select (Bio-Rad) show promise for the rapid isolation of MRSA. We compared MRSA-Select (Select) with mannitol salt agar with 8 μg/ml cefoxitin (MSFOX) (Quelab) for the detection of MRSA from clinical samples submitted for screening and surveillance for MRSA.
METHODS: A total of 6199 specimens from 1883 patients were evaluated, consisting of nares swabs (n=2483), perianal/rectal swabs (2312), catheter exit sites (647), skin/soft tissue (632), sputum (58) and urine (67). Specimens were planted onto Select and MSFOX media, incubated in ambient air at 35°C, and read independently at 24 h and 48 h by two technologists. Suspicious colonies (pink from Select; yellow from MSFOX) were worked up from both media for MRSA using standard methods, including a commercial latex agglutination test to detect PBP2a.
RESULTS: There were 181 MRSA isolated. Select detected a total of 177/181 (98%) MRSA compared with 164/181 (91%) with MSFOX (P=0.006). Of the 177 MRSA detected by Select, 169 (96%) grew after overnight incubation, while only 132/164 (81%) were detected on MSFOX after overnight incubation (P<0.0001). There were a total of 178 specimens planted on Select that yielded pink colonies after overnight incubation. All but one of these was confirmed as MRSA (177/178; 99.4%). The number of specimens with suspicious growth at 24 h and 48 h requiring further investigation and determined not to be MRSA was found more often with MSFOX (1302/6199; 21%) than with Select (468/6199; 8%) (P<0.001).
CONCLUSION: Our results showed that MRSA-Select was superior to MSFOX for the detection of MRSA from clinical screening specimens, allowing greater yield and more rapid and easier detection after overnight incubation. MRSA-Select was also less likely to support the growth of non-MRSA isolates.

C1
REAL-TIME PCR DETECTS VIRAL NUCLEIC ACIDS IN UNIVERSAL TRANSPORT MEDIUM (UTM-RT) WITH FLOCKED SWABS

S CASTRICIANO*, A PETRICH, M SMIEJA, MA CHERNESKY
McMaster University, St Joseph's Healthcare, Hamilton, Ontario

BACKGROUND: Nucleic acid amplification assays are becoming widely used for the laboratory diagnosis of viral infections. A versatile transport medium and collection system, effective for all laboratory technologies, is necessary.
OBJECTIVES: To compare a room temperature universal transport medium [UTM-RT] and flocked swabs [FS] (Copan) to M4-RT (Remel) transport medium and MicroGent Stainless Steel/Plastic swabs (MG) for the detection of HSV1, HSV2, VZV, CMV, Influenza A, Influenza B, and Echovirus 11 in contrived specimens. To test clinical samples collected into UTM-RT with FS in RT-PCR and by DFA/culture.
METHODS: Laboratory strains of HSV1, HSV2, VZV, CMV, Influenza A, Influenza B, and Echovirus 11 were serially diluted 10-fold in UTM-RT and M4-RT from 10^{-1} to 10^{-10}. One mL of each dilution for both media was aliquoted into sterile vials (contrived specimens). A swab, provided with each transport media, was added to the respective vials. Suspensions were vortexed, swabs were removed, and 0.2 mL was inoculated into two shell vial cell cultures each. For RT-PCR, nucleic acid was extracted from 0.2 mL using a manual magnetic silica method and extracted nucleic acids were tested in duplicate in the LightCycler using artus RealArt-LC PCR kits (Qiagen). A total of 48 culture-positive clinical samples collected by FS into UTM-RT were tested by RT-PCR, after freezing and thawing. Infected cell cultures were stained using FITC monoclonal antibodies.
RESULTS: End points of detection of contrived specimens were equivalent for the combination of FS with UTM-RT to the MG in M4-RT for HSV1 and 2, CMV, VZV and Influenza A. Endpoint determinations were enhanced 10-fold for Influenza B and Echovirus 11 by the FS/UTM-RT. The artus LC real-time PCR was more sensitive than shell vial culture with both swab/transport systems with contrived specimens. RT-PCR detected 46/48 clinical positives from UTM-RT.
CONCLUSION: The FS/UTM-RT could serve as a universal system for detecting viruses by RT-PCR.

C2
IS THE INCORPORATION OF AN INTERNAL CONTROL A TRUE TEST OF POLYMERASE CHAIN REACTION (PCR) INHIBITION?

L CHUI*, T CHIU
Provincial Laboratory, Edmonton, Alberta

OBJECTIVE: Achieving a high quality and quantity of nucleic acid extracted from clinical specimens is critical for molecular diagnostic laboratories. Inhibitory factors can be extracted with nucleic acid and result in PCR failure. The standard approach to detect inhibition is by incorporating an internal control (IC) in the PCR assay. The objective of this study was to evaluate if the absence of amplification of the IC is a true indicator of inhibition of the PCR reaction.
MATERIALS AND METHODS: Sixty-four decontaminated sputum samples and 20 stools samples were spiked with SARS cornavirus and the DNA was extracted with an automated extractor (PSS GC12). DNA was extracted manually by MagaZorb (Cortex BioChem) from 202 bovine feces following immunocapture for M *avium* ssp *paratuberculosis* (Map) using IgY-magnetic beads. Commercial real-time PCR assays (Artus) were used to detect the various targets and the internal control (IC) provided by each assay was included.
RESULT: The IC was amplified with the target in most of the positive samples. For the samples that tested negative, the IC amplification curve failed, suggesting the presence of PCR inhibition. However, when the post-amplification samples were analyzed by gel electrophoresis, various fragments of amplified DNA were observed for all sample types.
CONCLUSION AND DISCUSSION: Failure of IC amplification curve might not be a true indication of PCR inhibition, as nonspecified amplified products were present in some negative samples when analyzed by gel electrophoresis. The possible mechanisms include loss of the IC during the extraction step or the relatively lower amount of IC in comparison with non-specific targets that led to non-specific amplification. It is important to monitor IC amplification failure.

C3
DETECTION OF *ACANTHAMOEBA* SPECIES FROM CORNEAL ULCERS BY MULTIPLEX PCR AND SPECIATION BY SEQUENCING THE 18S rRNA GENE

D EISLER D, A MCNABB*, T LO, Q WONG, J ISAAC-RENTON
Laboratory Services, British Columbia Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: To determine if a multiplexed PCR assay can reliably detect *Acanthamoeba* species in corneal ulcers and if DNA sequencing of the 18S rRNA gene can act as a means to identify *Acanthamoeba* species.
METHODS: Fifteen corneal scrapings and related specimens submitted for the detection of *Acanthamoeba* were examined by standard culture techniques and PCR (Schroeder et al, J Clin Microbiol 2001;39:1903-11). Amplicons of appropriate size from positive specimens were sequenced and compared to a database of *Acanthamoeba* 18S rRNA gene sequences downloaded from GenBank supplemented with sequences from 24 *Acanthamoeba* isolates previously archived by our laboratory.

RESULTS: Of 15 specimens, eight were positive for the presence of *Acanthamoeba* species by culture (53%), four positive by PCR (27%). No specimen was culture-negative and PCR-positive. Nine PCR tests (60%) failed to detect the presence of human DNA, and were reported as indeterminate. Human DNA was detected in two PCR-negative but culture-positive specimens. The time to detection of *Acanthamoeba* species by culture averaged 2.5 days, and PCR detection was one day. Analysis of 18S rRNA sequences indicated that morphology-based identification is inadequate to identify *Acanthamoeba* species. Clinical isolates were related to isolates designated as *A castellani*, *A lugdunensis/thysodes*, *A palestinensis* or *A polyphaga* by the submitting authors.
CONCLUSIONS: Our data indicate that PCR is suboptimal to our culture technique for the detection of *Acanthamoeba* species from corneal scrapings. This is probably due to sample size and our preference to culture prior to processing samples for PCR detection. 18S rDNA gene sequencing appears to be useful for identification, but will not recognize its potential until further taxonomic clarification is achieved.

C4
EVALUATION OF THE ANTIBIOTIC SUSCEPTIBILITY PATTERN FOR TOXIGENIC *CLOSTRIDIUM DIFFICILE* ISOLATES FROM MANITOBA OVER THE PAST 14 YEARS

MJ ALFA*, GK HARDING, P DEGAGNE
St Boniface Hospital; University of Manitoba; Diagnostic Services of Manitoba, Winnipeg, Manitoba

OBJECTIVES: *Clostridium difficile* is the primary cause of nosocomial diarrhea (CDAD). Recently, nosocomial outbreaks of CDAD have been linked to widespread use of fluoroquinolones in healthcare and development of resistance to this class of antibiotic. The aim of this study was to determine the antibiotic susceptibility pattern of toxigenic *C difficile* over the past 14 years and to determine if the outbreaks were associated with fluoroquinolone-resistant strains.
METHODS: *C difficile* clinical isolates collected since 1991 were evaluated using the E-test method on Mueller-Hinton agar to determine their MIC to metronidazole, clindamycin, levofloxacin and ciprofloxacin, as well as their PFGE pattern. There were a total of 133 isolates analyzed. There were two major outbreaks, with 20 patients in one outbreak and 54 patients from a second outbreak. To determine the overall susceptibility pattern, only one isolate from each patient was used and only one isolate from each of the two outbreaks was included for a total of 78 isolates.
RESULTS: All 78 strains evaluated had MICs to ciprofloxacin that were >32 µg/mL. The levofloxacin MIC_{90} was >32 µg/mL (range 3 µg/mL to >32 µg/mL). All 78 strains were susceptible to metronidazole with an MIC_{90} that was ≤0.5 µg/mL. Only four of 78 strains were sensitive to clindamycin and the MIC_{90} was >256 µg/mL. The two clonal outbreak strains (C1 associated with 54 patients and NC26 associated with 20 patients) both had ciprofloxacin and levofloxacin MICs of >32 µg/mL.
DISCUSSION: The data from this analysis indicated that all the *C difficile* isolates as far back as 1991 had high MICs to both levofloxacin and ciprofloxacin. There has not been any significant shift in the fluoroquinolone or metronidazole resistance in our facility over the past 14 years. The strains associated with the two major outbreaks seen over this timeframe did not differ in their susceptibility patterns to clindamycin, metronidazole, ciprofloxacin or levofloxacin compared with the nonoutbreak strains.

C5
EVIDENCE OF SPREAD OF *CRYPTOCOCCUS GATTII* FROM VANCOUVER ISLAND TO THE BRITISH COLUMBIA MAINLAND

L MACDOUGALL*[1], E GALANIS[1], S KIDD[2], K BARTLETT[2]
[1]British Columbia Centre for Disease Control; [2]University of British Columbia, Vancouver, British Columbia

OBJECTIVE: To assess the spread of *Cryptococcus gattii*, an emerging infectious disease in BC, from its current endemic location on Vancouver Island (VI) to the mainland.
METHODS: Between January 2001 and June 2005, enhanced surveillance was conducted for all culture-confirmed cases of *C gattii* in humans and animals, regardless of residence. Human cases were interviewed with a standard questionnaire to assess history of travel and other risk factors for infection; owners were asked about their animal's exposure to VI. Environmental sampling was conducted near case homes.
RESULTS: Four animal cases (a llama, two cats and a dog) were detected between December 2003 and April 2004 with no known travel to VI. In the spring of 2005, three human cases from the lower mainland also reported no contact with VI during their incubation period. Prior to this, all human cases had either lived on VI or had travelled there within 12 months of onset. In BC, approximately 25 human cases of *C gattii* are detected per year in principally immunocompetent individuals. Surprisingly, two-thirds of these mainland patients had underlying health conditions. One patient was undergoing dialysis and the other reported a history of lung cancer and hepatitis C. Two air samples taken in the fall of 2004 from regions near pet homes were weakly positive at concentrations well below levels expected on VI. To date, environmental sampling from tree swabs or soil samples taken in areas of the lower mainland have been negative.
CONCLUSION: Surveillance findings suggest the acquisition of *C gattii* from the lower mainland of BC, representing a spread of the fungus from VI. Low concentrations of the fungus in air samples, its absence in tree and soil samples, and the detection of few numbers of human cases suggest that the organism is not yet fully established in this new environment.

D1
QuantiFERON-TB GOLD TESTING OF HIV PATIENTS FOR LATENT TB INFECTION

D KUNIMOTO*, T BIRSE, A LINDEMULDER, T MANNING, L MIEDZINSKI, L SAXINGER, S SHAFRAN, G TAYLOR, S HOUSTON
University of Alberta, Edmonton, Alberta

OBJECTIVE: HIV patients with a positive tuberculin skin test (TST) have one of the highest risks of reactivation of tuberculosis at 10% per year. However, the lower the CD4 count, the less reliable the TST. The objective of this study was to determine the usefulness of a new blood test for TB infection, the QuantiFERON-TB Gold test (QFT), in HIV patients.
METHODS: HIV patients attending the University of Alberta Hospital Infectious Diseases clinics were asked to have a QFT test in addition to their routine TST. Five millimitres of induration or greater was considered a positive TST. Additional information related to TB was also obtained. The CD4 count nearest in time to the QFT testing was noted. QFT testing (In-Tube method including a mitogen control) was performed as per the manufacturer's recommendations.
RESULTS: Fifty-seven HIV patients with CD4 counts ranging from 20 to 750 (median 330) had QFT In-Tube testing performed. Twenty-one patients had negative TST and QFT. One patient had a positive TST and negative QFT. Two patients had a positive QFT but negative TSTs. Three patients had positive QFT and no TST performed (one patient had a history of TB in the past). Seventeen (30%) patients had a TST planted but did not return for a reading; one of these patients was QFT positive. Only one patient did not respond to the mitogen control, resulting in an indeterminate result. Stratification by CD4 count did not demonstrate any decrease in the proportion of QFT-positive tests at lower CD4 counts.
CONCLUSION: QFT appears to be more sensitive than the TST in our HIV patients with the added advantage of not requiring a return visit for a reading. QFT appears to be useful even at low CD4 counts, with only one of 57 resulting in an indeterminate test.

D2

DISTINGUISHING *MYCOBACTERIUM TUBERCULOSIS* COMPLEX FROM ATYPICALS BY PCR IN SMEAR-POSITIVE PULMONARY SPECIMENS.

MC LEE*[1], L CHUI[2], G TYRELL[2], C SHANDRO[2], D KUNIMOTO[1]
[1]University of Alberta; [2]Alberta Provincial Laboratory for Public Health (Microbiology), Edmonton, Alberta

OBJECTIVE: Conventional identification of mycobacterial organisms is often delayed, hampering optimal clinical management decisions for patients with smears of respiratory samples positive for acid-fast bacilli (AFB). Published studies have shown improved cost-effectiveness, accuracy and early diagnosis of M *tuberculosis* (MTB) complex when polymerase chain reaction testing of respiratory specimens were added to the battery of initial TB screening tests. This study investigated the reliability of MTB complex-specific PCR of AFB-positive smears as part of routine laboratory testing.
METHODS: The first AFB-positive respiratory specimen from any suspect TB patient in Alberta submitted to the Alberta Provincial Lab for Public Health between 2004 and 2005 were routinely tested within one business day using the RealArt M *tuberculosis* LC PCR Kit and the Light Cycle PCR instrument (Roche Diagnostics). Results of PCR were compared to the conventional AFB smear and TB culture method.
RESULTS: For all 95 respiratory specimens, the sensitivity and specificity of LC PCR relative to the final culture were 97.01% and 98.78%, respectively, with a PPV of 98.48%, an NPV of 97.59%, a +LR of 79.6 and a –LR of 0.03. When 84 sputa alone were analyzed, the sensitivity and specificity improved to 98.25% and 98.46%, respectively, with a PPV of 98.25%, an NPV of 98.46%, a +LR of 63.9 and a –LR of 0.02. Further accuracy in sensitivity and NPV was achievable by limiting the PCR analyses to only those 73 sputa that displayed AFB staining of ≥1+. The resulting sensitivity was 100.00%, with an NPV of 100.00%. PCR was negative on the 21 sputa that grew other non-tuberculous mycobacteria (16 MAC, 2 fortuitum, 2 abscessus, 1 gordonae).
CONCLUSION: Real-time PCR testing of smear-positive sputa (≥1+ AFB) was 100% sensitive, which allows the clinician to confidently prevent patients from undergoing unnecessary isolation and treatment early on when sputum PCR is negative. For bronchoaveolar lavage specimens or sputa with rare AFB, PCR was less reliable.

D3

COMPARISON OF TWO MOLECULAR TYPING METHODS USING MYCOBACTERIAL INTERSPERSED REPETITIVE UNIT AND RESTRICTION FRAGMENT LENGTH POLYMORPHISM FOR *M TUBERCULOSIS*

L CHUI*, T CHIU, G TYRRELL
Provincial Laboratory, Edmonton, Alberta

OBJECTIVE: The gold standard for typing M *tuberculosis* (MTB) is by IS*6110*-restricted fragment length polymorphism (RFLP), and the technique has been used extensively for epidemiological studies. However, this method is tedious and requires cultures. Moreover, strains with low IS6110 copy numbers cannot be adequately analyzed. The lack of reproducibility from different labs is a limitation to this assay. An alternative method for fingerprinting is by PCR of Mycobacterial Interspersed Repetitive Units (MIRU). The first objective of this study was to compare MIRU with RFLP and the second was to examine the feasibility of performing MIRU on prospective sputum samples.
MATERIALS AND METHODS: MTB isolates were heat-inactivated and, after a lysis step in the presence of lysozymes, DNA was extracted with a commercial kit (Cortex BioChem). The DNA was quantified and a standard amount of DNA from each isolate was tested by both RFLP and MIRU. For the prospective sputum samples, DNA was extracted by PSS, an automated extractor and tested for MTB by a commercial real-time PCR assay (Artus). All MTB-positive samples were typed by MIRU.
RESULT: There was 100% concordance between RFLP generated by southern hybridization with IS*6110* and MIRU. MIRU-based typing was also successful with DNA extracted from decontaminated sputum without isolation of the bacteria by culture.
CONCLUSION: MIRU is an alternative method to IS*6110*-RFLP typing because it is less labour intensive, more cost efficient and has a shorter turn-around time. There is no requirement for culturing the MTB isolates with fingerprinting by MIRU. For diagnostic laboratories that use nucleic acid-based detection of MTB, the DNA extracted from sputum samples can be used directly for typing using MIRU.

D4

COMPARISON OF THE GENOTYPE *MYCOBACTERIUM* CM AND GENOTYPE *MYCOBACTERIUM* AS ASSAYS TO HPLC FOR IDENTIFICATION OF *MYCOBACTERIUM* SPECIES ISOLATED FROM CLINICAL SPECIMENS

AK PETRICH*[1], K LUINSTRA[1], P DALEY[1], P CHEDORE[2], F JAMIESON[2]
[1]St Joseph's Healthcare, Hamilton; [2]Ontario Ministry of Health, Etobicoke, Ontario

OBJECTIVES: To compare new commercial line probe assays, Genotype *Mycobacterium* CM and AS (Hain LifeScience GmbH), to HPLC for the identification of *Mycobacteria* at the species level.
METHODS: Ninety-five *Mycobacteria* isolated and identified by HPLC in a reference laboratory over the past 10 years were cultured until positivity in the MGIT (Becton Dickenson). An aliquot (200 µL) of cultured material was extracted using the miniMag (bioMérieux) and eluted into 50 µL. All samples were tested using GenoType *Mycobacterium* CM kit, which identifies 14 common *Mycobacterium* species including M *tuberculosis* complex. Isolates identified as *Mycobacterium* species but not one of the 14 common species were tested with the GenoType *Mycobacterium* AS kit. This assay identifies an additional 16 *Mycobacterium* species. For all assays, purified nucleic acid was diluted 1:10 and 5 µL was added to the reaction mixture, which included Qiagen HotStar Taq DNA polymerase. Total time of the assay excluding extraction was approximately 3.5 h.
RESULTS: The HPLC and Hain results were concurrent for 81 samples. One sample had a unique line pattern that was not described in the package insert. Of 14 discordant samples, DNA sequence analysis of the 16S ribosomal RNA gene (MicroSeq, ABI Applied Biosystems) confirmed the identification obtained by the Genotype assays for 8 samples. The remaining 6 samples had 3 different identification results for HPLC, the Genotype assays and DNA sequence analysis. Three samples contained *Tsukamurella* or *Nocardia* species and were correctly identified by the Hain assay as high GC gram-positive organisms but not mycobacteria.
CONCLUSIONS: The Genotype *Mycobacterium* CM and AS assays are a reliable method for the identification of 30 of the most common *Mycobacterium* species found in clinical specimens.

E1

PROSPECTIVE SURVEILLANCE OF BLOODSTREAM AND VASCULAR ACCESS INFECTIONS IN HEMODIALYSIS PATIENTS

S GOULET*, AM BOURGAULT, J GAGNÉ, P LAPLANTE, P ST-ANTOINE, A LAROSE
CHUM Unité de prévention des infections, Québec

OBJECTIVE: Bacteremia and its complications place the hemodialysis patient at particular risk. The risk of infection varies according to the type of vascular access (VA). As part of a quality assurance program, following the merger of three hospitals, we conducted a prospective surveillance to determine the rates and risk factors for VA infections.
METHODS: Standardized questionnaires were filled for each patient: demography, indications for dialysis, type of VA device and complications. Rates were calculated per 1000 VA-days and per 100 patient-months.
RESULTS: Surveillance was conducted during 52 weeks in two hospitals and 32 weeks in the third hospital. A total of 662 VA in 490 patients (mean age 60 years) were followed. Major risk factors were (number of patients, %): diabetes (181, 37%), BMI <20 or >27 (169, 35%) and immunosuppression (40, 0.8%). There were 14 infections : 12 bacteremias (10 associated with cuffed catheters [KT], two with PTFE) and three cellulitis (cuffed KT). *Staphylococcus aureus* was responsible for nine of the 12 bacteremias.

Type of VA	No	%	Duration (days) Mean	Median	Infection rate/1000 VA-days	Infection rate/ 100 patient-months
Native fistula	273	41	1154	767	0	0
Cuffed KT	221	33	168	63	0.63	1.79
Temporary KT	116	18	13	6	0	0
PTFE	52	8	760	576	0.21	0.58
Total	662		592	178	0.17	0.48

CONCLUSION: The incidence of infections was low as compared to the CDC surveillance data. The patient population, the proportion of the different types of VA, the indications, protocols and techniques for insertion and maintenance of cuffed and non-cuffed KT contribute to these very good results.

E2
ANTIMICROBIAL RESPIRATOR WITH ENHANCED VIRAL FILTRATION EFFICIENCY OFFERS HIGHER LEVEL OF RESPIRATORY PROTECTION TO HEALTH CARE WORKERS

S BOURGET*[1], J TANELLI[1], D OHAYON[1], AM GENDRON[2], A STAFFA[2], ME SANS CARTIER[2], PJ MESSIER[1]
[1]Triosyn Corp, Williston, Vermont, USA; [2]Triosyn Research, Mirabel, Québec

OBJECTIVES: Recent SARS outbreaks have seen N95-wearing Health Care Workers (HCWs) develop the disease; probable sources of infection included airborne and droplet transmission while performing procedures that may generate aerosols (such as intubations), as well as contact transmission when removing contaminated Personal Protective Equipment (PPE). N95-certified respirators filter 95% of 0.3 µm particles, while allowing up to 5% of a respiratory hazard to pass through.
Triosyn has developed an improved N95 respirator by combining the equivalent of a N95 facemask with an iodinated polymer, adding an antimicrobial property to the particulate filtration efficiency.
METHODS: Viral Filtration Efficiency (VFE) of the respirators was evaluated by third-party laboratories. A bioaerosol containing 10^7 to 10^9 of MS2 coliphage is delivered using a Henderson apparatus and Collison nebulizers, and then filtered through swatches of the respirator membrane at a flow rate of 6.5 LPM. Effluent air is recovered in All Glass Impingers operated in parallel. Efficacy of the antimicrobial finish was determined following the AATCC Test Method *Assessment of Antibacterial Finishes on Textile Materials*, modified for a viral challenge.
RESULTS: Assessment of the Triosyn respirator VFE showed a 6-log reduction of the viral challenge, translating into a protection factor of 99.9999%. For comparison, three commercially available N95 respirators yielded an average 3-log reduction. As per AATCC 100-1999, viral challenge suspensions were completely devitalized by the Triosyn antimicrobial filtering membrane over short contact periods.
CONCLUSIONS: While reducing the risk of cross-contamination after disposal by devitalizing microorganisms entrapped within the filtering membrane, the N95-certified Triosyn respirator achieves standard BFE and offers an added protection against aerosolized viral threats.

E3
ARE PAGERS PORTERS FOR PATHOGENS?

D BEER*[1], B VANDERMEER[1], C BROSNIKOFF[2], S SHOKOPLES[2], R RENNIE[2], S FORGIE[1]
[1]University of Alberta; [2]Medical Microbiology Laboratory, University of Alberta Hospital, Edmonton, Alberta

OBJECTIVES: Healthcare-acquired infections are a problem in many facilities. Could pagers be a source of pathogenic organisms? If so, how should they be cleaned?
METHODS: A convenience sample of healthcare providers from our facility were asked to complete a brief survey and submit their pagers for bacterial culture and cleaning. Statistical analysis showed that a sample of 100 pagers would be sufficient: 15 pagers needed to be contaminated to reject the null hypothesis ($P<0.05$) that fewer than 10% of pagers would be contaminated with pathogenic bacteria. To achieve 80% power, 18 pagers needed to be contaminated with pathogenic bacteria.
RESULTS AND DISCUSSION: All 100 pagers were contaminated with bacteria. 12 pagers grew 14 pathogenic organisms, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella* species. One pager grew methicillin-resistant *Staphylococcus aureus* (MRSA). Demographic analysis revealed that no specific population was more likely than another to have a pager contaminated with pathogenic bacteria. The questionnaire revealed that 8% of healthcare workers cleaned their pager "at least occasionally" and 91% "never cleaned their pagers". The 12% contamination rate, although greater than our projected 10%, was not enough to achieve statistical significance.
The pagers randomized to cleaning with 0.5% chlorhexidine/70% isopropyl alcohol swabs, 70% isopropyl alcohol swabs or CaviWipes quaternary ammonium wipes all had reduced amounts of bacterial contamination. The efficacy of the chlorhexidine wipes was significantly greater than the other agents used (P value in comparison with second most efficacious cleaning agent was 0.00058).
CONCLUSIONS: This study did not attempt to document transmission of organisms or infection between individuals, but this remains a concerning possibility. Given the pathogens found on pagers and the efficacy, ease and safety of cleaning one's pager, it is reasonable to recommend regular cleaning of pagers with chlorhexidine swabs.

E4
EPIDEMIOLOGY OF NOSOCOMIAL BACTEREMIA: A SEVEN-YEAR REVIEW

AM BOURGAULT*, A LAROSE, S GOULET, C GAUDREAU, J VINCELETTE, P TURGEON, F LAMOTHE
Hôpital Saint-Luc du CHUM, Québec

OBJECTIVE: The rapid progress of medical science and technology has a major impact on health care practices. The objective of our study was to review the epidemiology of nosocomial bacteremias (NB) over the last seven years to analyze the trends and risk factors.
METHODS: A prospective surveillance program of NB based on the CDC NNIS System has been instituded in 1997. Standardized questionnaires are filled for each episode and data analyzed with the EPI info system.
RESULTS: During the last seven years, there were 1056 episodes of NB: 117 to 178 episodes per year with annual rates ranging from 6.8 to 10.3 per 1000 admissions. There were no significant changes in the rates, gender (60.8% male), and age (mean 59 years, median 61 years) over time. The frequence of NB differed among services, with very high rates in hepatology (67.3) and in hepatopancreaticobiliary surgery (45.7). The sources of infection, which did not vary significantly over time, were: primary bacteremia (47.1%) (catheter related 25.7% of the 1056 episodes); surgical site infections (20.9%); intraabominal infections (15%); urinary tract infections (9.9%); respiratory infections (4.5%), and others (2.5%). Rates of polymicrobial bacteremia remained stable, with an average of 15.7%. The germs isolated were *S aureus* (207), *Enterococcus* species (193), *E coli* (171), *Candida* species (110), *S epidermidis* (106), and *Klebsiella* species (105). More than 70% of enterococcal bacteremias were associated with liver or pancreatic diseases and surgeries. The proportion of methicillin-resistant *S aureus* (MRSA) increased from <10% in 1997 to >33% in 2004. NB was associated with a direct mortality of 12.4% and an overall mortality of 31%.
CONCLUSION: The high incidence of *Enterococcus* species bacteremia is linked to importance of the liver and pancreatic referal units. The percentage of catheter-related bacteremia has remained stable. The percentage of MRSA is constantly increasing. The profile of NB is related to the hospital clientele, hence the importance of local analysis as part of a quality assurance program.

F1

TRICHINELLOSIS OUTBREAK IN VICTORIA, BRITISH COLUMBIA

L MCINTYRE[1], S POLLOCK[2], M FYFE[3], J FUNG[1], Y SIMPSON[1], J ISAAC-RENTON[1], M MORSHED[1], A GAJADHAR[4]
[1]BC Centre for Disease Control, Vancouver; [2]University of British Columbia, Vancouver; [3]Vancouver Island Health Authority, Victoria, British Columbia; [4]Canadian Food Inspection Agency, Saskatoon, Saskatchewan

OBJECTIVES: Case identification of trichinellosis in Victoria, BC, led to an outbreak investigation associated with the consumption of wild bear meat.

METHODS: Interviews were conducted and food was retrieved for analysis. Cases were defined as those consuming bear meat with symptoms (fever, muscular and gastrointestinal symptoms). Compressed, raw, previously frozen bear meat was examined microscopically, and parasite levels were enumerated after enzymatic digestion. Species identification was determined by multiplex PCR, and infectivity was assessed by mouse bioassay.

RESULTS: Interviews were conducted on 63 individuals attending 3 separate barbeque (BBQ) events when the bear meat was consumed on May 28th, June 11th and July 3rd, 2005. 43 (68%) consumed bear meat, of which 27 (63%) reported illness (some of which were confirmed by serology). Encapsulated larvae were detected microscopically in muscle cells, and digestion assay performed in two laboratories revealed more than 300 non-motile larvae per gram of tissue. Multiplex PCR on 21 individual larvae consistently identified genotype T2 (*T nativa*). No parasites were observed in mice infected with the bear meat.

DISCUSSION: Consumption of game meat (bear meat, in this case) was linked to over 25 illnesses in Victoria, BC. Although the meat was frozen at least once for a minimum of 72 hours prior to any of the BBQ events, *T nativa* is known to be resistant to cold. The genotyping of all 21 individual larvae as T2 suggest that only *T nativa* was involved in this outbreak. Initial freezing and cooking practices were inadequate to prevent infection, although repeated freezing of the bear meat probably rendered the *Trichinella* larvae non-infective in the mouse assay.

F2

QUALITY ASSURANCE FOR THE DIAGNOSIS OF MALARIA USING TWO REAL-TIME PCR ASSAYS

A MCNABB*, R CHEN, T LO, Q WONG, J ISAAC RENTON
Laboratory Services, BC Centre for Disease Control, Vancouver, British Columbia.

OBJECTIVE: To investigate the feasibility of using a published real-time PCR assay for the detection and identification of *Plasmodium* species to function as a QA/QC program to aid in the ongoing optimization for the diagnosis of malaria in a low prevalence setting.

METHODS: Two real-time PCR assays (de Monbrison et al [Trans Roy Soc Trop Med Hyg 3003;97:387-390] and Mangold et al [J Clin Microbiol 2005;43:2435-2440]) were compared using 65 positive malarial samples (*P falciparum* [32], *P malariae* [6], *P ovale* [3] and *P vivax* [24]) and 10 negative plasma samples to determine assay performance in our laboratory.

RESULTS: Both assays confirmed all 32 *P falciparum*-positive and 10 *Plasmodium*-negative samples. The PCR assay of de Monbrison et al confirmed 1/6 *P malariae*, 2/3 *P ovale*, 16/24 *P vivax*, failed to detect 6 *P vivax*, 4 *P malariae* and 1 *P ovale*, and identified 1 *P malariae* as *P vivax*, 1 *P vivax* as *P falciparum* and only detected *P falciparum* from a mixed *P malariae* and *P falciparum* specimen. The PCR assay of Mangold et al confirmed 1/6 *P malariae*, 3/3 *P ovale*, 20/24 *P vivax*, identified 4 *P malariae* as *P ovale*, 3 *P vivax* as *P ovale* and only detected *P malariae* from a mixed *P malariae* and *P falciparum* specimen.

CONCLUSIONS: During this study, we found the PCR assay method of Mangold et al easier to perform and more accurate than the PCR assay published by de Monbrison et al and recommend that the assay can be used as a QC/QA aid to confirm the presence of malarial species from specimens in a low prevalence area, but should not be considered as a replacement for current detection methods.

F3

CLINICAL CHARACTERISTICS AND OUTCOMES IN PATIENTS WITH PULMONARY BLASTOMYCOSIS

D KRALT*[1], J EMBIL[2], B LIGHT[2], M CHEANG[3], T CRAMPTON[4], L WIEBE[5], B LIMERICK[5], P SARSFIELD[5], K MACDONALD[6]
[1]Faculty of Medicine; [2]Department of Medicine, Section of Infectious Diseases; [3]Department of Community Medicine; [4]Department of Diagnostic Imaging, University of Manitoba, Winnipeg, Manitoba; [5]Northwestern Health Unit; [6]Department of Pathology, Lake of the Woods District Hospital, Kenora, Ontario

OBJECTIVES: To review the outcomes of patients with pulmonary blastomycosis, focusing on the epidemiological characteristics of patients with pulmonary blastomycosis, the role of seasonal variation in clinical presentations, and the physiological/hemodynamical characteristics of patients admitted to the ICU with respiratory failure.

METHODS: A retrospective study of 353 patients with blastomycosis identified in hospitals in Manitoba and northwestern Ontario revealed 318 patients for whom a complete data set was available, of which 296 had pulmonary blastomycosis.

RESULTS: The majority of patients were Caucasian (62.3%), male (60.7%), and from Ontario (65.7%). An over-representation of Aboriginal patients was observed (32.1%). The majority of patients had localized lung disease (≤3 quadrant involvement on chest radiograph). Of 294 patients requiring hospitalization, 90.5% received all their care on an inpatient ward, and 9.5% received care in the intensive care unit (ICU). The factors associated with ICU admission included diffuse pulmonary disease (4 quadrant involvement on chest radiograph), receipt of antecedent antimicrobial therapy, and diabetes. Patients who died were more likely to be older (59.6±19.2 versus 37.7±19.0; $P<0.001$) and be of aboriginal ethnicity. For the 28 ICU patients, the 10 (35.7%) who died had higher initial severity of illness scores. Haemodynamic parameters for the ICU patients were indistinguishable from bacterial sepsis. The acute respiratory distress syndrome was observed in 21 (75%) patients, of which 8 (38.1%) died. The overall mortality for pulmonary blastomycosis in this group was 8.1% (24/318). A distinct seasonal variation was observed in clinical presentations, with those presenting in autumn and winter having localized pneumonia, while diffuse pulmonary disease mainly occurred in spring and summer, suggesting "primary", and "post-primary" or "reactivation" presentations similar to tuberculosis.

CONCLUSION: Blastomycosis must be considered a cause of serious pulmonary infection in our geographic region.

F4

A NINE-YEAR, SINGLE-CENTRE EXPERIENCE WITH CANDIDEMIAS

AC LABBÉ, C PATINO, C RESTIERI, M LAVERDIÈRE*
Hôpital Maisonneuve-Rosemont, Montréal, Québec

OBJECTIVES: To review the clinical and microbiologic features of candidemias that have occurred at Hôpital Maisonneuve-Rosemont between April 1996 and August 2004.

METHODS: Isolates from episodes at least seven days apart were retrieved from the microbiology laboratory. Antifungal susceptibility was determined by the CLSI M27A2 method.

RESULTS: *C albicans* was identified in 58% of the 174 different episodes, followed by *C glabrata* (14%), *C parapsilosis* (11%), *C krusei* (8%), *C tropicalis* (7%), and other *Candida* species (2%). 77 episodes (44%) were sustained with an average of 6 positive cultures (range 2 to 32) over 7 days (range 2 to 23). The most common underlying conditions were hemato/oncology diseases in 43% of the episodes, recent abdominal surgery (24%) and presence in intensive care unit (15%). Intravenous central line, corticosteroid therapy and colonization at two non-contiguous sites were present in 87%, 47% and 11% of the episodes, respectively. All-cause mortality at 30 days was 30%. MIC 90 (mg/L) of amphotericin B (AmB), fluconazole (F), itraconazole (I), voriconazole (V), ravuconazole (R) posaconazole (P), micafungin (M) and anidulafungin (A) were as follows:

Species (n)	AmB	F	I	V	R	P	M	A
C albicans (101)	1	2	0.12	0.06	0.06	0.12	0.5	0.06
C glabrata (25)	2	128	32	2	4	4	0.25	0.12
C parapsilosis (19)	2	4	0.5	0.12	0.12	0.25	16	4
C krusei (13)	2	64	1	0.5	1	1	1	0.25
C tropicalis (12)	1	2	1	0.25	0.12	0.25	0.5	0.5

All 32 azole (F and/or I)-resistant isolates were inhibited by ≤0.25 mg/L of A compared to 25/32 for M.

CONCLUSIONS: Frequency, species distribution, and sensitivities of candidemic isolates remain stable over the years. Newer antifungal agents have a high in vitro activity against bloodstream isolates.

G1
A FATAL CASE OF TRANSFUSION-TRANSMITTED *SERRATIA MARCESCENS*

SM RAMIREZ-ARCOS*[1], I CHIN-YEE[2], H HUME[1], M FEARON[1], M GOLDMAN[1], K ECKERT[2], I MARTINCIC[1], G PETERS[2], D KOVACH[3], SE RICHARDSON[3]
[1]Canadian Blood Services, Ottawa; [2]London Health Sciences Centre, London; [3]Hospital for Sick Children, Toronto, Ontario

BACKGROUND AND OBJECTIVE: Bacterial contamination of blood products poses the highest risk of post-transfusion infection. We report a fatal septic shock case occurring after transfusion of an apheresis platelet unit in spite of a negative BacT/ALERT culture performed 29 h after collection. Within 30 minutes of starting the transfusion, the three-year-old patient was transferred to the intensive care unit, where she died of multisystem organ failure. The platelet product and patient's autopsy samples were positive for *Serratia marcescens*. The objective of this study was to investigate the source of *Serratia* contamination.

METHODS: Samples obtained during platelet-volume reduction as well as several items having the same lot number as the items used during collection of the transfused unit were analyzed for *S marcescens* contamination. *S marcescens* isolated from the implicated platelet unit and from the patient's autopsy samples were genotyped by pulsed-field gel electrophoresis (PFGE). To investigate whether initial low bacterial concentration was the cause of the missed cultures, apheresis platelet units were spiked with low concentrations of *S marcescens* and sampled for BacT/ALERT cultures at 29 h after spiking.

RESULTS: While samples obtained during platelet-volume reduction were positive for *S marcescens*, collection items were negative for this bacterium. Blood and urine samples provided by the donor were also negative and the donor confirmed that he was not ill prior to or after the transfusion. PFGE genotyping of *S marcescens* isolated from different samples showed they had identical band patterns. Spiked platelets with low bacterial concentrations resulted in positive BacT/ALERT cultures.

DISCUSSION: We did not identify the source of contamination in this case. It is possible that the platelets were contaminated during sampling or that the bacterium may have been present at the time of collection, but was missed due to very low bacterial concentration or sampling error. There is therefore an urgent need to develop bacterial detection systems with a rapid turn around time that can be used close to the time of transfusion.

G2
LABORATORY PRACTICES FOR PRENATAL GROUP B STREPTOCOCCUS (GBS) SCREENING IN ONTARIO

CA FLEMING*,C RUTHERFORD, F JAMIESON, B TOYE, L BORYCHESKI, S POUTANEN, A SARABIA, J GUN-MUNRO, H RICHARDSON
Quality Management Program-Laboratory Services, Toronto, Ontario

OBJECTIVE: To assess the level of compliance in Ontario labs with published guidelines for prenatal GBS screening.

METHODS: The 91 Ontario labs licensed for bacteriology in April 2005 were required to complete a questionnaire on practice with prenatal genital specimens for GBS screening. Results were compared to the 2002 recommendations of the Centers for Disease Control and Prevention (CDC), Atlanta, and the 2004 guidelines published by the Society of Obstetricians and Gynaecologists of Canada (SOGC).

RESULTS: Of 89 responding labs, 85 (96%) performed prenatal GBS screening. Of these, 82 processed combined vaginal/anorectal swabs, 64 processed vaginal swabs and 9 processed endocervical swabs. On initial processing, 26/85 (31%) used selective enrichment broth only, 35 (42%) used a combination of selective broth and agar media and 24 (28%) used culture protocols that were not in keeping with the current guidelines. 30 labs subcultured the selective broth to BA, 23 to CNA, 4 to BA and CNA, 2 to BA and PEA, 1 to PEA, and 1 to BA with neomycin. Protocols for GBS bacteriuria during pregnancy were as follows: 35 labs reported GBS from urine when pregnancy was noted, 20 from all women of child-bearing age, 5 on all urines from female patients and 21 on all urines regardless of sex or age. 46 reported any amount of GBS present in these urine specimens, 30 if the count was $\geq 10\times10^6$ CFU/L, 5 if $\geq 100\times10^6$ CFU/L, 1 if $\geq 50\times10^6$ CFU/L and 1 if 'significant growth'. 51/85 (60%) routinely performed antibiotic susceptibility testing (AST) on all GBS isolates from prenatal screening specimens and 19 (22%) did so only on request. Of the 70 labs that performed AST, all tested erythromycin, 68 tested clindamycin and 32 tested vancomycin. 31 labs automatically reported clindamycin as resistant if macrolide-resistant, and 36 performed the disk approximation test to screen for inducible clindamycin resistance.

CONCLUSION: A significant number of microbiology labs in Ontario are not in full compliance with the CDC and SOGC guidelines. QMP-LS has released these findings to Ontario labs along with a summary of the current guidelines encouraging labs to update their protocols.

G3
AN OUTBREAK OF *SALMONELLA* IN BRITISH COLUMBIA, ALBERTA AND WASHINGTON LINKED TO PET TREATS

L MCINTYRE*[1], S BRISDON[2], L WILCOTT[1], E GALANIS[1], L MACDOUGALL[1], L GUSTAFSEN[2], D EVERETT[4], L CHUI[3], D MACDONALD[5], R BAER[6], R COLINDRES[7], A ELLIS[5], L CROWE[4]
[1]BC Centre for Disease Control, Vancouver; [2]Fraser Health Authority, Surrey, British Columbia; [3]Provincial Laboratory, Edmonton; [4]Calgary Health, Calgary, Alberta; [5]Health Canada, Guelph, Ontario; [6] Washington State Department of Health, Shoreline, Washington, USA; [7]Centres for Disease Control and Prevention, Atlanta, Georgia

OBJECTIVES: A case of *Salmonella thompson* in an Alberta resident, and further case identification in British Columbia, led to an investigation of pet treats.

METHODS: Cases were comprised of persons living in the US or Canada with *S thompson* isolated from stool between August 2004 and June 2005 and a PFGE national designation pattern of STHXA1.0034. Retrospective case findings and case interviews were conducted in British Columbia (BC), Alberta (AB) and Washington (WA). Trace-back of pet treats led to inspections of pet food facilities and collection of pet treat samples from cases' homes, pet stores, distributors and manufacturers in BC and WA. Human and animal fecal samples and pet treats were tested for *Salmonella*, and serotypes and Pulsenet standardized PFGE was performed.

RESULTS: Nine cases of *S thompson* PFGE STHXA1.0034 were identified (2 in AB, 4 in BC and 3 in WA). Six cases from which information was available (75%) had exposure to pet treats; in five of these instances, treats were manufactured in a BC or WA plant. Two other cases had dogs. The implicated treats were made from raw dehydrated salmon, shrimp and/or beef. The salmon treats had elevated counts of *Salmonella* ranging from less than 100 CFU/gram to 80,000 CFU/gram. *S thompson* was found in the salmon, shrimp and beef jerky treats, including 5 other distinct PFGE patterns. Further *Salmonella* serotypes identified included *S montevideo*, *S newport*, *S give*, *S meleagridis*, *S cero*, and *S muenster*.

DISCUSSION: Pet treats are not processed and regulated in the same manner as human foods. These pet treats were not subjected to any heat treatment or kill step, which resulted in high levels of *Salmonella* contamination. Even though the treats were not consumed, handling of pet treats or infected pets caused human illness.

G4
MICROBIOLOGY AND EPIDEMIOLOGY OF *STAPHYLOCOCCUS LUGDUNENSIS* INFECTIONS IN A REGIONAL HEALTHCARE SYSTEM

S ELSAYED*, K ZHANG
[1]University of Calgary; [2] Calgary Laboratory Services, Calgary, Alberta

OBJECTIVES: *Staphylococcus lugdunensis* is a highly virulent coagulase-negative staphylococcus with a spectrum and severity of human infection mimicking that of *Staphylococcus aureus*. However, our understanding of the microbiology and epidemiology of infections caused by this pathogen has been based on isolated case reports or small case series. We describe our 6-year analysis of *S lugdunensis* clinical isolates and human infections in a large, integrated, Canadian, urban healthcare region.
METHODS: A centralized laboratory provides virtually all acute care diagnostic microbiology services in our health region, including hospitals, nursing homes, and doctors' offices. Phenotypically well-characterized clinical isolates of *S lugdunensis* recovered from patients in our health region during the 6-year period from December 1999 to November 2005 were retrospectively studied. Patient demographic and clinical information, along with antimicrobial susceptibility profile data of individual isolates, were obtained and analyzed accordingly.
RESULTS: A total of 26 non-duplicate clinical isolates of *S lugdunensis* were recovered during the study period. The average age of affected patients was 57.5 years (range 12 to 82 years) with no gender predominance. Of these, 12 were recovered from lower extremity wounds/abscesses, 5 from blood cultures, 2 from breast abscesses, 1 from synovial fluid, 1 from cornea, 1 from gortex graft, and 4 from other wounds. Of these, 21 were recovered in pure culture. Susceptibility results were available for 22 strains of which 18 (81.8%) were beta-lactamase negative. Resistance to macrolides and lincosamides was noted in only 4 isolates, while all strains were susceptible to sulfonamides and quinolones. No isolates were methicillin-resistant.
CONCLUSIONS: Our study provides important data on the microbiology and epidemiology of *S lugdunensis* infections in a regional healthcare system and may serve useful in guiding the management of human infections caused by this microbe.

G5
PNEUMOCOCCI-LIKE *STREPTOCOCCUS MITIS* BACTEREMIA IDENTIFIED IN AN IMMUNOCOMPROMISED HOST

SX ZHANG*[1], BM WILLEY[2], L LOUIE[3], DE LOW[2], A MCGEER[2], M MINDEN[4], SM POUTANEN[2]
[1]University of Toronto; [2]Toronto Medical Laboratory and Mount Sinai Hospital; [3]Sunnybrook and Women's College Health Science Centre; [4]University Health Network and Univeristy of Toronto, Toronto, Ontario

OBJECTIVES: Pneumococci-like *Streptococcus mitis* have recently been reported from our group from respiratory specimens submitted to a cross-Canada *Streptococcus pneumoniae* (SPN) surveillance program. No such isolates have been identified from sterile site specimens. Recently, a patient presenting with febrile neutropenia had an alpha-hemolytic, optochin-susceptible (OPT-S) streptococcus isolated from a blood culture along with a similar optochin-resistant isolate. SPN AccuProbe (Gen-Probe) was negative for both isolates and 16S rDNA sequencing identified both to be *S mitis*. Characteristics of these isolates are described.
METHODS: Both isolates were characterized base on colony morphology, plate bile solubility, OPT-S, SPN Phadebact agglutination (Boule), and antibiogram. PCR for autolysin and pneumolysin genes (*lytA* and *ply*) and PFGE using SmaI were completed.
RESULTS: Colonies from both isolates showed alpha-hemolysis. Neither had typical SPN morphology and both were plate bile-insoluble. However, one of the two was OPT-S and both were positive by Phadebact and contained *ply* and neither had *lytA*. PFGE revealed a 2-band difference between the isolates; the OTP-S isolate appeared to have a DNA insertion in an existing fragment. Both were non-susceptible to erythromycin but susceptible to penicillin and levofloxacin.
CONCLUSIONS: The identification of an OPT-S, Phadebact-positive, *ply*-positive *S mitis* from a clinically significant blood culture demonstrates that these test characteristics should not be used for definitive identification of SPN. The identification of SPN phenotypic features such as OPT-S and SPN virulence factors such as *ply* in *S mitis* illustrates the genome plasticity between SPN and *S mitis*. Further studies are needed in order to determine the potential clinical importance of *S mitis* that have acquired SPN DNA and the need for clinical microbiology laboratories to identify these pneumococci-like *S mitis* as distinct from *S mitis* and SPN.

G6
PSEUDO-OUTBREAK OF METHICILLIN-SUSCEPTIBLE *STAPHYLOCOCCUS AUREUS* BACTEREMIA IN PATIENTS RECEIVING COMBINATION THERAPY FOR CHRONIC HEPATITIS C VIRUS (HCV) INFECTION

D WEBSTER*[1], R AHMED[1], L CHUI[2], R MCDONALD[3], K DOUCETTE[1]
[1]Division of Infectious Diseases; [2]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta; [3]Provincial Laboratory, Saskatchewan Health, Regina, Saskatchewan

BACKGROUND: Bacteremia, particularly *S aureus* bacteremia, has rarely been reported in patients receiving HCV therapy.
OBJECTIVE: The purpose of this report is to describe the features and investigation of a pseudo-outbreak of *S aureus* bacteremia in 4 patients receiving combination therapy for chronic HCV infection.
METHODS: Between November 3rd 2004 and January 10th 2005, we recognized *S aureus* bacteremia in 4 patients receiving pegylated interferon alpha-2b (Peg-IFN) and ribavirin for treatment of chronic HCV infection. An epidemiologic investigation was carried out and molecular typing methods using pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP) to generate fingerprinting patterns were used to further characterize relatedness of the *S aureus* strains.
RESULTS: The mean time of bacteremia was week 10 of Peg-IFN and ribavirin treatment. No patient had preceding neutropenia. The average duration of bacteremia was 2.6 days and complications included acute renal failure (2/4), coagulopathy (2/4), sepsis syndrome (1/4), septic arthritis (1/4), spinal epidural abscess (1/4), and endocarditis (1/4). No further cases were identified despite contacting HCV treaters locally and nationally. Two patients were in the same weight class for dosing, but no other epidemiologic links were found. One patient admitted to and another was suspected of injection drug use (IDU). The other two patients were cirrhotic, but had no other risk factors for *S aureus* bacteremia. All isolates were methicillin-susceptible with an identical antibiogram. By PFGE, two were found to be indistinguishable; however, AFLP analysis demonstrated distinct molecular fingerprints of all 4 isolates.
CONCLUSION: This case series describes a cluster of *S aureus* bacteremia in patients receiving combination therapy for the treatment of chronic HCV infection. As no epidemiologic link or molecular evidence of a common strain was found, this appears to have been a 'pseudo-outbreak', with IDU and cirrhosis identified as risk factors.

H1
HIV, SEXUALLY TRANSMITTED INFECTIONS AND MASSAGE PARLOUR WORKERS: APPLICATION OF SOCIAL NETWORK ANALYSIS TO DETERMINE THE POTENTIAL FOR DISEASE PROPAGATION

VP REMPLE*[1], A JOLLY[2], DM PATRICK[1], MW TYNDALL[3], C JOHNSTON[4]
[1]UBC Centre for Disease Control, Vancouver, British Columbia; [2]Public Health Agency of Canada, Ottawa, Ontario; [3]BC Centre for Excellence in HIV/AIDS; [4]Asian Society for the Intervention of AIDS, Vancouver, British Columbia

OBJECTIVES: To determine the structure and characteristics of massage parlour workers' sexual networks, and the potential for HIV and STI transmission within the networks, and to lower risk in the general populations.
METHODS: In-depth interviews elicited sociodemographic, sexual

health, sexual behaviour, and egocentric network data from parlour workers and proxy data on their most recent 5-10 sex partners.

RESULTS: Interviews were conducted in seven parlours with 39 women and included 226 sexual dyads, 202 of whom were with commercial clients. Workers reported a median of 25 (IQR 12 to 48) sex partners per month, and provided proxy network data for a median of 6 (IQR 4.5 to 7) partners. Ninety-one per cent (95% CI 72 to 99) and 37% (95% CI 16 to 62) reported consistent condom use with clients and intimate partners, respectively. Age difference was greater than 5 years in 67% (95% CI 62 to 75) of dyads, and the proportion of race discordance was 66 (95% CI 59 to 72). The total giant component size was 428, with a density of 1.5% and high frequency of microstructures. Twenty-one per cent of clients were identified as "house regulars" (sex with all workers) who were highly central to the total network and bridged between sex venues and/or to the general population. Approximately 62% (95% CI 55 to 68) of the dyads were concurrent, and 51% (95% CI 41 to 54) of clients had a wife or girlfriend. Thirty-nine per cent (95% CI 30 to 49) of clients bridged between sex venues, and 52 (95% CI 43 to 61) of clients and parlour workers combined bridged to the general population.

DISCUSSION: Social network analysis provides valuable tools with which to examine HIV and STI risk at the individual, dyadic and network levels. The potential for transmission of STI throughout the massage parlour networks and to the lower risk general population is high, particularly for STIs for which condom use does not provide adequate protection. Outreach education should focus on condom use with non-paying partners, and reinforcement of 100% condom use with clients, with particular emphasis on "house regulars".

H2

INCIDENCE OF RESPIRATORY CORONAVIRUS INFECTION IN A SYMPTOMATIC PAEDIATRIC POPULATION

C DANIS[1], C ROUSSEAU[2], C LAFERRIÈRE[2], H CHAREST*[1]
[1]Laboratoire de santé publique du Québec, Sainte-Anne-de-Bellevue; [2]Hôpital Sainte-Justine, Montreal, Québec

BACKGROUND: Coronaviruses (CoV) are usually associated with the common cold. However, recent evidence suggests they can cause more severe infections such as life-threatening pneumonia.

OBJECTIVES: To evaluate the incidence and the clinical importance of CoV infection in respiratory specimens of a symptomatic paediatric population over a full year period and to describe the seasonal distribution and clinical manifestations of the infection.

METHODS: A total of 1132 specimens, representing almost all respiratory samples submitted throughout the year 2001 at the virology laboratory of a large paediatric tertiary care centre, were screened for CoV-specific RNA by RT-PCR. Primer pairs used were selected to specifically amplify common CoV such as prototypes 229-E and OC-43, or the newly described New Haven isolate (CoV-NH). Clinical data were compiled from medical records according to a pre-established scheme.

RESULTS: CoV were detected in 28 of all clinical samples tested (2.5%); CoV-NH represented 29% (8/28) of positives. The majority of infections (73%) occurred during the respiratory disease season (November to March); 19% were found in spring, and three isolated cases were detected in the summer. For CoV-positive paediatric cases reviewed (n=18), 83% were from infants younger than four years of age. Symptoms and signs included fever (56%), cough (67%), and gastrointestinal symptoms (44%). Diagnoses included pneumonia (28%), bronchiolitis (17%), and Kawasaki disease (5%); 94% of cases were hospitalized cases. An important proportion of co-infections was observed (39%).

CONCLUSIONS: CoV may cause severe infections in infants and children, or exacerbate infections caused by other respiratory viruses. The gastrointestinal symptoms observed in CoV-infected patients have significant implications for clinical presentation and transmission prevention efforts in hospital settings.

H3

DECREASE IN *CAMPYLOBACTER* RATES COINCIDING WITH AN AVIAN INFLUENZA OUTBREAK IN POULTRY, BRITISH COLUMBIA, 2004

E GALANIS*, L MACDOUGALL, K LABERGE, B RADKE
BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: To determine if the drop in *Campylobacter* infection rates in BC in 2004 was significant and associated with the H7N3 poultry avian influenza outbreak.

METHODS: Time series analysis of *Campylobacter* and *Salmonella* cases reported provincially from January 1995 to June 2005 was conducted using SPSS. Data on production of poultry, poultry imports and stocks of frozen poultry in BC from 2000 to June 2005 were reviewed using Microsoft Excel.

RESULTS: In 2004, 1472 cases (3.5/10,000) of *Campylobacter* infection were reported in BC, a drop of 31% as compared to an average of 2134 cases per year (5.2/10,000) between 2000 and 2003. There was a significant decrease in *Campylobacter* infection rates from May to October 2004 as compared to previous years. In 2004, *C jejuni* accounted for 57% of the *Campylobacter* isolates, whereas it accounted for 96% between 2000 and 2003. There was no decrease in the rate of *Salmonella* or *S heidelberg* (exclusive to poultry) in 2004. Poultry production decreased 15-fold and poultry imports increased 15-fold in BC from May to July 2004, as compared to previous years. Frozen poultry stocks increased by 52% in BC in 2004, starting in April, and remained high until March 2005.

CONCLUSIONS: During the AI outbreak in BC, *Campylobacter* rates dropped significantly. The time frame when this occurred does not exactly coincide with any of the poultry indicators. Potential explanations include a decreased availability and consumption of poultry, safer handling of poultry and consumption of poultry with a lower load of *Campylobacter* (imported, frozen and processed poultry). A better understanding of the contribution of these factors may lead to better control of this disease.

H4

COMPARISON OF THE CLINICAL PRESENTATION OF NOSOCOMIAL LINE-RELATED BACTEREMIA WITH MSSA (S.A) AND MRSE (S.CN)

A MUKHERJI*[1], C THOMPSON[2]
[1]Dalhousie University, Halifax, Nove Scotia, and AHSC, Saint John, New Brunswick; [2]University of Louiseville, School of Public Health, Louiseville, Kentucky, USA

BACKGROUND: Nosocomial *Staphylococcus* bacteremias are common.

METHODS: Retrospective chart review of the peri and bacteremia period.

RESULTS: For S.A/S.CN: mean age was 62.6 versus 58.74 (P=0.136), males 58.25% (60/103) versus 57.58% (38/66) (P=0.931). **Clinical:** Asymptomatic 0% versus 1.5%; fever (>38.3°C) 85.4% versus 75.8%; Tmax 38.4 for both groups; chills/rigours 35.0% versus 21.2%; Lethargy 28.2% versus 16.7%; confusion 16.5% versus 6.1%; tachypnea (RR>22) 3.9% versus 15.2%; tachycardia (P>100) 6.8% versus 6.1%; shock (SystBP<90) D-1 13.90% versus 21.21% (P=0.004); Day O 10.68% versus 21.21% (P=0.06). Increased WBC 12.6% versus 18.2%; WBC max 13.5 versus 12.6. The mean number of initial + BC bottles was 2.49 (SE mean=0.14) versus 2.67 (SE mean=0.15). At Day 7, the mean number + BC was 2.63 (SE mean=0.15) versus 3.03 (SE mean=0.18). At Day 14, the mean number + BC was 2.68 (SE mean=0.15) versus 3.14 (SE mean). The difference between the two groups was not significant initially (P=0.391), and only weakly significant at both day 7 (P=0.092) and day 14 (P=0.061). Risk analysis (dialysis/DM/pacemaker/orthoimplants) were not significantly different between groups. Overall, 15.79% (15/95) of the pts had risk of complicated bacteremia (fever >72 h) versus 33.33% (21/63) of S. CN. This difference was statistically significant (P=0.010). Using logistic regression, none of the risk factors above showed any clear association with risk of complicated bacteraemia. **S.A:** Of patients with complications, 29.63% (8/27) had fever >72 h. Of patients without complications, 10.29% (7/68) had fever >72 h (P=0.02). **S.CN:** Of patients with complications, 30.0% (6/17) had fever >72 h. Of patients without complications, 30.43% (14/46) had fever >72 h. **Initial complications:** None 73.7%

versus 72.7%; endocarditis 22.3% versus 13.6%; Xray c/w vertebral osteomyelitis 5.8% versus 3.0%; septic thrombophlebitis 2.9% versus 9.1%. Complications after 6 months: 10.7% % versus 4.6%.

CONCLUSION: Our study patients had greater number of S.CN with shock. Patients who were febrile for >72 h had increased risk of complications in both S.A and S.CN grps. Low power, selection/information bias and single centre are some study limitations with the limitations of selection/information bias and external generalizability.

H5
THE RAPID GROUP B STREPTOCOCCUS PCR TEST PROVIDES A METHOD FOR RELIABLE DETECTION OF GROUP B STREPTOCOCCUS CARRIAGE IN WOMEN IN LABOUR

MJ ALFA*, GK HARDING, P DEGAGNE, B SHEARD
[1]St Boniface Hospital; [2] University of Manitoba; [3] Diagnostic Services of Manitoba, Winnipeg, Manitoba

OBJECTIVES: Current National guidelines indicate that screening of pregnant women for *Streptococcus agalactiae* (Group B streptococcus) is recommended in week 35-37 of pregnancy. Women often present in labour and their GBS status is unknown. The aim of this study was to compare the rapid PCR-based test to enrichment culture to determine if it accurately predicts women who carry GBS when in labour.

METHOD: Combined vaginal/rectal samples were collected for 200 women who presented in labour at SBGH and gave informed consent. Each sample was eluted from the swab and the eluate was evaluated by the IDI Rapid GBS PCR test (Somagen Diagnostics) as well as by culture using Todd-Hewitt enrichment broth containing 10 µg/mL Colistin and 15 µg/mL Naladixic Acid. Chart reviews were also done.

RESULTS: The sensitivity, specificity, PPV and NPV for the rapid GBS screening test was 90.7%, 96.2%, 86.7% and 97.4%, respectively. The discordant results included 4 that were culture-positive but PCR-negative and 6 that were culture-negative but PCR-positive. There was one culture-positive, PCR-negative sample that was PCR-positive when repeat testing was performed after a freeze/thaw (as recommended by manufacturer); however, the other 3 discordants were non-resolvable by repeat testing. For the PCR-positive, culture-negative discordants, one grew GBS after re-subculture, 4 were from patients who received intra-partum antibiotics, and one was non-resolvable. After resolution of the discordant results, the rapid GBS sceening test sensitivity, specificity, PPV and NPV were 93.8%, 99.3%, 99.7% and 98.1%, respectively.

DISCUSSION: The rapid GBS PCR test provides excellent sensitivity, specificity, PPV and NPV when used to screen pregnant women in labour. Based on the performance of this test in our labour and delivery area, we would recommend a strategy of targeted implementation where routine screening in week 35-37 is performed by standard culture methods and the PCR test is made available for those women in labour for whom the GBS status is not known.

I1
A LONGITUDINAL STUDY OF MACROLIDE RESISTANCE IN PHARYNGEAL ISOLATES OF GROUP A STREPTOCOCCUS (GAS) IN NOVA SCOTIA

SJ CAMPBELL*[1], S MACDONALD[2], D HALDANE[1], K FORWARD[1], RJ DAVIDSON[1]
[1]Queen Elizabeth II Health Sciences Centre; [2]Dalhousie University, Halifax, Nova Scotia

OBJECTIVE: For penicillin-allergic patients, macrolides are frequently used as an alternative for the treatment of non-invasive GAS. Erythromycin (Ery) and clindamycin (Clin) resistance in GAS has been reported with increasing frequency. This study was performed to determine the prevalence of Ery, Clin and telithromycin (Tel) resistance in pharyngeal isolates of GAS in Halifax, Nova Scotia.

METHODS: GAS isolates were collected in Halifax between November 1998 and June 1999, October 1999 and February 2000, February 2002 and February 2003, October 2003 and March 2004, and June and December of 2005. Susceptibilities to Ery, Clin and Tel were performed using agar disk diffusion and the phenotype determined using a double disk diffusion method. PFGE was performed on a subset of isolates.

RESULTS: In total, 4368 GAS isolates were collected. Between 1998 and 2000, 4.5% of isolates were resistant to Ery. Of these, 80% exhibited MLS_B resistance. 7.7% had a constitutive phenotype ($cMLS_B$) and 72% had an inducible phenotype ($iMLS_B$). The remaining strains (20.3%) displayed the M phenotype. In 2002, Ery resistance increased significantly to 25.3%. High-level resistance accounted for 65% of the resistant isolates. Two clones accounted for 95% and 85% of the MLS_B and M phenotypes, respectively. For strains collected in the 2003 to 2004 and 2005 time periods, Ery resistance decreased to approximately 19%. High-level resistance (MLS_B) accounted for >85% of the resistant strains, primarily the $iMLS_B$ phenotype. We failed to find a single isolate expressing Tel resistance in the entire collection.

CONCLUSION: Significant increases in resistance have been observed over the last 5 years. Unlike *S pneumoniae*, where macrolide resistance is primarily low-level, high-level resistance predominates in GAS. Although this study examined pharyngeal isolates of GAS, it underscores the importance of susceptibility testing, particularly in invasive GAS.

I2
VANCOMYCIN-RESISTANT *ENTEROCOCCI* DETECTION IN STOOL SAMPLES SUBMITTED FOR *CLOSTRIDIUM DIFFICILE* TOXIN TESTING

BJ MACK*, S HENWICK, DB PURYCH, JL TOMBLIN, C WONG, A LAU
Medical Microbiology Committee, Fraser Health, British Columbia

OBJECTIVE: Patients who develop antibiotic-associated diarrhea (AAD) from *Clostridium difficile* often have risk factors for colonization with vancomycin-resistant *Enterococci* (VRE). The present investigation was carried out to determine if testing for VRE on stool samples submitted for CD toxin (CDT) testing would lead to an increased detection of VRE-colonized patients. In addition, stool samples were tested for *S aureus*, which has been associated with treatment-resistant AAD.

METHODS: For 6 one- to two-week periods throughout 2005, all stool samples that were tested in our laboratories for CDT were also tested for VRE. A commercial cytotoxicity assay was used to detect CDT. To test for the presence of VRE, stool samples were plated onto BEAV screening plates containing 6 mg/L of vancomycin; suspicious colonies were identified by biochemical methods and susceptibility testing was performed using Microscan and vancomycin E-test. To test for the presence of *S aureus*, swabs of samples were plated onto CNA plates, and suspect colonies were confirmed by the tube coagulase method.

RESULTS: CDT testing was performed on 675 stool specimens. CDT was detected in 142 samples (21%). VRE was detected in 12 samples (1.8%), including 5 samples that were positive for CDT and 7 samples that were negative for CDT. This represented 12 patients, 6 of whom were newly identified as VRE-colonized. *S aureus* was tested for in 520 samples, and was detected in 36 samples (6.9%), including 11 samples that were positive for CDT and 25 samples that were negative for CDT. The treatment of these patients was reviewed.

CONCLUSION: Testing for VRE on stool samples submitted for CDT testing may detect colonized patients who are at high risk of spreading VRE in the hospital and who may not be detected by routine infection control screening measures.

I3
SEVEN-YEAR SURVEILLANCE OF *STAPHYLOCOCCUS AUREUS* SUSCEPTIBILITY TO FUSIDIC ACID IN A CANADIAN HOSPITAL

R RENNIE*[1], C BROSNIKOFF[1], S SHOKOPLES[1], L TURNBULL[1], P KIDSON[2]
[1]Medical Microbiology Laboratory, University of Alberta Hospital, Edmonton, Alberta; [2]Leo Pharma, Thornhill, Ontario

OBJECTIVE: The objective of this study was to perform semi-annual surveillance of fusidic acid susceptibility of *Staphylococcus aureus* strains in a Canadian tertiary care hospital over an extended period of time.

METHODS: *S aureus* strains were collected from routine cultures submitted to the laboratory for one month every six months over a seven-year period. Only one strain per patient was tested in each test period. Routine antimicrobial susceptibility testing was performed in the laboratory by an automated susceptibility method (either bioMerieux Vitek or BD Phoenix). Testing for fusidic acid susceptibility was performed by disk diffusion (Oxoid, Nepean, Ontario) on Mueller-Hinton agar plates. The plates were incubated at 350°C for 18-20 hours in ambient air. A zone diameter of >20 mm was used to indicate susceptibility of the *S aureus* strains to fusidic acid. Fusidic acid-resistant strains were stored frozen at –20°C in double-strength skimmed milk.
RESULTS: 2242 *S aureus* strains were tested for fusidic acid susceptibility between 1999 and 2005, including 230 methicillin-resistant (MRSA) strains. Among all strains tested, 62 (2.8%) were resistant to fusidic acid (range 0.7% to 5% annually). Only 11 of the MRSA (4.5%) were resistant to fusidic acid. Eight of these 11 were discovered in 2005. Although from different patients, a number appeared to be clonally related.
CONCLUSIONS: Fusidic acid is the most widely prescribed topical antimicrobial in Canada. Our ongoing surveillance data show that there has been no trend towards increasing fusidic acid resistance in our hospital over this seven-year period even among MRSA that are unrelated to defined outbreaks of infection. These data support the importance of continuing surveillance and suggest that fusidic acid has maintained its efficacy over many years.

I4
MOLECULAR DIAGNOSTIC ASSAY FOR THE DETECTION OF CTX-M-TYPE EXTENDED-SPECTRUM BETA-LACTAMASES

JDD PITOUT*[1], N HAMILITON[1], DL CHURCH[1], P NORDMANN[2], L POIREL[2]
[1]University of Calgary, Calgary, Alberta; [2]South Paris Medical School, Paris, France

OBJECTIVES: Enterobacteriaceae producing CTX-M beta-lactamases have recently emerged concomitantly in the community and hospital settings from different parts of the world. Five clusters of CTX-M enzymes have been described sharing <90% amino acid identity between them. Because of the significant public health implications, the spread of organisms producing CTX-Ms merits close monitoring with enhanced efforts in surveillance surveys.
METHODS: A molecular diagnostic assay using two different sets of primers simultaneously for the detection of all bla_{CTX-M}-like beta-lactamase genes was developed, verified, and validated. This test was then used to investigate the prevalence of CTX-M enzymes among 240 clinical isolates of Enterobacteriaceae from the Calgary Health Region isolated during 2003-2004.
RESULTS: The duplex PCR using primers CTXMA1/A2 amplified a 450 bp amplicon in strains producing CTX-M-1, -2, -3, -5, -9, -10, -14, -15, -16, -17, -19, -30 and Toho-1 and primers CTX825F/R amplified a 307 bp amplicon in strains producing CTX-M-8 and CTX-M-25. No amplified product was obtained from strains producing TEM- and SHV-type ESBLs, or plasmid-mediated AmpC enzymes. This assay repeatedly demonstrated 100% sensitivity and specificity. The majority (55%) of the clinical isolates were positive for bla_{CTX-M} genes.
CONCLUSION: We report on a study that developed a duplex PCR for the simultaneous detection of all bla_{CTX-M} genes. This assay is easy to use and can be introduced in a reference laboratory to track and monitor the spread of organisms producing CTX-M-enzymes in the community and hospital settings.

I5
EPIDEMIOLOGY OF *HAFNIA ALVEI* INFECTIONS: A POPULATION-BASED LABORATORY SURVEILLANCE STUDY.

KB LAUPLAND*, DL CHURCH, T ROSS, JDD PITOUT
University of Calgary, Calgary Laboratory Services, Calgary Health Region, Calgary, Alberta

OBJECTIVES: Hospital-based case series have characterized *Hafnia alvei* primarily as an agent of polymicrobial nosocomial infections in middle-aged males with underlying illness. The objective was to define the incidence, demographic risk factors, and anti-microbial susceptibilities of *Hafnia alvei* infections in a non-selected population.
METHODS: Population-based laboratory surveillance was conducted in a large Canadian health region during 2000-2005.
RESULTS: A total of 129 patients with a *Hafnia alvei* infection were identified for an annual incidence of 1.9 per 100,000 population. The majority (87; 67%) were community-onset infections. The median age was 69 years (interquartile range 49 to 79 years) and there was a significant increase in the incidence associated with advancing age with the highest rate of 55 per 100,000 per year observed in those aged 90 years and older. Seventy-six per cent (98) of patients were female and the risk for infection was significantly higher among females as compared to males (2.9 versus 0.9 per 100,000; RR 3.15; 95% CI 2.08 to 4.88; P<0.0001). As compared to females, males were much more likely to have hospital onset infections (18/31 [58%] versus 24/74 [24%], RR 2.4, 95% CI 1.50 to 3.75; P<0.001). The most common focus of infection was urine in 106 (82%), followed by lower respiratory tract in 9 (7%), and soft tissue infection in 5 (4%). Eight patients were bacteremic. The majority of the infections (88; 68%) were mono-microbial in etiology. The majority of isolates were non-susceptible to ampicillin (102; 79%), cephalothin (97; 75%), amoxicillin/clavulanate (89; 69%) and cefazolin (86; 67%) but remained fully susceptible to imipenem and ciprofloxacin.
CONCLUSIONS: *Hafnia alvei* most commonly causes mono-microbial community-onset urinary tract infections in women. This study highlights the importance of population-based studies in accurately defining the epidemiology of an infectious disease.

ORAL PRESENTATIONS
Friday, March 17, 2006

J1
DIAGNOSIS OF NOROVIRUS INFECTIONS BY ELECTRON MICROSCOPY AND THE DETECTION OF ANTIGENS OR NUCLEIC ACIDS

S CASTRICIANO*, A PETRICH, K LUINSTRA, M SMIEJA, C LEE, M CHERNESKY
McMaster University, St Joseph's Healthcare, Hamilton, Ontario

BACKGROUND: Noroviruses cause severe outbreaks of gastroenteritis in all age groups. A rapid and sensitive screening method would improve timely diagnosis and facilitate infection control measures in hospitals.
OBJECTIVES: To compare Electron Microscopy (EM), two enzyme immune assays (EIA) and an in-house reverse transcription-polymerase chain reaction (RT-PCR) for the detection of Norovirus in stool samples.
METHOD: Frozen stool samples (n=240) from hospitalized or outpatient adults and children during outbreaks of diarrhoea in the spring and fall of 2004 were studied. Samples, previously tested by EM and held at –70°C were tested by RIDASCREEN EIA (R-Biopharm), the Dako Norovirus GI and GII EIA and in an in-house RT-PCR, using the published JV12 and JV13 primers of Vinje et al. Nucleic acids were extracted using magnetic extraction, and 5 µl tested by RT-PCR with random primers and 40 cycles, followed by electrophoresis. The negative staining method was used for EM testing, reading four grid squares. An expanded reference standard, where two different tests were required for positivity, was used to calculate sensitivity and specificity. A commercial RT-PCR (Argene) was used to confirm samples only positive by JV 12/13 primers.

RESULTS: Out of 240 samples, EM detected 29 positives and R-Biopharm EIA, Dako GI and GII EIA and RT-PCR detected 53, 0 and 40 and 78 positives, respectively. After repeat testing and picture examination, EM had five false positives. Of 25 samples only positive by RT-PCR, 12 were available for retesting in the Argene RT-PCR and were positive. Elimination of the other 13 from the analysis determined 65 positives and 162 negatives. Percentage sensitivity and specificity of the methods were as follows: R-Biopharm EIA (81.5 and 100, respectively), Dako GII EIA (61.5 and 100, respectively); EM (36.9 and 96.9, respectively) and RT-PCR (100 and 100, respectively).
CONCLUSION: The R-Biopharm EIA demonstrated significantly higher sensitivity compared to Dako and specificity than EM. Supplemental testing of the additional RT-PCR positives is warranted.

J2
COMPARISON OF ARTUS REALART INFLUENZA RT PCR WITH VIRAL CULTURE FOR THE DETECTION OF INFLUENZA

R GARCEAU*[1], M MALLET[2], T HATCHETTE[3], R DAVIDSON[3], M MAILLET[1]
[1]Hopital Dr GL Dumont, Moncton, New Brunswick; [2]Pêche et Océans Canada, Moncton, New Brunswick; [3]Queen Elizabeth II Hospital, Halifax, Nova Scotia

OBJECTIVE: Viral culture (VC) for Influenza surveillance is labour intensive and requires 2 to 7 days of incubation. Novel commercial real-time PCR assays may simplify and speed up Influenza detection. A panel of previously tested samples was assembled. We used this panel to evaluated the performance of the commercially available Artus RealArt Influenza real-time PCR kit (Artus) using a panel of previously tested specimens.
METHODS: 96 samples previously tested for respiratory viruses by viral culture (VC) were selected. The panel contained 26 samples positive for Influenza A (IA), 25 for Influenza B (IB), 23 for other viruses and 26 showing no virus by viral culture. Processed samples were thawed once and extracted manually with Qiagen columns (cat #59602). Samples were tested according to Artus kit instructions (cat #3102) on a LightCycler 2. Samples yielding discrepant results (Artus [+]/VC [–]) were referred and retested by a conventional, in-house PCR (PCR-2). Influenza (+) (Inf +), IA (+) or IB (+) were defined as either VC (+) or PCR2 (+). IA/IB (–) were defined as VC (–) and PCR-2 (–).
RESULTS: We obtained the results shown below:

	Inf (+)	Inf (–)		IA (+)	IB (+)	IA/IB (–)
Artus (+)	50	1	Artus (+)	25	25	1
Artus (–)	1	44	Artus (–)	1	0	44

No sample was positive for both Influenza A and B. One IA/IB (–) growing RSV was Artus (+). Artus stats are given below:

	Sens	Spec	PPV	NPV	Pos likelihood	Neg likelihood
Influenza	98	98	98	98	44	0.02
Influenza A	96	98	98	98	43.27	0.04
Influenza B	100	98	96	100	45	0

Cohen's kappa calculation gave a value of 0.958 for Artus.
CONCLUSION: Results obtained by Artus correlated well with those obtained by VC and PCR2. We foresee Artus as a promising assay for the surveillance and diagnosis of Influenza in respiratory samples.

J3
MOLECULAR DIAGNOSTIC OF HIGHLY PATHOGENIC H5 TYPE STRAINS OF INFLUENZA A

C YEA[1], D ADACHI[1], G JOHNSON[1], E NAGY[2], S RICHARDSON[1], M PETRIC[3], R TELLIER*[1]
[1]Microbiology, Hospital for Sick Children, Toronto; [2]University of Guelph, Guelph, Ontario; [3]BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: Concerns about the likely occurrence of an influenza pandemic in the near future are increasing. The highly pathogenic strains of Influenza H5 circulating in Asia have become the most feared candidate for giving rise to a pandemic strain. Our objective was to develop a RT-PCR assay able to specifically and definitively detect highly pathogenic H5 asian strains with high sensitivity.
METHODS: Primers targeting regions of the influenza genome conserved among highly pathogenic H5 strains were designed. The targeted regions included defining genetic signatures of highly pathogenic H5 asian strains. A sequence alignment including 20 different sequences from asian H5N1 highly pathogenic strains with representatives of clades 1, 1', 2 and 3 showed extremely high homology in the targeted segments. We use a single tube RT-PCR protocol and tested the assay on RNA from several different H types (verified by sequencing).
RESULTS: After optimization of the assay, we showed that our assay can detect RNA from a 2004 asian strain of H5N1 virus (RNA obtained from the CDC). We conservatively estimate the analytical sensitivity to be 10-100 genome copies. Using this method, no amplicons were synthesized from a low pathogenicity strain of H5 influenza or from influenza strains of type H1, H2, H3, H6, H7 and H9.
CONCLUSION: We developed a single tube RT-PCR assay that can detect with high sensitivity and specificity all the highly pathogenic H5 asian strains, yet the assay does not generate amplicon from any of the other H types tested. Such an assay would provide a very rapid and convenient diagnostic tool for the clinical laboratory, should a pandemic strain of influenza H5 emerge from strains currently circulating in Asia.

J4
MOLECULAR CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST THE SPIKE (S) PROTEIN OF SARS CORONAVIRUS (SARS-COV)

A ANDONOV*, S SAMSON, M DREBOT, J BERRY, M GUBBINS, M ANDONOVA, E GRUDESKI, S TYLER
National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba

OBJECTIVE: Monoclonal antibodies (Mabs) against SARS-CoV were developed at NML in 2004 and their utility for diagnostic development demonstrated. The goal of this study is further characterization and mapping of Mabs against the spike protein, which is a major antigenic determinant inducing protective immunity.
METHODS: A panel of 9 Mabs has been characterized by competitive ELISA, Western Blot, immunofluorescence and neutralization assays.
RESULTS AND DISCUSSION: The nine anti-spike SARS-CoV MABs belong to three different groups:
(1) F26G6 and F26G8 are non-neutralizing spike reactive antibodies with near 100% epitope overlap. The data strongly suggests that this is a linear epitope located at the N-proximal end of the S protein. A single amino acid change in the putative epitope TAFSPAQDIWG*TSAAAYFVGY (aa G245C) abolishes the reactivity of these two Mabs in Western blot;
(2) F26G9, F26G10, F26G18 and F26G19 are all neutralizing antibodies with a nearly 100% epitope overlap among the first three and around 90% overlap with F26G19. Among these, F26G18 has a very high neutralizing titre of around 1:120,000. We believe that the linear epitope recognized by F26G6 and F26G8 Mabs may be part of a complex conformational epitope for F26G18 neutralizing Mab judging from the loss of reactivity of F26G18 in Western blot with a strain of SARS-CoV carrying the G245C amino acid exchange;
(3) F26G3, F26G4 and F26G7 belong to a third group which is difficult to classify at present; preliminary data suggests that the first two may be similar.

An important aspect of this study is that F26G18 has very high affinity to a major immunodominant domain of the spike protein which does not seem to coincide with the ACE-2 receptor binding region of the spike protein. The precise mapping of this domain could prove useful for a wide range of potential applications, including therapy and designing SARS vaccines.

J5

AN INDIRECT IMMUNOFLUORESCENCE ASSAY FOR THE DETECTION OF HMPV DIRECTLY FROM NASOPHARYNGEAL SPECIMENS AND IN R-MIX CELL CULTURE

S CASTRICIANO*, M BOOTH, C ROBINSO, M MACPHERSON, A CAMPANELLA, R SERVEDIO, K LUINSTRA, M CHERNESKY, M SMIEJA, A PETRICH

McMaster University/St Joseph's Healthcare, Hamilton, Ontario

BACKGROUND: Human Metapneumovirus (hMPV) accounts for 3% to 10% of respiratory infections. Testing for hMPV has been hampered by the difficulty of isolating virus in cell culture and lack of availability of an indirect fluorescence assay (IFA).

OBJECTIVE: To develop and evaluate an IFA for the detection of hMPV directly from nasopharyngeal (NPS) specimens and in culture.

METHODS: The virus was obtained from Dr Boivin (Quebec City). 0.2 mL of virus dilutions were inoculated into LL-MK2 and R-Mix cells shell vials, then fixed and stained at 24, 48, and 72 hours. Cell type, incubation time and virus dilution were determined. The hMPV monoclonal antibody, from Dr Gerna (Pavia, Italy) was diluted to obtain the best IFA with a clean background. Cell smears were prepared with 24 hours infected hMPV R-mix cells. For this study, 208 NPS from children ≤5 years of age, submitted from January to April 2005, were used. All NPS were tested by nucleic acid amplification. The NPS cell pellet was used to prepare cell smears for routine respiratory DFA and for hMPV IFA. After fixation, the smears were IFA stained for 30 minutes with the hMPV antibody, and 30 minutes with goat anti-mouse FITC. The NPS fluid was inoculated in R-Mix cells. After 48 hours, the cells were fixed and stained for routine respiratory DFA and IFA for hMPV as described above.

RESULTS: The LL-MCK2 and the R-Mix cells detected hMPV growth equally well; 48 hours was the optimal incubation time, hMPV stock at 10^{-2} gave 50-75% fluorescing cells and hMPV antibody, diluted at 1:80, exhibited clear IFA. The same IFA conditions were used for both hMPV antigen detection in NPS cell smears and R-Mix cells culture isolation. Out of 208 NPS, 84 were negatives, 118 positives for other respiratory viruses and 6 were positives for HMV by IFA antigen detection and virus isolation in R-Mix cells and RT-PCR.

CONCLUSION: The newly developed IFA assay can be used to routinely test NPs for hMPV direct antigen detection and virus isolation in R-Mix cells shell vials. The hMPV antibody is specific only to hMPV; no cross-reaction was noted with any of the other respiratory viruses.

K1

MRSA*SELECT*: PROLONGED INCUBATION OF SPECIMENS INCREASES RECOVERY OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

A WOLFE*[1], AU CHANDRAN[1,2], R VERITY[1,2], K CRAIG[1], S ALVOLEDO[1], J NIGRIN[1,2]

[1]Dynacare Kasper Medical Laboratories; [2]University of Alberta, Edmonton, Alberta

OBJECTIVE: MRSA*Select*, a selective chromogenic agar, enables isolation and identification of MRSA within 24 h. A previous comparison in our laboratory showed MRSA*Select* to perform well compared to oxacillin mannitol salt agar (OMSA), the screening medium already in use. We assessed the performance of MRSA*Select* for nasal and groin MRSA screening specimens at 24 h and 48 h post-inoculation to assess any differences in the isolation rates of MRSA.

METHODS: In June and July 2005, nasal and groin MRSA screening specimens from local hospitals, nursing homes and the community were inoculated to MRSA*Select* and examined for pink colonies after 18 h to 28 h incubation at 35°C in O_2. Although the package insert recommends a single read at 18 h to 24 h, culture plates were reincubated and re-examined at 42 h to 48 h total incubation time, similar to the protocol already in place for OMSA. After species identification was confirmed, methicillin resistance was confirmed by cefoxitin Kirby-Bauer disk diffusion.

RESULTS: In a two-month period, a total of 12665 MRSA screening samples were examined, with 491 positive specimens. Of the total positive specimens, 91.2% (448/491) were detected at 18 h to 28 h, while 8.8% (43/491) were detected at 42 h to 48 h. Of the MRSA isolates detected after prolonged incubation, 88.4% (38/43) were repeat isolates from patients already known to be colonized with MRSA. The incidence of false-positive colonies increased with prolonged incubation.

CONCLUSION: MRSA*Select* is a new selective chromogenic medium for detection of MRSA. Although the manufacturer recommends a total incubation time of 24 h, we suggest that a prolonged incubation time of up to 48 h be considered for optimal recovery of MRSA isolates, thus preventing any negative impact on infection control efforts.

K2

TEMPORAL EVOLUTION OF STAPHYLOCOCCAL CASSETTE CHROMOSOME *mec* (SCC*mec*) TYPES AND ANTIMICROBIAL RESISTANCE PROFILES OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

K ZHANG*[1], J MCCLURE[1], S ELSAYED[3], L GOLDING[1], T LOUIE[2], J CONLY[1]

[1]Centre for Antimicrobial Resistance; [2]University of Calgary; [3]Calgary Laboratory Services, Calgary, Alberta

OBJECTIVE: SCC*mec* is a mobile genetic element serving as a vehicle of transfer for genetic markers, including genes mediating antimicrobial resistance. We conducted a retrospective study on a natural population of MRSA clinical isolates in a large Canadian health region over a 16-year period to determine the temporal evolution of SCC*mec* types and their degree of correlation with antimicrobial susceptibility profiles.

METHODS: Randomly selected MRSA clinical isolates from 1989-2004 were analyzed for the presence of SCC*mec* types I, II, III, IVa-d or V using a multiplex PCR assay. Further characterization of representative isolates by PFGE, *spa* and MLST typing were performed to confirm clonality. For each strain tested, SCC*mec* type was compared with the profile of susceptibility to multiple antistaphylococcal agents using Vitek.

RESULTS: Of 543 isolates tested, 317 (58.4%), 116 (21.4%), 92 (16.9%), 8 (1.5%), 3 (0.6%), and 7 (1.3%) were found to be SCC*mec* types II, III, IVa, IVb, IVc, and unknown (non-typeable), respectively. No isolates with SCC*mec* types I, IVd, or V were found. Prior to 1994, type III (92.4%) was dominant, followed by types II and IVc (3.3% and 1.1%) respectively. Since 1999, type II has been dominant, with types IVa and IVb emerging and increasing yearly. The overall prevalence for types II, III, IVa, IVb and IVc between 1999 to 2004 was as follows: 69.3%, 6.8%, 20.3%, 1.8% and 0.4%. Regardless of time period or genetic diversity (PFGE, spa, MLST), the same SCC*mec* types exhibited similar antibiotic-resistant profiles. The SCC*mec* type correlated with typical susceptibility patterns and the type IV isolates were more often community-associated, whereas types II and III could be both hospital- and community-associated.

CONCLUSION: Our analysis indicates that temporal evolution of SCC*mec* types has occurred among genetically diverse SA isolates over a 16-year period in a single health region. SCC*mec* type was also associated with specific susceptibility patterns and could serve as a marker for understanding the evolution and molecular epidemiology of MRSA.

K3

MOLECULAR AND PHENOTYPIC ANALYSIS OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (CA-MRSA) STRAINS IN A LARGE CANADIAN URBAN HEALTHCARE REGION

S ELSAYED*[1,2], K ZHANG[1,2,3], J MCCLURE[3], D GREGSON[1,2], T LOUIE[1], J CONLY[1,3]
[1]University of Calgary; [2]Calgary Laboratory Services; [3]Centre for Antimicrobial Resistance, Calgary Health Region, Calgary, Alberta

BACKGROUND: CA-MRSA are emerging as important pathogens worldwide and demonstrate a remarkable propensity for causing pyogenic skin/soft-tissue infections and necrotizing pneumonia, particularly in marginalized populations. Recent outbreaks of CA-MRSA infections in our health region have led to intensive efforts to determine the molecular and phenotypic properties of infecting strains. We describe our laboratory-based analysis of outbreak-associated clinical CA-MRSA strains recovered in a large Canadian urban healthcare region.

METHODS: CA-MRSA clinical isolates recovered from infected patients within a correctional facility (Jan 2004 to May 2005) or cases identified during a community-wide outbreak (in 2004) in our health region were included for study. All MRSA strains were identified using a duplex PCR assay for *nuc* and *mecA* genes. Susceptibility to anti-staphylococcal agents was determined using Vitek, Etest and *mupA* PCR. Pulsed-field gel electrophoresis (PFGE) patterns were determined using a nationally standardized protocol. SCC*mec* types and presence of Panton-Valentine leukocidin (PVL) genes were determined by PCR.

RESULTS: A total of 110 MRSA isolates were recovered from the study population. Virtually all isolates were from skin/soft tissue sites and had PFGE patterns and SCC*mec* types (IVa) identical to those of USA300 or USA400 strains, although PVL was notably absent in the latter group. Antimicrobial susceptibility testing revealed virtually uniform susceptibility to vancomycin, TMP-SMX, tetracyclines, rifampin, aminoglycosides, mupirocin and fusidic acid. Virtually all strains resembling USA400 demonstrated fluoroquinolone susceptibility and inducible clindamycin resistance, in contrast to USA300-like strains.

CONCLUSIONS: CA-MRSA strains in our region demonstrate unique molecular and phenotypic characteristics compared with those reported from other locales. Favourable susceptibility profiles to mupirocin and fusidic acid suggest that they may be considered as alternative agents for empiric therapy of CA-MRSA skin/soft tissue infections in our region.

L1

ASSOCIATION OF MACROLIDE USE AND MACROLIDE RESISTANCE IN *S PNEUMONIAE* IN A CANADIAN SURVEILLANCE SYSTEM, 1994-2005

O VANDERKOOI[1], A MCGEER[2], J RABOUD[3], B WILLEY*[2], D LOW[1]
[1]Alberta Children's Hospital, Calgary, Alberta; [2] Mount Sinai Hospital, Toronto, Ontario; [3] Samuel Lunenfeld Research Institute, Toronto, Ontario

BACKGROUND: Since 1993, the rate of macrolide resistance in Canadian strains of *S pneumoniae* has been increasing steadily. We asked whether a prior hypothesis that resistance was primarily associated with long-acting macrolide use was supported by data from national surveillance.

METHODS: Macrolide resistance in *S pneumoniae*, by province and year (from 1994 to 2005), was derived from the Canadian Bacterial Surveillance Network. Macrolide prescription data was obtained from IMS Health, Canada. Rates of use (scripts/1000 pop/year) were calculated using population data from Statistics Canada. Poisson generalized estimating equation models were used to examine the relationship between resistance rates and prescription rates of all macrolides, erythromycin, clarithromycin and azithromycin. Odd ratios are expressed as the multiplicative increase in the resistance rate associated with an increase in the prescription rate of 1 script per 100 persons per year.

RESULTS: 24,678 isolates were tested by the surveillance system. Macrolide resistance in Canada increased from 1.9% in 1993 to 19.3% in 2005. Rates in different provinces were not different between 1993 and 1997; from 1998 on, there were statistically significant differences in rates of macrolide resistance in different provinces. The overall rate of outpatient macrolide prescribing in Canada did not change significantly from 1994 to 2004; however, there were significant changes between provinces and over time in individual provinces (from 96 to 196 scripts per 1000 persons per year). In univariate analysis, clarithromycin (OR 1.18, 95% CL 1.14 to 1.22) and azithromycin (OR 1.20, 95% CL 1.15 to 1.26), but not erythromycin use or total macrolide use, were associated with resistance. In multivariable analysis, only azithromycin use was associated with resistance (OR 1.13, 95% CL 1.02 to 1,18; P=0.01).

CONCLUSIONS: These data support previous data from European and Canadian surveillance suggesting that use of long-acting macrolides is a more important predictor of resistance than use of other macrolides.

L2

CONSUMPTION OF NEW MACROLIDES AND RESISTANCE TRENDS IN GROUP A STREPTOCOCCI, 1995 TO 2004

G QUAN*, D ROSCOE, DM PATRICK, M CHONG, F MARRA
University of British Columbia, Vancouver, British Columbia

OBJECTIVES: This study determined trends in macrolide susceptibility of Group A streptococci from invasive infections (iGAS) and pharyngitis (pGAS) and compared them with trends in new macrolide consumption.

METHODS: Retained community-acquired iGAS isolates from patients at one site (1995-2004) were retrieved and tested for macrolide susceptibility using disk diffusion methodology and CLSI guidelines. Antimicrobial susceptibility profiles of pGAS from the same facility were obtained from a national surveillance study. Antimicrobial consumption trends were monitored by analysis of all outpatient prescriptions collected through a provincial pharmacy system. Statistical analyses used the Chi squared test and Spearman rank correlation coefficient in SPSS.

RESULTS: Macrolide resistance remained stable in iGAS isolates (1995-2004), but an increase in resistance among pGAS isolates was observed and correlated with an increase in consumption of new macrolides (Spearman's rho=0.87; P=0.05).

Year	95	96	97	98	99	00	01	02	03	04
pGAS, n	–	–	–	–	30	30	20	20	19	20
pGAS-resistant, %	–	–	–	–	3	5	5	10	11	25
iGAS, n	13	16	14	16	14	19	18	22	16	16
iGAS-resistant, %	31	12	14	19	28	0	39	13	12	25
Macrolide DDD/ 1000 persons per day	–	–	0.6	0.7	1.0	1.4	1.7	1.9	2.3	–

DISCUSSION: An increase in macrolide resistance among throat pGAS isolates is observed and correlates with increasing consumption of newer macrolides.

L3

UTILIZATION OF COLISTIN FOR TREATMENT OF METALLO-BETA-LACTAMASE-PRODUCING *PSEUDOMONAS AERUGINOSA*

DM SABUDA*[1], BR DALTON[1], KL LAUPLAND[1,2,3], J PITOUT[3], TJ LOUIE[1,2], HR RABIN[1,2], JM CONLY[1,2,4]
[1]Calgary Health Region; [2]University of Calgary; [3]Calgary Laboratory Services; [4]Centre for Antimicrobial Resistance, Calgary, Alberta

OBJECTIVE: *P aeruginosa*-producing metallo-beta-lactamase (MBL) re-appeared in our health care region in 2001-2005. Screening, identification and colistin susceptibility testing of this organism are now routine in our laboratory. Given our local experience, we sought to describe our usage of colistin in the management of these patients.

METHODS: Adult patients who received colistimethate sodium (colistin) between January 2000 and December 2005 were identified via pharmacy records, and their medical records were reviewed. Patient demographics, clinical course, and relevant laboratory data were reviewed

and extracted from the charts. Imipenem resistant strains of *P aeruginosa* (MIC ≥8 µg/mL) were identified using the Vitek system. Production of MBL was evaluated using the combination of an EDTA screen test and MBL E-Test.

RESULTS: A total of 33 courses of colistin therapy was received by 25 patients. In all but two cases, the implicated pathogen was an MBL-producing *P aeruginosa*. Intravenous (IV) colistin was administered to 14 patients for an average of 12.8 days (SD 13.5; range 1-46). The other 11 patients received nebulized colistin. The highest IV dose regimen was 125 mg q6h (5 mg/kg/day). One patient was receiving dialysis at the time of IV colistin therapy and two patients received IV colistin on an outpatient basis through a Home Parenteral Therapy Program. Eight evaluable patients had an average increase in creatinine of 50 µmol/L (range 1-124). Adverse effects noted that were possibly due to colistin included drug fever, nephrotoxicity, drug interactions, neurotoxicity and anorexia due to altered taste sensation. Eight of 12 patients treated with IV colistin responded either fully or partially.

CONCLUSION: Our patients received both IV and nebulized colistin for multidrug-resistant *P aeruginosa*. The use of IV colistin contributed to nephrotoxicity in 63% of patients. Despite this undesirable side effect, colistin may be a useful therapeutic agent in the setting of multi-resistant *P aeruginosa* infections.

L4

ANTIMICROBIAL RESISTANCE IN PATHOGENS ISOLATED FROM CANADIAN INTENSIVE CARE UNITS: RESULTS OF THE CANADIAN NATIONAL INTENSIVE CARE UNIT (CAN-ICU) STUDY 2005

M DECORBY*[1], N LAING[1], B WESHNOWESKI[2], M EDEL[2], K KARLOWSKY[1], JA KARLOWSKY[2], R VASHISHT[1], C SIEMENS[1], M MCCRACKEN[1], M MULVEY[3], DJ HOBAN[2], GG ZHANEL[1]

[1]University of Manitoba; [2] Health Sciences Centre; [3]National Microbiology Laboratory, Winnipeg, Manitoba

OBJECTIVES: Antibiotic resistance is a global concern, and patients in intensive care units (ICU) are at high risk of acquiring nosocomial infections caused by resistant pathogens. The CAN-ICU study assessed the types of pathogens causing ICU infections as well as the prevalence of resistance to antimicrobials used in ICUs in Canada.

METHODS: 20 medical centres from across Canada were asked to submit up to a maximum of 250 isolates from patients in the ICU. Isolates were tested using CLSI (2005) methods.

RESULTS: To date, 2260 isolates have been collected. 56.8% were from respiratory specimens. 16.0%, 14.9%, 12.3% were from blood, tissue/wound and urine specimens, respectively. The top 10 pathogens isolated were:

Ranking	Organism	# of isolates	% of total
1	*S aureus*	400	17.7
2	*P aeruginosa*	260	11.5
3	*E coli*	251	11.1
4	*Enterococcus* spp	164	7.3
5	CNS	156	6.9
6	*H influenzae*	148	6.5
7	*K pneumoniae*	120	5.3
8	*S aureus* – MRSA	107	4.7
9	*S pneumoniae*	102	4.5
10	*E cloacae*	90	4.0

Antimicrobial resistance rates for all ICU isolates were 9.2% for piperacillin/tazobactam, 9.4% for meropenem, 20.5% for levofloxacin, 21.5% for gentamicin and 23.2% for ceftriaxone.

DISCUSSION: Respiratory tract infections are the predominant infections in Canadian ICUs. The most common pathogens include *S aureus*, *P aeruginosa*, Enterobacteriaceae, *Enterococcus* spp, *H influenzae*, MRSA, and *S pneumoniae*. Antibiotic resistance is lowest with piperacillin/tazobactam and meropenem.

M1

PREVALENCE OF OCCULT HEPATITIS B VIRUS IN A COHORT OF HIGH-RISK HIV- AND HCV-INFECTED INDIVIDUALS IN BRITISH COLUMBIA

C OSIOWY*[1], R RAHMANIAN[2], J BORLANG[1], M PETRIC[2], M KRAJDEN[2]

[1]Public Health Agency of Canada, Winnipeg, Manitoba; [2]BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: Occult HBV infection (defined as HBsAg-negative with or without serological markers of a previous HBV infection) has been reported to occur frequently in individuals infected with hepatitis C virus, particularly injection drug users. To investigate the prevalence of occult HBV in high-risk individuals infected with HIV and HCV, serum samples from individuals having an "HBc only" HBV serological profile were analyzed for HBV DNA.

METHODS: Serum samples from an anonymized cohort of 149 high-risk HIV- and HCV-infected individuals were analyzed. All sera were positive for antibody to HBV core and were negative for HBsAg. Although all samples were considered non-immune for antibody to HBsAg (anti-HBs <10 mIU/mL), sera were stratified based on anti-HBs reactivity (non-reactive, <1.0 mIU/mL, and >1.0 mIU/mL) with the HBV AUSAB assay (Abbott Laboratories). HBV DNA was extracted and amplified by real-time PCR and nested PCR specific for the core promoter/precore and surface antigen region of the virus genome. Any resulting amplicons were gel purified and sequenced.

RESULTS: Following amplification analysis, 12/149 (8%) samples were HBV DNA-positive. All samples were verified as distinct HBV isolates by sequence analysis. At the time of writing, HBV genotype data was available for 11 samples: genotype D (5); genotype B (4); genotype A (1); and genotype G (1). HBV DNA-positive samples grouped dependent on the level of anti-HBs present in the sample: 0 mIU/mL (2.6% DNA positive); <1.0 mIU/mL (12.3% DNA positive); and >1.0 mIU/mL (7.4% DNA positive). Mutations within the HBsAg coding region were not observed, other than an sT125M mutation observed in genotype D samples. Further mutation analysis will also be reported.

CONCLUSION: The prevalence of occult HBV in high-risk individuals infected with HIV and HCV was lower than that reported previously for this cohort. Occult HBV was observed more frequently in non-immune individuals having very low anti-HBs reactivity by the AUSAB assay.

M2

PREVALENCE ANTI-HBC AS THE SOLE MARKER OF HBV INFECTION IN INDIVIDUALS CO-INFECTED WITH HCV AND/OR HIV

A MAK*[1], R RAHMANIAN[2], M ALVAREZ[1], K CHU[1], J BURGOYNE[1], G MCNABB[1], P TSANG[1], M KRAJDEN[1], M PETRIC[1]

[1]British Columbia Centre for Disease Control; [2]University of British Columbia, Vancouver, British Columbia

INTRODUCTION: Hepatitis B virus (HBV) infection is generally established by the presence of viral antigen and antibody markers. In rare cases, the only marker of HBV infection is reactivity to Anti-HBc. The objective of this study was to examine the prevalence of Anti-HBc as the sole marker of HBV infection in individuals co-infected with HCV and/or HIV.

METHODS: A cohort of subjects at high risk for blood-borne infections in Vancouver, British Columbia, were tested by commercial immunoassays for Anti-HIV, Anti-HCV, Anti-HBc, Anti-HBs and HBsAg to determine the proportion for whom Anti-HBc was the only marker of HBV infection.

RESULTS: Out of 1043 individuals, 178 (17%) were Anti-HIV Western Blot reactive, of which 110 (96%) were also reactive for Anti-HCV. Of these HIV/HCV co-infected individuals, 70 (78%) were reactive for Anti-HBc, and in 22 (33%) cases, this was their only marker of HBV infection. In contrast, 859 (82%) of the 1043 cases tested were Anti-HIV-1, two were negative and 698 (82%) of those cases were also HCV reactive. Anti-HBc reactivity occurred in 376 (57%) subjects, and in 37 (10%) cases, this was their only marker of HBV infection. Seventeen per cent (137) of the HIV-negative cases were not reactive for HCV. Of these individuals,

18 (14%) were Anti-HBc core reactive, and in one (0.06%) case, this was the sole marker of HBV.

CONCLUSION: Among the normal population, the presence of Anti-HBc as the only marker of HBV infection has been reported to be between 1% and 4%. Our findings suggest that people infected with HCV and HIV may exhibit much higher proportions of Anti-HBc as the only marker of HBV infection than reported in the normal population.

M3

DETECTION OF MUTATIONS ASSOCIATED WITH RESISTANCE TO ADEFOVIR DIPIVOXIL IN CHRONIC HEPATITIS B VIRUS PATIENTS BY THE INNO-LIPA HBV DR V2 ASSAY

C OSIOWY*[1], JP VILLENEUVE[2], EJ HEATHCOTE[3], E GILES[1], J BORLANG[1]

[1]Public Health Agency of Canada, Winnipeg, Manitoba; [2]Hôpital Saint-Luc du CHUM, Montréal, Québec; [3]Toronto Western Hospital, Toronto, Ontario

OBJECTIVE: The nucleotide analog adefovir dipivoxil (ADV) is an effective antiviral treatment for chronic hepatitis B virus infection. The detection of ADV resistance mutations is necessary during therapy to monitor and anticipate possible treatment failure. The INNO-LiPA HBV DR v2 (LiPA; Innogenetics, Ghent, Belgium) is a DNA hybridization line probe assay for the detection of HBV polymerase mutations associated with resistance to lamivudine and adefovir. Evaluation of this assay to detect ADV resistance mutations was performed.

METHODS: A total of 124 serum samples were analyzed from 38 chronic hepatitis B patients currently undergoing treatment with ADV. Extracted HBV DNA was analyzed by LiPA and sequencing. The HBV genotype was determined for each sample. Translated polymerase protein coding sequences were aligned with genotype-specific sequences from the GenBank database to determine polymorphisms or mutations.

RESULTS: By LiPA analysis, 12 patients (31.5%) were found to have mutations associated with resistance to ADV (rtA181V/T and/or rtN236T). This contrasted with sequence analysis, which found 9 patients (24%) having either or both mutations. Twice as many samples were rtN236T positive by LiPA (18/124) compared to sequence analysis (9/124). LiPA detected the rtN236T mutation at least 6 months earlier than its detection by sequencing in those patients for whom consecutive serum samples were available. Several novel and ADV-resistance associated codon substitutions in the HBV polymerase of most patients were observed by sequence analysis.

CONCLUSION: The LiPA assay performed well and, compared to sequencing, was able to sensitively and specifically detect the mutations rtA181V/T and rtN236T associated with ADV-R, thus allowing anticipation for changes in treatment requirements. Although less sensitive, sequencing has the advantage of providing information on other polymerase mutations not represented on LiPA strips.

M4

COMPARISON OF BIO-RAD GENETIC SYSTEMS HBSAG VERSUS BAYER ADVIA CENTAUR HBSAG FOR CADAVERIC SPECIMENS

G MCNABB*, J BURGOYEN, P TSANG, L DIFRANCESCO, M PETRIC, M KRAJDEN

Provincial Health Services Authority and BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: To compare the use of Bio-Rad Genetic Systems (licensed by Health Canada in January 2003 for cadaveric specimens) versus Bayer ADVIA Centaur in testing HBsAg for cadaveric sera collected from the Eye Bank of BC/BC Tissue Bank.

METHODS: In a retrospective study, 32 cadeveric sera were tested by two methods. The Genetics Systems HBsAg test is a manual, plate-based enzyme immunoassay. The Bayer ADVIA Centaur HepBsAg is an automated chemiluminescent enzyme immunoassay. In an ongoing prospective study (September 2005 and to date), 69 cadaveric sera from the Eye Bank of BC/BC Tissue Bank were compared by the two methods.

RESULTS: Among the 32 retrospective sera, there were 4 discordant findings. Three sera were positive by the Genetic Systems test and nonreactive by the Bayer ADVIA Centaur assay; one serum was positive by Bayer ADVIA Centaur assay and nonreactive by Genetic Systems assay. In the prospective study, 69 cadaveric sera were nonreactive by both methods.

CONCLUSIONS: The Bio-Rad Genetic Systems HBsAg test for HBsAg is a time consuming manual method that was licensed by Health Canada for testing cadaveric specimens for transplantation. It is only through such comparative studies that the validity of the automated systems can be validated.

N1

THE UTILIZATION OF URINE CULTURES FOR SYMPTOMATIC IN-PATIENT GERIATRIC POPULATION: A PROSPECTIVE STUDY

A MUKHERJI*[1], E MACDONALD[2], S FAIRWEATHER[2], C THOMPSON[3]

[1]Dalhousie University, Department of Medicine and AHSC, Saint John, New Brunswick; [2]Dalhousie University, Department of Geriatric Medicine and AHSC, Saint John, New Brunswick; [3]University of Louisville, Bepartment of Biostatistics and Bioinformatics, Louisville, Kentucky, USA

BACKGROUND: UTIs are a significant diagnostic challenge for clinicians.

METHODS: To characterize the rationale for utilizing urine c/s, between between February 2003 and March 2004, we prospectively asked clinicians (MD/RN) ordering urine c/s on geriatric rehabilitation unit in-pts to complete a questionnaire. Data were verified by chart audit retrospectively.

RESULTS: Complete data was available in 91 cases. The mean age was 83.8 years, and 68.1% of cases were female. A reasonable hx was probable from the majority, as suggested by mean baseline MMSE of 24.6. No concurrent foci of infection or other new dx were identified at the time of positive urine c/s. The risk factors for UTI were: prostate disease (44.8%), recent bedrest (40.7%), constipation (30.8%), cancer (27.5%), DM (26.4%), prior UTI (25.3%), poor hygiene (24.2%), CRF (14.3%) and foley (13.2%). Clinicians suspected UTI in 44% of cases, and their reasons were: 1) acute cognitive change (35.4%) (ie, behaviour change or LOC or acute confusion); 2) localized GU sx (25.5%) (ie, frequency, urgency, new incontinence, suprapubic pressure and hematuria). 7.3% c/s were ordered due to foul or cloudy urine, or elevated WBC or positive urinalysis. Urine c/s was positive in 32 symptomatic cases. Individual sx independently associated with positive c/s were: change in LOC (positive c/s 85.7%, P=0.006), and back pain (positive c/s in 60%, P=0.031). Many pts with positive c/s had combination of sx, and certain combinations were significantly associated with positive c/s. *Change in LOC and fatigue* (+ c/s 83%, P=0.019), *back pain and poor appetite* (+ c/s 75%, P=0.011), *back pain and fatigue* (+ c/s 77.8%, P=0.038), *back pain and change in behaviour* (+ c/s 62%, P=0.019) and *urinary urgency and fatigue* (+ c/s 77.8%, P=0.008). It is interesting to note that increased urinary frequency, decreased urinary output, suprapubic pressure, hematuria, new incontinence, foul smelling urine, and elevated WBC were not predictive of + urine c/s.

CONCLUSION: In our cohort of elderly in-pts with hx of recent functional decline, CNS sx, back pain, fatigue, and poor appetite were more predictive of + urine c/s than sx localized to GU tract.

N2

TREATMENT OF HEPATITIS C IN LUNG TRANSPLANT CANDIDATES

K DOUCETTE*[1], J WEINKAUF[2], D LIEN[2]

[1]Division of Infectious Diseases; [2] Division of Pulmonary Medicine, University of Alberta, Edmonton, Alberta

BACKGROUND: Hepatitis C virus (HCV)-related liver disease is accelerated following renal transplant with an increase in mortality of HCV-positive recipients, primarily due to liver disease; thus, HCV treatment may be considered prior to renal transplant. Lung transplant (LTx) candidates, however, have generally not been considered candidates for therapy,

although no data exist regarding the safety and efficacy of HCV therapy in this group.

OBJECTIVE: To describe our single-centre experience with the treatment of HCV in LTx candidates.

METHODS: Based on clinical, virologic and histologic data, 5 LTx candidates with chronic HCV were considered candidates for and underwent HCV treatment.

RESULTS: 3 women and 2 men with a median age of 52 years (range 37 to 54 years) were treated for HCV. The indication for LTx was talcosis in 4 and emphysema in 1 patient and all were on home O_2 at 2.5 L/min to 4 L/min. The HCV genotype was 3a in 3 patients and 1a in 2 patients. Liver biopsy was done in 3 cases and documented grade 1-2 activity and stage 1-2 fibrosis. Four patients received pegylated interferon (Peg-IFN) plus ribavirin and 1 was treated with standard IFN and ribavirin. Four patients have completed treatment (24 weeks) and one is 10 weeks into a planned 48-week course of therapy. There were no unexpected side effects of HCV treatment. All had a slight increase in O_2 requirements and 3 have had infectious exacerbations of lung disease while on treatment. SVR has been documented in 3 patients (genotype 1a [1] and 3a [2]); one of these patients has undergone LTx and 2 remain on the waitlist. An end of treatment response has been documented in an additional patient (genotype 3a) with SVR to be assessed March 2006.

CONCLUSION: Selected LTx candidates can be safely and successfully treated for HCV. Further studies are needed to further clarify both the impact of HCV following LTx and the effect of HCV treatment prior to LTx.

N3

SCREENING FOR HIV-ASSOCIATED ANAL CANCER: TEST CHARACTERISTICS OF ANAL CYTOLOGY AND ONCOGENIC HPV FOR THE DETECTION OF HIGH-GRADE DYSPLASIA

IE SALIT*[1], J TINMOUTH[1], A LYTWYN[2], J RABOUD[1], W CHAPMAN[1], T DARRAGH[3], B WINKLER[4]

[1]University of Toronto, Toronto; [2]McMaster University, Hamilton, Ontario; [3]UCSF, San Francisco, California; [4]Quest Diagnostics, Teterboro, New Jersey, USA

OBJECTIVES: We initiated an anal cancer screening study in HIV-positive men using anal cytology, HPV detection and high-resolution anoscopy with directed anal biopsy. The aim was to determine the test characteristics of anal cytology and oncogenic HPV for detection of histologic high-grade anal intra-epithelial neoplasia (AIN 2/3).

METHODS: Subjects were HIV-positive men with anal receptive intercourse. Samples for anal cytology were collected by rotating a swab in the anal canal and processing by ThinPrep. Oncogenic HPV was determined using Hybrid Capture. Anal cytology and biopsies were independently assessed by at least 2 blinded pathologists and analyses done using these consensus diagnoses. The reference standard was consensus diagnosis of AIN 2/3.

RESULTS: Results are presented on 246 pt. visits (median age=44, CD4=403, viral load and lt;50, 89% on HAART). Anal cytology was abnormal in 73%: HSIL in 7%, low-grade changes (LSIL) in 50% and ASCUS in 16%. Anal biopsies were abnormal in 71%: AIN 2/3 in 28% and AIN 1 in 43%. The sensitivity (Sn) of any abnormality on the anal cytology in detecting AIN 2/3 was 84% and the specificity (Sp) was 32%. Negative predictive value (NPV) was 84% and positive predictive value (PPV) was 33%. Abnormal anal cytology missed only 11/70 (16%) AIN 2/3 lesions. HSIL on anal cytology was not strongly predictive of AIN 2/3. Of 18 pts with HSIL on anal cytology, only 12 (67%) had AIN 2/3; Sn was 17% and Sp was 97%. Oncogenic HPV was found in 86% of pts. The presence of HPV had Sn of 99%, Sp of 19%, NPV of 97% and PPV of 28%. Detection of HPV missed only 1/67 (1.5%) AIN 2/3.

CONCLUSIONS: High rates of anal dysplasia have been detected. Abnormal anal cytology was sensitive but not specific in detecting AIN 2/3. Oncogenic HPV detection had similar performance to anal cytology.

N4

PSEUDOMEMBRANOUS COLITIS SECONDARY TO *STRONGYLOIDES STERCORALIS*: A CASE REPORT

J JANVIER*[1,4], S KUHN[2,4], D CHURCH[1,3]

[1]Foothills Medical Centre; [2]Alberta Children's Hospital; [3]Calgary Laboratory Services; [4]University of Calgary, Calgary, Alberta

PRESENTATION: A 43-year-old female presented with a two-day history of acute left lower quadrant pain, non-bloody diarrhea and bilious emesis. Past history included systemic lupus erythematosis complicated by end-stage renal disease requiring hemodialysis, and a remote history of pulmonary TB. Community-acquired pneumonia was treated 4 months prior with a 10-day course of levofloxacin. She immigrated to Canada from Cambodia in 1988 but had no recent travel history, infectious contacts, or suspicious foods consumed; there was no family history of inflammatory bowel disease. Medications included prednisone, ASA, azathioprine, levothyroxine, carvedilol, pantoprazole, and pravastatin.

LABORATORY INVESTIGATIONS: Investigations found an elevated WBC count and creatinine, but normal liver enzymes and lipase. Abdominal X-rays showed mural thickening in the splenic flexure and descending colon but no evidence of obstruction. An abdominal CT scan revealed pancolitis. Toxin assays for *Clostridium difficile* (CD) were negative, and sigmoidoscopy revealed biopsy-confirmed pseudomembranes. Stool examinations subsequently revealed *Strongyloides stercoralis* larvae.

TREATMENT: Combination therapy with albendazole (400 mg BID for 7 days) and ivermectin (200 mcg/kg OD for 2 days) resulted in clinical response. Symptoms relapsed 5 weeks later, but resolved with 7 days of the same medications. She is symptom-free after 10 months of follow-up.

DISCUSSION: Severe strongyloidiasis occurs principally in immunocompromised hosts. This presentation is an example of hyperinfection syndrome, consisting of symptoms attributable to accelerated auto-infections. Pseudo-membranous colitis is a rare manifestation of hyperinfection, having been reported only once previously. Immigrants from endemic countries should be screened with *Strongyloides* serology before receiving immunosuppressive therapy to prevent this potentially life-threatening infection. A high index of suspicion should be maintained as long as such patients remain immunosuppressed for a broad array of presentations.

O1

INVESTIGATION OF CALCIUM AND MAGNESIUM ION REQUIREMENTS FOR MINIMUM INHIBITORY CONCENTRATION TESTING IN MUELLER-HINTON BROTH

R RENNIE*[1], C BROSNIKOFF[1], S SHOKOPLES[1], D CALLIHAN[2], L TURNBULL[1]

[1]Medical Microbiology Laboratory, University of Alberta Hospital, Edmonton, Alberta; [2]ID/ASTResearch & Development, BD Diagnostic Systems, Sparks, Maryland, USA

BACKGROUND: The CLSI M7 standard concentrations of divalent cations in Mueller-Hinton Broth (MHB) of 20 mg/L to 25 mg/L for calcium (Ca) and 10 mg/L to 12.5 mg/L for magnesium (Mg) were established before many newer agents were developed. This study was undertaken to re-evaluate the ranges for Ca and Mg in MHB that are needed to produce MICs within acceptable QC limits for drug-organism combinations for which divalent cation concentration is critical.

METHODS: Tobramycin, gentamicin, imipenem, tetracycline, oxacillin, and daptomycin were tested against *E coli* 25933, *P aeruginosa* 27853, *E faecalis* 29212 and *S aureus* 29213 (all ATCC strains) in frozen microdilution trays prepared by TREK Diagnostic Systems as described in CLSI M7 A6. Trays were prepared using two MHB lots (Oxoid and BD) initially containing <10 mg/L Ca and <5 mg/L Mg. For each drug concentration, Ca:Mg was varied in 5:2.5 mg/L increments over a range 10:5 to 55:27.5 mg/L.

RESULTS: For *P aeruginosa*, aminoglycoside MICs remained acceptable up to 45:22.5. *S aureus* MICs were all in the mid-QC range over the entire cation range for gentamicin and tobramycin. For *E faecalis*, gentamicin yielded mid-QC range MICs over the entire Ca:Mg series, but tobramycin MICs were all at the low end of the QC limits. For all agents, the only

out-of-range results with *E coli* were at the highest Ca:Mg concentrations (≥50:25). For *E coli* and tetracycline, MICs were in mid-QC range over a Ca:Mg range of 15:7.5 to 40:20, although acceptable over the entire range of Ca:Mg. For both *E faecalis* and *S aureus*, daptomycin yielded mid-QC range MICs at Ca:Mg concentrations as low as 40:20, even though CLSI recommends 50 mg/L Ca.

CONCLUSIONS: These observations indicate that for selected drug-organism combinations that are thought to be affected, it appears that maintaining a 2:1 ratio of Ca:Mg in MHB may be more important than the specific concentrations of each. Alternatively, the concentration of these ions *in toto* may be the critical element. These preliminary data suggest that the concentration range of Ca and Mg required to meet CLSI criteria for acceptable performance may be broader than current specifications.

O2

IDENTIFICATION OF *NOCARDIA* SPECIES BY PARTIAL SEQUENCING OF THE *hsp*65 GENE

A MCNABB*, K ADIE, G GEDDES, C SHAW, S MITHANI, JI RENTON
Laboratory Services, BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: To determine if partial sequencing of the 65 KiloDalton Heat Shock Protein Gene (*hsp*65) is a valid means to identify *Nocardia* species causing, or isolated from, human infections.

METHODS: An *hsp*65 sequence database was created using 24 *Nocardia* species type strains and 3 select clinical isolates previously identified by 16S rRNA gene sequencing. 175 clinical *Nocardia* isolates identified by biochemical test panels, whole cell fatty acid analysis, and confirmed by partial sequencing of the 16S rRNA gene using the criteria of Mellmann et al had a 441 nt region of their *hsp*65 gene sequenced and compared to the *hsp*65 sequence database for identification purposes.

RESULTS: All *hsp*65 type strain sequences were unique, with some sequences more discriminatory than 16S rRNA sequence, for example, separating *N asiatica* from *N abscessus*. Overall, *hsp*65 sequences had a 63% correlation with the 16S rRNA identifications. Failure to corroborate the identification of some isolates was due mainly to the presence of groups of unknown validity within *N nova* (2), *N cyriacigeorgica* (4) and *N otitidiscaviarum* (3), or the lack of a corresponding species' sequence entry or difficulty in data interpretation.

CONCLUSIONS: Partial sequencing of the *hsp*65 gene appears to have great potential for the identification of *Nocardia* species from clinical samples and has the advantage that it can be used in mixed cultures. Our current identification database has limitations due to the presence of a limited number of *Nocardia* species and therefore must be used with caution. The presence of groups within defined species requires further investigation to corroborate their validity, but *hsp*65 sequencing confirms the findings of 16S rRNA gene sequencing that *N asteroides* is a rare cause of human infections in British Columbia and probably Canada.

O3

CREATING A NATIONAL CULTURE COLLECTION FOR CANADA

K BERNARD*[1], A LEVESQUE[2], C BABCOCK[2], K SEIFERT[2]
[1]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba; [2]Agriculture and Agrifoods Canada, Ottawa, Ontario

OBJECTIVES: Repositories for permanent, secure, long-term storage of live cultures of bacteria, viruses, fungi and other biomaterials exist around the world, including all G-7 countries except Canada. We outline an approach to create a national network of collections to ensure permanent repository of valuable biological materials in secure locations.

METHODS: Interested partners formed a working group in 2004, obtained a CRTI grant [BIO 021AP] to investigate this proposal, and held a consensus conference to decide how to initiate creation of a national collection. Three initiatives were undertaken: 1) purchase equipment to enhance or maintain collections; 2) conduct an inventory of existing collections; 3) conduct a feasibility study of resources and funding required to create a national collection for Canada.

RESULTS: The consortium consisted of scientists from diverse disciplines from Canadian federal government departments. Equipment was purchased to assist partners with enhancing collections or improve capacity for long-term storage of organisms. A new inventory of existing Canadian culture collections was launched [December 2005]. It was decided that key culture collections located in different parts of the country under individual expert curators would be linked together to form a single network, with support by a funded umbrella organization. A feasibility study, where start-up and maintenance requirements for a national collection would be detailed, would focus on multi-site models successfully used by several European collections, rather than a single geographic location model like the ATCC.

DISCUSSION: When funded, creation of a national culture collection should: 1) ensure long-term storage of valuable cultures in secure sites that meet biosafety regulations; 2) allow researchers to acquire strains/biomaterials in Canada, avoiding cross-border import issues; 3) preserve collections in imminent danger of being lost due to retirements or mandate changes; 4) allow for deposit of type strains of *species nova*, required by the International Committee on Systematics of Prokaryotes.

O4

COMPARISON OF TRIAGE AND TECHLAB TESTS FOR DETECTION OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA (CDAD) IN STOOL SAMPLES

MJ ALFA*[1], GK HARDING[1], P PANG[1], A WOLD[3]
[1]St Boniface Hospital; [2]University of Manitoba; [3]Red River College; [4]Diagnostic Services of Manitoba, Winnipeg, Manitoba

OBJECTIVES: The recent increased incidence and disease severity has focused attention on rapid and accurate diagnosis of CDAD in healthcare facilities. The aim of this study was to evaluate two antigen detection tests: the Triage test and the TechLab test.

METHODS: Ninety consecutive stool samples submitted for assessment of CDAD were included in the evaluation. The CPE assay using HFF cells was used to resolve discordants for the Triage test and when the results of the two methods being compared were different. For TechLab testing, the GD card was tested first and testing with the Toxin A/B card was only performed for those stools that were positive for the GD test card. For Triage testing, there is only one test card that detects both GD and Toxin A; however, additional testing with the CPE assay was done for discordant test results (i.e. GD [+], ToxA [–] or GD [–], ToxA [+]).

RESULTS: The TechLab results showed sensitivity, specificity, PPV and NPV of 80%, 100%, 100% and 92.9%, respectively. For unresolved Triage data, the sensitivity, specificity, PPV and NPV were 96%, 82%, 66.7% and 98.2%, respectively. Once discordants were resolved using the CPE assay, the sensitivity, specificity, PPV and NPV for Triage testing were 96%, 100%, 100% and 98.5%, respectively.

DISCUSSION: The TechLab test advantages include: no discordants testing needed so the TAT for positives was faster; no centrifugation step required; results easy to read as most were strong reactions; and the sample volume needed was small. The Triage test advantages include: one card to complete assay; filtered sample used so less biohazard risk; and the sensitivity was excellent (i.e. does not miss many true positives). If discordants were not resolved for the Triage test, then the TechLab test provided superior PPV (100% vs 66.7%) and specificity (100% vs 82%) but lower sensitivity (80% vs 96%) compared to the Triage test. If the CPE assay was used in conjunction with the Triage test, then the Triage test provided optimal sensitivity, specificity, PPV and NPV compared to TechLab testing.

POSTER PRESENTATIONS
Thursday, March 16, 2006

P1.01
ANTIMICROBIAL EFFECT OF LIPOSOMAL ANTIBIOTICS ON CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* FROM CYSTIC FIBROSIS PATIENTS

M ALIPOUR, C MUGABE*, A OMRI
Laurentian University, Sudbury, Ontario

OBJECTIVE: Cystic fibrosis is the most common lethal autosomal recessive disease found in the Caucasian population, with a frequency of approximately one in 2,500 births. Chronic respiratory infections with *P aeruginosa* are the leading cause of morbidity and mortality in such individuals. Once *P aeruginosa* colonizes the cystic fibrosis patient's lung, it cannot be eradicated even by the most aggressive antibiotic therapy. The aim of this work is to evaluate bactericidal activity of liposomes-entrapped aminoglycosides against clinical isolates of *P aeruginosa* from cystic fibrosis patients.
METHODS: Gentamicin, amikacin and tobramycin were incorporated into liposomes prepared by the modified dehydration-rehydration method. The sizes and encapsulation efficiencies of these vesicles were determined. The minimum inhibitory concentrations and the time-killing curves of free and liposomal drugs against clinical isolates of *P aeruginosa* were assessed.
RESULTS: The average liposomal size was below 400 nm in diameter. The encapsulation efficiency ranged from 10%±1% for gentamicin to 36%±2% for amikacin and 15%±2% for tobramycin. The minimum inhibitory concentrations of liposomal aminoglycosides for clinical isolates of *P aeruginosa* were lower compared to the corresponding free drugs. The most notable difference was seen for a highly resistant strain PA-48912-2, which displayed a minimum inhibitory concentration of 16 mg/L for liposomal amikacin compared to 256 mg/L for the free drug. In addition, the time-killing values for liposomal aminoglycosides were either equivalent to or better than that of the free antibiotics.
CONCLUSION: Liposomal aminoglycosides are more potent anti-pseudomonal antibiotics, with improved killing time and prolonged antimicrobial activity, and warrant further preclinical investigations into the use of these formulations for the treatment of chronic pulmonary infections.

P1.02
ANTIVIRAL EFFECT OF AURINTRICARBOXYLIC ACID (ATA) ON WEST NILE VIRUS (WNV) AND SARS-CORONAVIRUS (SARS-COV) INFECTION: DIFFERENT MECHANISMS OF ACTION?

M ANDONOVA*, R HE, J BERRY, X LI, A ANDONOV, M DREBOT
[1]National Microbiology Laboratory, Winnipeg, Manitoba; [2]Centre for Biologics Research, Ottawa, Ontario

OBJECTIVE: WNV and SARS-CoV are emerging pathogens that can significantly impact public health. In this study, we investigated the antiviral activity of aurintricarboxylic acid (ATA) on WNV and SAR-CoV replication and show that the target of drug action appears to differ between the two RNA viruses.
METHODS: Inhibition of viral replication was evaluated by: 1) decreased production of infectious virus progeny by plaque titration of the extracellular virus; 2) quantitative reduction of total viral load by real-time PCR. Experiments were performed with a VERO E6 (VE6) cell line, inoculated with SARS-CoV and WNV prior to or after treatment with several different concentrations of ATA (800 mg/L, 400 mg/L and 200 mg/L). A molecular docking (MD) analysis was used to determine probable sites of interaction between ATA and various viral proteins.
RESULTS: Various concentrations of ATA (400 mg/L to 800 mg/L) inhibited WNV replication by over 500-fold if the compound was incubated with virus before infection or added during virus adsorption to the cell monolayer. No significant antiviral effect was observed when ATA was added after viral adsorption. The drug showed similar inhibitory effects on SARS-CoV but the addition of ATA after virus adsorption led to a more pronounced inhibition of viral replication. MD analysis indicated that ATA probably binds most tightly to the envelope protein of WNV and may effect viral entry into cells. In contrast, MD showed that the primary interaction of SARS–CoV with ATA may be at the level of viral RNA polymerase substructure
CONCLUSION: Our results show that ATA is a potent inhibitor in vitro against SARS-CoV and WNV. Intial characterization of the possible modes of antiviral action indicated that ATA may target different aspects of replication for the two viruses.

P1.03
A GENOTYPING STUDY OF COMMUNITY ACQUIRED-METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (CA-MRSA) ISOLATES FROM BRITISH COLUMBIA

L JANZ[1], L WANG[1], J BORN[1,2], A MCNABB[1], D CHAN[1], AH LEUNG[2], T RAHIM[1], C SHAW[1], J ISAAC RENTON[1,2], SH GOH*[1,2]
[1]BC Centre for Disease Control; [2]University of British Columbia, Vancouver, British Columbia

OBJECTIVE: To determine the genotype of circulating MRSA strains in British Columbia with respect to the pulsed-field gel electrophoresis type, and the presence or absence of SCC*mec* IV cassette and the Panton-Valentine Leucocidin-coding genetic locus (PVL).
METHODS: PCR methods developed by Lina et al (PVL detection), Oliveira and de Lencastre (SCC*mec* typing) and Bergland et al (SCC*mec* IV subtyping) were used for genotyping the MRSA strains. The MRSA PFGE protocol developed by the National Microbiology Laboratory (NML), Winnipeg, Manitoba, was used for subtyping. PFGE subtype strains were assigned NML numbers using the NML CNISP MRSA database.
RESULTS: A total of 182 random isolates representative of 12 different British Columbia health care facilities were tested. 100 MRSA isolates were both PVL and SCC*mec* IVa positive. 97 isolates clustered as Canadian MRSA 10 (CMRSA 10), while 3 were CMRSA 7. The majority of the CMRSA 10 strains belonged to the NML 0473 type. Two PVL-positive strains were SCC*mec* negative and did not cluster within any of the current CMRSA types in the NML database. The remaining 80 MRSA strains were PVL-negative, with the majority having SCC*mec* II and III cassettes and clustered mainly as CMRSA 2 and 6, respectively.
CONCLUSION: The CA-MRSA strains in British Columbia are predominantly derived clonally as CMRSA 10, NML 0473 type. All strains are PVL-positive and exclusively harbour the SCC*mec* IVa cassette.

P1.04
EVALUATION OF ACCURACY LIMITS OF COUNTABLE COLONY-FORMING UNITS (CFU) ON AGAR PLATES

JC ARBIQUE*, AF RENDELL, KR FORWARD
QEII Health Sciences Centre, Halifax, Nova Scotia

OBJECTIVES: Accurate colony counts are an essential component of many microbiology processes. The suggested range of accuracy of CFUs extends from 30 to 300. This recommendation dates to 1907, and fails to adequately address the numerous sources of inter- and intra-variability. Without more detailed analysis, it is difficult to estimate the sample size and number of replicates necessary to ensure reproducible results. The purpose of this study was to determine the validity of CFU accuracy limits on agar plates.
METHODS: ATCC strains of *Escherichia coli* and *Staphylococcus epidermidis* were used to prepare series of four organism densities ranging from approximately 40 to 500 CFU, on 3 different days. On each day, each of the 4 densities for both organisms was plated on SBA, and CFU were counted following incubation. An average of the margins of error obtained over the 3 days of testing was used to determine the reproducibility of plate counts and to estimate the optimum number of replicate plates required for each organism at each concentration.

RESULTS: Margins of error for both organisms were greatest with suspensions yielding approximately 40 CFU, and lowest for suspensions yielding 300 to 500 CFU. Nine replicate plates were required for a suspension of *S epidermidis* yielding 40 CFU, to achieve the same margin of error obtained with 3 replicate plates at concentrations yielding 100 to 300 CFU. Similarly, 7 replicates plates were required for a suspension of *E coli* yielding 40 to 100 CFU, whereas, only 4 replicate plates were required at concentrations yielding 300 CFU, and 3 replicate plates at concentrations yielding 500 CFU.
DISCUSSION: We found that the greater the concentration (300 to 500 CFU), the fewer replicate plates were necessary to reproducibly estimate organism concentrations. At lower organism densities (40 CFU), more plates were required to reliably estimate CFUs. Contrary to the recommendations described in Standard Methods for the Examination of Water and Wastewater, CFUs of 500 were reliably reproducible. For greatest accuracy, experiments should be conducted to assure that colony counts are in the range of 300 to 500 CFU.

P1.05
EVALUATION OF THE COPAN M40 AND STARPLEX AMIES TRANSPORT SYSTEMS′ POTENTIAL TO PREVENT OVERGROWTH OF AEROBIC ORGANISMS DURING SIMULATED TRANSPORT

JC ARBIQUE*, AF RENDELL, KR FORWARD
QEII Health Sciences Centre, Halifax, Nova Scotia

INTRODUCTION: CLSI document M40-A addresses quality control issues for microbiological swab transport devices. Although it is recognized that microorganism overgrowth during transport and storage at ambient temperatures (RT) may adversely affect pathogen recovery from mixed cultures, this issue is not addressed in M40-A. Here, we challenge two commercial products to evaluate the effect of contaminating organisms on the ability to recover *Neisseria gonorrhoeae* (NG).
METHODS: Two suspensions, *Pseudomonas aeruginosa* [PA] (10^6 as described in M40-A), and NG and *Escherichia coli* (EC) were used to challenge 3 lot numbers of Amies without charcoal (Copan Diagnostics and Starplex Scientific Inc). The NG/EC suspension (approximately 10^4 CFU) was chosen to represent organism concentrations likely to be recovered from genital samples. Suspensions were inoculated to swabs in triplicate. Swabs were then held at RT or refrigeration for periods of 0, 6, 24 or 48 hours, and then plated onto solid media. PA CFUs were counted on SBA; NG CFUs were counted on Thayer-Martin (TM), and EC on CHOC. Recovery was determined using the M40-A swab elution technique.
RESULTS: All products tested met the overgrowth acceptance criteria described in M40-A for PA. Refrigeration prevented overgrowth of EC, and NG was successfully recovered from both products after 48 hours. At RT, NG was recovered from all 3 Copan lots, and 2 of the 3 Starplex lots, after 24 hours. EC concentrations increased approximately 1×10^2, and $>2\times10^3$ after 24 and 48 hours, respectively, on the Copan product; and 5×10^2 and 5×10^4 on the Starplex product.
DISCUSSION: We demonstrated recovery of NG from both products after holding for 24 hours at RT or refrigeration. However, swab elution is not the standard inoculation protocol used in most clinical laboratories, and further studies are required using clinical practice (roll-plate technique) to determine the ability of commercially available swab transport devices to recover pathogens such as NG from specimens contaminated with less fastidious organisms.

P1.06
EVALUATION OF THE COPAN M40 AND STARPLEX AMIES TRANSPORT SYSTEMS′ POTENTIAL TO MAINTAIN THE VIABILITY OF AEROBIC FASTIDIOUS ORGANISMS DURING SIMULATED TRANSPORT

JC ARBIQUE*, AF RENDELL, KR FORWARD
QEII Health Sciences Centre, Halifax, Nova Scotia

INTRODUCTION: CLSI document M40-A, Quality Control of Microbiological Transport Systems, addresses quality control issues related to microbiological swab transport devices, and provides methods and acceptance criteria for manufacturers and laboratorians to evaluate and compare products. Here, we describe our experience using M40-A methodologies to evaluate 2 commercial swab transport systems.
METHODS: ATCC suspensions of *Haemophilus influenzae* (HI) and *Neisseria gonorrhoeae* (NG) were inoculated in triplicate to 3 lot numbers of Amies without charcoal (Copan Diagnostics and Starplex Scientific Inc) using the roll-plate technique described in M40-A. Transport devices were held at ambient or refrigeration temperatures for periods of 0, 6, 24, 48 or 72 hours, and then plated on CHOC media. CFU were counted following incubation. Baseline counts were obtained from media inoculated at zero time.
RESULTS: The Copan product demonstrated superior performance, despite much higher baseline counts obtained with the Starplex product. Refrigeration greatly improved recovery of organisms; both products held at refrigeration met or exceeded M40-A minimum acceptance criteria for both organisms, although recovery of HI was superior at 48 and 72 hours with the Copan product. At RT, all 3 Copan lots exceeded minimum acceptance criteria for NG and HI, while 2 of the 3 Starplex lots failed for NG and 1 failed for HI. NG was not recovered from either manufacturer's products at 48 hours RT. Considerable lot-to-lot variability was observed with the Starplex product, with 1 lot in particular demonstrating much lower baseline recovery than the other 2 lots.
CONCLUSION: Organism recovery was improved at simulated refrigeration transport temperatures; however, transportation and holding of microbiology specimens frequently does not currently involve refrigeration. Therefore, worst-case transportation scenarios must be considered when choosing transport devices. We demonstrated superior recovery with the Copan product over the Starplex product at both refrigeration and RT.

P1.07
EVALUATION OF A RAPID AMPLIFICATION-DETECTION ASSAY FOR THE IDENTIFICATION OF VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE)

J FULLER*, L TURNBULL, S SHOCKOPLES, B LUI, L ROSMUS, R RENNIE
Medical Microbiology, University of Alberta Hospitals, Edmonton Alberta

OBJECTIVE: Routine identification of vancomycin-resistant enterococci (VRE) often yields a lengthy turn-around time that may impede infection control efforts, particularly in an outbreak situation. We evaluated the GenoType Enterococcus assay (Hain Lifescience, Germany), which provides both species and *van* gene identification for VRE, and compared the results to conventional methods.
METHODS: Forty clinical enterococcal strains isolated on VRE-screen agar media were selected for study. *Lactococcus* and *Pediococcus* were used as negative controls. Conventional testing involved basic culture and identification tests, E-test susceptibility testing for vancomycin and teichoplanin, and PCR for *van*A, *B*, and *C* genes. The GenoType Enterococcus assay involved multiplex DNA amplification and reverse hybridization of amplified product on an immobilized DNA strip-blot containing probes for *E faecium*, *E faecalis*, *E casseliflavus*, *E gallinarum*, *van*A, *van*B, *van*C1 and *van*C2/3.
RESULTS: The GenoType Enterococcus assay produced correct species and *van* gene identification for all 40 (100%) VRE isolates, including 7 *E faecalis vanB*, 12 *E faecium vanA*, 12 *E faecium vanB*, 6 *E gallinarum vanC1*, 1 *E gallinarum vanA-vanC1*, and 2 *E casseliflavus vanC2/3*. The only minor discrepancy was an *E casseliflavus* that hybridized very weakly with the *vanC1* probe in addition to the expected *vanC2/C3* probe. The costs per specimen were comparable for each test method. However, the GenoType Enterococcus assay could be completed within a normal working day in contrast to conventional testing, which required a minimum of two days from the point of isolation on the vancomycin-screen media.
CONCLUSION: From this preliminary evaluation, the GenoType Enterococcus amplification-detection assay provides VRE species and *van* genotype identification in a rapid and cost-effective manner, superior to conventional culture methods. Although further study is required, this kit may have clinical utility during a VRE outbreak.

P1.08
BIOCHEMICAL, ANTIGENIC AND GENETIC ANALYSIS OF INVASIVE *HAEMOPHILUS INFLUENZAE* IN MANITOBA, CANADA IN THE POST-VACCINATION ERA

ML SILL*[1], S MUBAREKA[2], DKS LAW[1], J WYLIE[3], S SKINNER[2], RSW TSANG[1]
[1]Public Health Agency of Canada, National Microbiology Laboratory; [2]University of Manitoba, Department of Medical Microbiology and Infectious Diseases; [3]Cadham Provincial Public Health Laboratory, Winnipeg, Manitoba

OBJECTIVE: Invasive *Haemophilus influenzae* (Hi) isolates collected in Manitoba, Canada, from 2000 to 2004 were studied according to their serotype, biotype, and genotype to assess the impact of vaccination on the type of strains causing invasive Hi disease.
METHOD: Serotyping was done by slide agglutination using serotype-specific antisera as well as PCR detection of serotype-specific genes and the capsule transport gene *bexA*. The clonal nature of the Hi isolates was analysed by pulsed-field gel electrophoresis (PFGE) using *SmaI*, and multi-locus sequence typing (MLST) was performed on seven housekeeping loci to determine sequence types (STs). Antibiotic susceptibility was determined by the disk diffusion method and detection of beta lactamase was done using a commercial dry slide nitrocefin test kit.
RESULTS: During the five-year period from 2000 to 2004, 53 invasive Hi cases were identified and 52 isolates were successfully retrieved for further characterization. Twenty six (50%) of the Hi isolates were found to be serotype a and another 38.5% (20 isolates) were found to be non-serotypeable (NST). There were only 3 serotype b (Hib) strains and one isolate each for serotypes c, d and f. All 26 serotype a isolates belonged to biotype II, demonstrated identical or highly similar DNA fingerprints by PFGE, and were identified by MLST as belonging to the clonal complex ST-23. In contrast, the 20 NST isolates were found to belong to 18 different STs, and most were unrelated when analyzed by PFGE. Two (6.25%) of the 32 serotypeable isolates (1 serotype a and 1 serotype b) and 6 (30%) of the 20 NST isolates were resistant to ampicillin due to beta lactamase production.
CONCLUSION: *H influenzae* serotype a is emerging as the predominant serotype associated with invasive disease in the Hib post-vaccination era in Manitoba. This has implications for surveillance for cases of invasive Hi and the development of control strategies.

P1.09
CHARACTERIZATION OF A UNIQUE CLONE OF SEROGROUP B *NEISSERIA MENINGITIDIS* CAUSING INVASIVE MENINGOCOCCAL DISEASE IN QUEBEC

DKS LAW*[1], M LORANGE[2], L RINGUETTE[2], R DION[2], AM HENDERSON[1], J STOLTZ[1], RSW TSANG[1]
[1]National Microbiology Laboratory, Winnipeg, Manitoba; [2]Laboratoire de sante publique du Québec, Institut national de sante publique du Québec, Sainte-Anne-de-Bellevue, Québec

OBJECTIVE: A cluster of invasive meningococcal disease (IMD) cases due to serogroup B *Neisseria meningitidis* were detected in the south shore of Québec City. The objective of this poster is to present laboratory findings that show that the outbreak strain is an emerging clone first identified to cause IMD in Québec in 2003.
METHODS: Laboratory confirmation of IMD cases was achieved by either culture or PCR detection of *N meningitidis*-specific DNA from normally sterile body fluids of patients. Serotyping and serosubtyping of *N meningitidis* strains were done by indirect whole-cell ELISA using monoclonal antibodies. DNA sequencing of antigen and housekeeping enzyme genes as well as pulsed-field gel electrophoresis were performed to characterize the outbreak strain and to identify the emerging clone.
RESULTS: A total of 38 isolates with the antigenic formula of B:17:P1.19 were identified from IMD cases in Québec during the last 30 months, including 11 cases from the first 6 months of 2005. Multi-locus sequencing typing identified the clone as ST-269. A search of our records at both the Laboratoire de santé publique du Québec and the National Microbiology Laboratory did not identify any B:17:P1.19 (ST-269) isolate prior to 2003.
CONCLUSION: An emerging clone of ST-269 with the antigenic formula of B:17:P1.19 was identified to cause recent IMD cases in Québec. Hypothesis for the emergence of this clone is discussed.

P1.10
EVALUATION OF THREE COMMERCIAL LATEX AGGLUTINATION KITS FOR SEROGROUPING BETA-HEMOLYTIC STREPTOCOCCI

P KORNHERR*, D DOWHANIUK, D RAYMONDO
Gamma-Dynacare Laboratories, Ottawa, Ontario

OBJECTIVE: There is a clinical need to rapidly and accurately diagnose whether pharyngitis is attributable to Group A Streptococcus (GAS), as it is the only form of pharyngitis that requires antibiotic treatment. Several significant variables in the rapid detection of GAS from culture exist, one of which is the accuracy and reliability of Strep grouping latex kits. This study compares 3 latex agglutination kits, namely Phadebact (Boule), PathoDx (Remel) and Prolex Blue (Pro-Lab Diagnostics), in terms of accuracy, ease of use and speed of obtaining results.
METHOD: This study includes 279 strains of Beta Hemolytic Streptococci (BHS). All isolates were grown on 5% sheep blood incubated anaerobically for 18 to 24 hours. Large colony BHS were tested for Lancefield groups A, B, C, F, G in accordance with manufacturer's written instructions. Testing with Phadebact and PathoDx kits incorporated the optional direct colony procedures, whereas the Prolex Blue kit utilized modified acid extraction procedures.
RESULTS: Of the 279 throat swabs, 191 were Group A, 28 were Group B, 26 were Group C, 26 were Group G, 4 were Group F and 4 were unknown (not Group A, B, C, F or G). Rare cross reactions did occur with each kit, but had occurred close to the one minute test time restriction.
CONCLUSION: All the latex test kits tested acceptably well. With the required number of isolated colonies, the Phadebact and PathoDx kits provided faster results than the Prolex kit, as Prolex requires the addition and manipulation of 3 reagents. Overall, the PathoDx provides the easiest kit to use in terms of easy to read instructions, layout and favourable numbers of tests per disposable card – an aid to high-volume laboratories. The direct kits offer an advantage in providing faster results when using proper smearing procedures. The Prolex kit required more preparation time but was more sensitive when using lower colony numbers.

P1.11
THE IDENTIFICATION OF *CLOSTRIDIUM DIFFICILE* CONTAINING BINARY TOXIN IN SASKATCHEWAN

RR MCDONALD*[1], PN LEVETT[1], R KITZUL[1], KL MONTGOMERY[1], T DU[2], AD BOYD[2], NA ANTONISHYN[1], GB HORSMAN[1], MR MULVEY[1]
[1]Provincial Laboratory, Regina, Saskatchewan; [2] National Microbiology Laboratory, Winnipeg, Manitoba

OBJECTIVE: *Clostridium difficile* is a major cause of antibiotic-associated diarrhea. Strains of increased virulence have recently been reported. *C difficile* containing binary toxin genes has caused outbreaks of infection in several cities. The objective of this study was to describe the presence of binary toxigenic *C difficile* in Saskatchewan.
METHODS: In Saskatchewan, 33 *C difficile* isolates from 2000 and an additional 29 isolates collected in 2004/2005 were tested for the presence of clostridial toxin genes *tcdA*, *tcdB*, and the two binary toxin subunit genes (*cdtA* and *cdtB*) by PCR. Isolates positive for binary toxin genes were subtyped using fbAFLP and PFGE, and the PaLoc negative regulation gene, *tcdC*, was sequenced.
RESULTS: All 62 isolates contained *tcdA* and *tcdB*. Binary toxin genes were detected in nine of the 62 isolates tested. PFGE clustered four of these with the epidemic strain NAP1/027 and three produced identical patterns. fbAFLP supported this clustering that included isolates from both the 2000 and 2004/2005 collection periods. The remaining five isolates were not similar to the epidemic strain cluster. Seven of the nine isolates contained a deletion in *tcdC* of varying lengths. The *tcdC* sequence of two isolates contained the 18 bp deletion described in the epidemic strain as well as a 1 bp deletion causing a predicted truncated amino acid sequence.

A third isolate contained a 36 bp deletion that would produce a truncated protein. Typing of this isolate did not show relatedness to the epidemic cluster. The three isolates were not related by location or time collected.
CONCLUSIONS: Strains of *C difficile* containing binary toxin genes that genetically resemble the epidemic strain have been circulating in Saskatchewan for at least five years and have not caused a major outbreak thus far. Truncated *tcdC* may be responsible for increased virulence. The combination of binary toxin PCR with molecular typing in Saskatchewan may be necessary for increased surveillance for this strain of *C difficile*.

STUDENT POSTER PRESENTATIONS
Thursday, March 16, 2006

SP.01
LIPOSOMAL ANTIBIOTICS AND BACTERIA INTERACTIONS: A MECHANISTIC STUDY

C MUGABE*, A OMRI
Laurentian University, Sudbury, Ontario

OBJECTIVES: *Pseudomonas aeruginosa* is a Gram-negative bacterium that is inherently resistant to almost all conventional antibiotics. The mechanism of resistance of this organism is mainly associated with its low outer-membrane permeability to these agents. The bactericidal efficacy of liposomes-entrapped aminoglycosides against clinical isolates of *P aeruginosa* and the mechanism of liposome-bacteria interactions were assessed.
METHODS: Aminoglycosides were incorporated into liposomes by the modified dehydration-rehydration method. The bactericidal efficacies of both free and liposomal drugs were evaluated by performing time-killing curves toward resistant strains of *P aeruginosa*. To define the mechanism of liposome-bacteria interactions, transmission electron microscopy, flow cytometry, lipid mixing assay and immunocytochemistry were employed.
RESULTS: Liposomal antibiotics were significantly more effective in killing bacteria than the corresponding free drugs. Electron microscopy observations of liposome-bacteria interactions showed that liposomes interact intimately with the outer membrane of *P aeruginosa,* leading to membrane deformation, an indication of liposome fusion with bacterial membranes. The fusion of liposomes with bacterial membranes was further confirmed by flow cytometry and lipid mixing assay. The fusion between liposomes and *P aeruginosa* resulted in significantly increased amount of antibiotics penetrated inside the bacterial cells, as demonstrated by immunocytochemistry studies.
CONCLUSION: Encapsulation of aminoglycosides into liposomes significantly increased the bactericidal efficacy of these agents against clinical resistant strains of *P aeruginosa*. In addition, these data suggest that liposomes interact with pseudomonal membranes by fusion, leading to an increased amount of drugs inside the bacteria cells. Thus, these liposome-entrapped antibiotics could overcome the bacterial resistance of *P aeruginosa* associated with its low outer-membrane permeability.

SP.02
HEMOTOXICITY OF LIPOSOMES: AN IN VITRO STUDY

S BLOMME*, C MUGABE, A OMRI
Laurentian University, Sudbury, Ontario

OBJECTIVE: Various liposomal formulations developed by our team were shown to promote the antibacterial activity of aminoglycosides and macrolides against clinical resistant strains of *P aeruginosa* and *B cepacia* from cystic fibrosis patients. In this study, the aim was to examine the effects of the concentration and composition of these liposomes on the toxicity of human blood cells.
METHODS: Liposomes composed of 1,2 Dimyristoyl–*sn*–glycero–3–phosphocholine (DMPC); 1,2 Dipalmitoyl–*sn*–glycero–3–phosphocholine (DPPC); 1,2 Distearoyl–*sn*–glycero–3–phosphocholine (DSPC) and cholesterol in the molar ratio of 2:1 were prepared by the modified dehydration-rehydration method. After size characterization, four concentrations (50 µg/mL to 500 µg/mL) of each formulation were added to a human erythrocyte suspension and incubated at 37°C for 2 hours. Aliquots of these suspensions were removed from incubation at 20 min intervals and the amount of haemoglobin released was measured spectrometrically at 540 nm. Values are expressed as a percentage of complete haemolysis.
RESULTS: The average liposomal size was determined to be 47.9±28.6 nm for DMPC; 55.3±29.7 nm for DPPC; and 57.4±32.5 nm for DSPC, with differences not being significant. Haemolytic activity increased as function of liposomal concentration and the maximum haemolysis was observed at the highest concentration tested (500 µg/mL). DSPC was found to have the lowest haemolytic activity with 5.3±0.1% haemolysis at 500 µg/mL, and DPPC to have the highest haemolytic activity, with 12.4±0.2% haemolysis at the same concentration. DMPC had a haemolytic activity of 11.1±0.6%. Over the 2 hour incubation period, there was no significant change in haemolytic activity in all the formulations tested.
CONCLUSION: The haemolysis assay revealed that even at extremely elevated liposomal concentrations, minimal toxic tendencies are observed. These data further attests to the safe use of liposomes in preclinical investigations.

SP.03
ANTIMICROBIAL RESISTANCE AND SEROTYPE DISTRIBUTION FOR REPORTED CASES OF INVASIVE PNEUMOCOCCAL DISEASE IN BRITISH COLUMBIA FROM 2001 TO 2005

M WINTERS*[1], DM PATRICK[1], F MARRA[2], J BUXTON[1], M CHONG[2], JL ISSAC-RENTON[2], G TYRRELL[3], M LOVGREN[3]
[1]University of British Columbia; [2]University of British Columbia Centre for Disease Control, Vancouver, British Columbia; [3]National Centre for Streptococcus, Edmonton, Alberta

OBJECTIVE: A linkage of two population-based surveillance programs for invasive pneumococcal disease (IPD) was conducted to determine the proportion of reported cases referred for microbiological testing, and to examine trends in serotype distribution and antimicrobial susceptibility.
METHODS: Reported cases of IPD from 2001 to 2005 from the reportable disease database at the BC Centre for Disease Control (iPHIS) (n=1148) were linked with 802 laboratory reports from the National Centre for Streptococcus (NCS) by surname and birthdate. Matched records contained patient demographics and isolate serotype and susceptibility to 8 antibiotics (defined according to NCCLS breakpoints).
RESULTS: 577 (50%) IPD iPHIS reports had matching NCS records. Matched records were not different than unmatched records by age, gender or resistance profile. Serotype data was available for 97.9% of isolates; overall 60% of serotypes were those included in the 7-valent vaccine (80% for ages <6 years and 51% for ages ≥6 years). 9.7% of isolates were resistant to penicillin and 10.1% to erythromycin. The proportion of isolates with resistance to penicillin and erythromycin was highest in children <15 years of age (13.6% [P=0.002] and 15% [P=0.027], respectively). From 2001 to 2005, there was a trend toward increasing resistance to penicillin (from 0% to 29% of isolates, P=0.008) and to erythromycin (from 3% to 29%, P=0.02).
CONCLUSION: Microbiological testing of referred IPD isolates indicates increasing rates of antimicrobial resistance in BC but no significant changes in serotype distribution. While the highest rates of resistance were in children, the new conjugate vaccine may reduce the overall disease burden in this population. Testing of all IPD isolates will provide more complete evidence for use in the evaluation of new immunization programs and antibiotic usage policies.

SP.04

ACTIVITY OF THE GLYCYLCYCLINE-TIGECYCLINE AGAINST RESISTANT PATHOGENS OBTAINED FROM CANADIAN INTENSIVE CARE UNITS: RESULTS OF THE CANADIAN NATIONAL INTENSIVE CARE UNIT (CAN-ICU) STUDY 2005

GG ZHANEL[1], M DECORBY*[1], N LAING[1], B WESHNOWESKI[2], M EDEL[2], K KARLOWSKY[1], JA KARLOWSKY[2], R VASHISHT[1], C SIEMENS[1], M MCCRACKEN[1], M MULVEY[3], DJ HOBAN[1]
[1]University of Manitoba; [2]Health Sciences Centre; [3]National Microbiology Laboratory, Winnipeg, Manitoba

OBJECTIVES: Tigecycline is a new glycylcycline with documented activity against resistant gram-positive and gram-negative pathogens. The CAN–ICU study assessed the activity of tigecycline against antimicrobial-resistant pathogens from ICUs in Canada.

METHODS: 20 medical centres from across Canada were asked to submit up to a maximum of 250 isolates from patients in the ICU. Susceptibility testing was performed using CLSI (2005) methods. Tigecycline susceptible (S), intermediate (I) and resistant (R) breakpoints (FDA) were (μg/mL): *Staphylococcus aureus* and MRSA ≤0.5 (S); *Enterococcus* species ≤0.5 (S); *Enterobacteriaceae, Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Acinetobacter* species ≤2.0 (S), 4 (I) and ≥8 (R).

RESULTS: Of the 2260 isolates collected to date, the susceptibility of tigecycline against selected resistant pathogens is listed below:

Organism	# of isolates	% of total	% susceptible
S. aureus	400	17.7	98.6
P. aeruginosa	260	11.5	2.1
E. coli	251	11.1	100
Enterococcus species	164	7.3	98.2
K. pneumoniae	120	5.3	97.4
MRSA	107	4.7	100
S. maltophilia	57	2.5	76.5
Acinetobacter species	18	0.8	85.7

Tigecycline was very active against MRSA, *Enterococcus* species, including (VRE), and *Enterobacteriaceae,* including ESBL producers.

DISCUSSION: Tigecycline is a new glycylcycline that is active against antibiotic-resistant ICU pathogens including MRSA, *Enterococcus* species including (VRE), *Enterobacteriaceae* including ESBL producers and *S maltophilia* and *Acinetobacter* species. Tigecycline is a promising agent for the treatment of infections caused by antibiotic-resistant pathogens.

SP.05

FIRST-GENERATION CEPHALOSPORIN SUSCEPTIBILITY TESTING OF *ESCHERICHIA COLI* BY BD AUTOMATED PHOENIX SYSTEM: QUESTIONABLE UTILITY OF CEPHALOTHIN AS A PREDICTOR OF CEPHALEXIN SUSCEPTIBILITY

SX ZHANG*[1], F PARISIAN[2], Y YAU[2], J FULLER[2], SM POUTANEN[3], S RICHARDSON[2]
[1]University of Toronto; [2] Division of Microbiology, Hospital for Sick Children; [3]Toronto Medical Laboratories and Mount Sinai Hospital, Toronto, Ontario

OBJECTIVE: Cephalexin (CX) is the empiric oral antibiotic of choice for the treatment of urinary tract infections (UTIs) in children at our hospital. After implementation of the BD Automated Phoenix System (PHX), we noted an unusual susceptibility pattern in our *E coli* isolates: ampicillin (AM) susceptible (S), cefazolin (CZ) S, cephalothin (CF) resistant (intermediate [I] or resistant [R]). CF R was 86% with 52% AM R and 5% CZ R. Since CX was not on the PHX panel, CLSI recommendations to predict CX from CF resulted in the loss of this drug for empiric UTI treatment. In this study, we compared PHX to reference antimicrobial susceptibility testing (AST) for AM and first-generation cephalosporins.

METHODS: 125 *E coli* clinical isolates (89% urinary) were selected based on their susceptibility patterns to AM, CF and CZ by PHX. AST was repeated by PHX and reference broth microdilution (BMD) as per CLSI simultaneously for AM, CF, CZ and CX (PHX UK panel for CX).

RESULTS: No discrepancy was observed between PHX and BMD for AM and CZ (68 S and 57 R to AM, and all but 2 S to CZ) by both methods. For CX, 88% isolates tested by PHX correlated with BMD; 11% were falsely R (all I) by PHX. However, PHX and BMD were discrepant for CF: 57/81 (70%) CF I strains by PHX were actually S by BMD. Moreover, BMD showed that 50% of isolates were S to CF, whereas 98% of isolates were S to CX, indicating that CF overcalls R to CX by 48% when used to predict CX as per CLSI. In contrast, CZ and CX agreed for 96% of strains, suggesting that CZ is a better predictor of CX susceptibility. In addition, it is notable that 26 isolates were found to be AM, CZ and CX-S but CF I/R by both methods.

CONCLUSION: A pattern of AM S, CZ S, CX S, CF I/R was observed in 59 *E coli* tested by PHX. Of these, 43 (73%) were explained by PHX overcall of CF or CX resistance compared to reference BMD. In addition, we found that CF was a poor predictor for CX susceptibility using BMD and CLSI recommendations. In the absence of specific CX testing, CZ is a better drug for predicting CX than CF.

SP.06

CHARACTERIZATION OF FLUOROQUINOLONE-RESISTANT EXTENDED SPECTRUM BETA-LACTAMASE-PRODUCING *ESHERICHIA COLI*

PRS LAGACÉ-WIENS*, KA NICHOL, M MCCRACKEN, M ALFA, MR DECORBY, LE NICOLLE, MR MULVEY, GG ZHANEL
University of Manitoba, Public Health Agency of Canada, National Microbiology Laboratories, Nosocomial Infections, Winnipeg, Manitoba

OBJECTIVE: Multi-drug resistant, extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* are increasingly common. As a cause of infections, they pose therapeutic dilemmas. The purpose of this study is to characterize the susceptibility, genetic similarity and ESBL genotypes of fluoroquinolone-resistant, ESBL-producing *E coli*.

METHODS: We determined the antimicrobial susceptibility, genetic similarity and ESBL genotype of 29 fluoroquinolone-resistant, phenotypically confirmed ESBL strains and 9 fluoroquinolone-susceptible ESBLs from a variety of body sites obtained from the clinical microbiology laboratories of two teaching hospitals and the national microbiology laboratory between January 2000 and December 2004. Susceptibilities were determined by the microdilution broth method, pulsed-field gel electrophoresis was performed, and the ESBL genotype was determined by PCR and sequencing of bla_{CTX-M}, bla_{SHV} and bla_{TEM} genes.

RESULTS: Of the 29 fluoroquinolone-resistant ESBLs, 8 (27.6%) were genotype CTX-M-14, 20 (69.0%) were genotype CTX-M-15 and 1 (3.4%) was genotype CTX-M-24. Among the 9 fluoroquinolone-susceptible strains, 4 (44.4%) expressed SHV-type enzymes, 4 (44.4%) expressed TEM-type enzymes and one (11.1%) expressed CTX-M-9. Of 29 TMP/SMX-resistant strains, 4 (13.8%) were CTX-M-14, 19 (65.5%) were CTX-M-15, 3 (10.3%) were SHV types, 1 (3.4%) was CTX-M-9, 1 (3.4%) was CTX-M-24 and 1 (3.4%) was TEM-11. 82.8% of fluoroquinolone-resistant isolates were co-resistant to TMP/SMX, while 55.6% of fluoroquinolone-susceptible strains were TMP/SMX-resistant. PFGE analysis of the 38 isolates revealed clonality within CTX-M-15 isolates but not between different ESBL genotypes.

CONCLUSIONS: The CTX-M-15 genotype in *E coli* is strongly associated with both fluoroquinolone and TMP/SMX resistance compared to other ESBL genotypes (P≤0.001). CTX-M-14 is associated with fluoroquinolone resistance only (P=0.002). There is a trend for co-resistance to TMP/SMX among fluoroquinolone-resistant ESBLs.

SP.07

PEPTIDOGLYCAN BIOSYNTHETIC ENZYMES PBP1A AND 1B ARE REQUIRED FOR OPTIMAL EXPRESSION OF *PSEUDOMONAS AERUGINOSA* VIRULENCE PHENOTYPES

J LEUNG*, CV GALLANT, LL BURROWS
McMaster University, Hamilton, Ontario

OBJECTIVE: Peptidoglycan (PG) is a semi-rigid structural component of the bacterial cell wall that maintains cell shape and provides protection against osmotic lysis. Many of the proteins involved in PG biosynthesis and maintenance, such as autolysins and endopeptidases, are the targets of beta-lactam antibiotics and are therefore known as penicillin-binding proteins (PBPs). Our objective was to determine how inactivation of the nominally redundant high molecular weight PBPs 1A and 1B in *Pseudomonas aeruginosa* impacts on cell-wall penetrating macromolecular structures involved in bacterial virulence.
METHODS: Genes PA5045 (*ponA*) and PA4700 (*mrcB*) encoding PBPs 1A and 1B, respectively, were disrupted in the wild type strain PAO1. The resulting mutants were screened for virulence-related phenotypes, including flagellar swimming motility, pilus-mediated twitching motility, susceptibility to the type IV pilus-specific C5 bacteriophage, type II secretion of proteases and lipases, and efflux of antibiotics. In addition, both static and dynamic biofilm assays were performed, and confocal laser scanning microscopy was used to visualize morphology of the dynamic biofilms grown in flow cells.
RESULTS: The PBP1A and PBP1B mutants exhibited type IV pili twitching defects, reduced swimming motility, reduction of type II secretion activity and impaired static and dynamic biofilm formation compared to the wild type. Alteration of dynamic biofilm morphology was more pronounced in the PBP1A mutant than in the PBP1B, demonstrating that these proteins do not have redundant activities.
CONCLUSIONS: Since the mutants showed deficiencies in phenotypes involving macromolecular structures that must traverse the PG in order to be correctly expressed, our findings suggest that specific glycosyltransferase and/or transpeptidase activities are critical for virulence factor expression in *P aeruginosa*.

SP.08

FLAGELLIN-MEDIATED IL-8 RELEASE IS ATTENUATED BY MONOCLONAL ANTIBODIES AGAINST THE PROXIMAL CONSERVED DOMAIN OF FLAGELLIN

S REYNOLDS*, L SHOBAB, T STEINER
Department of Infectious Diseases, University of British Columbia, Vancouver, British Columbia

OBJECTIVE: Many gram-negative bacteria express flagella that are used for propulsion and adherence. The protein flagellin, which is the major constituent of flagella, induces an inflammatory response via Toll Like Receptor (TLR) 5. Flagellin is composed of a variable domain flanked by two conserved domains, proximal (C1) and distal (C2), which are highly conserved among flagellated organisms. A region in the C1 domain is believed to mediate TLR 5 binding. Our goal was to determine whether isolated C1 would elicit an in vitro inflammatory response in Caco-2 cells, and whether flagellin-mediated inflammatory responses in these cells can be attenuated by monoclonal antibody directed against C1.
METHODS: The first 516 nucleotides of the H18 flagellin from enteroaggregative *E coli* strain 042, corresponding to the C1 domain, were cloned by PCR into pCR-NT-T7-Topo, and the His-tagged C1 protein was expressed in BL21 (DE3) pLysS cells and purified by metal chromatography. Monoclonal antibodies raised against full-length H18 flagellin were screened for binding to the proximal conserved domain via ELISA. C1 alone and flagella with serially decreasing antibody concentrations were added to Caco-2 cells grown post-confluence in 24 well plates for three hours, after which wells which contained Caco-2 cells. IL-8 release was quantified by commercial EIA.
RESULTS: The proximal conserved domain (C1) alone did not stimulate IL-8 release in the Caco-2 cells. Flagella produced a strong IL-8 release, which was attenuated in a concentration-dependent fashion by the addition of a C1-specific monoclonal antibody.
CONCLUSION: The isolated proximal conserved domain of flagellin does not induce IL-8 release in Caco-2 cells and, by inference, does not activate TLR 5. However, flagellin-mediated IL-8 release by Caco-2 cells can be attenuated by monoclonal antibodies directed against this proximal conserved domain of flagellin. These findings reinforce the importance of this domain in TLR 5 activation and raise the possibility that blocking antibodies may be used to inhibit flagellin responses.

SP.09

TREATMENT OF LOWER URINARY TRACT INFECTION CAUSED BY MULTI-DRUG-RESISTANT EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING *ESCHERICHIA COLI* WITH AMOXICILLIN-CLAVULANATE

PRS LAGACÉ-WIENS*[1], KA NICHOL[1], LE NICOLLE[1], MR DECORBY[1], M MCCRACKEN[1], MR MULVEY[2], GG ZHANEL[1]
[1]University of Manitoba; [2]Public Health Agency of Canada, National Microbiology Laboratory, Nosocomial Infections, Winnipeg, Manitoba

OBJECTIVE. Extended-spectrum beta-lactamase (ESBL)-producing *E coli* is an increasingly common cause of nosocomial and community acquired infections. Limited information is available on the treatment of lower urinary tract infections caused by ESBL-producing *E coli* with oral beta-lactam/beta-lactamase inhibitors. This study reports outcomes of amoxicillin/clavulanate treatment in two patients with ESBL urinary infections and describes the genetics and microbiology of the isolates.
METHODS: Cure of two patients with persistent urinary infection caused by an ESBL-producing *E coli* treated with amoxicillin-clavulanate are described. Antimicrobial susceptibilities of the organisms were determined. One isolate was available for further testing. Pulsed-field gel electrophoresis (PFGE) was performed and compared to PGFE patterns of multiple local ESBL isolates and the ESBL genotype was determined by PCR. A review of relevant literature was undertaken to identify data supporting the treatment of lower urinary tract infections caused by ESBL-producing organisms.
RESULTS: A 78-year-old man with clinical evidence of cystitis and prostatitis and a 55-year old woman with persistent bacteriuria with urine cultures positive for multi-drug resistant ESBL *E coli* are described. Treatment with amoxicillin-clavulanate resulted in resolution of symptoms and microbiological eradication of the organisms in both cases. Additional analysis of one isolate revealed a unique PFGE pattern compared to local ESBL isolates and the presence of ESBL CTX-M-15 ESBL. In vitro data show that clavulanate is a potent inhibitor of most ESBLs, including CTX-M-15. ESBLs may be resistant to amoxicillin-clavulanate by in vitro testing but may respond clinically due to high urinary concentrations of amoxicillin-clavulanate.
CONCLUSION: A trial of amoxicillin-clavulanate for the treatment of outpatients with urinary tract infections of mild to moderate severity caused by ESBL *E coli* may be reasonable when alternative oral agents cannot be used. Further clinical trials should be considered.

SP.10

GENDER, BUT NOT RESISTANCE TO METHICILLIN, AFFECTS SURVIVAL OUTCOME AT ONE YEAR IN PERSONS WITH INVASIVE *STAPHYLOCOCCUS AUREUS* INFECTION

W WOBESER, J BOSEOVSKI*
Queen's University, Kingston, Ontario

OBJECTIVE: *Staphylococcus aureus* is a leading microbial cause of morbidity and mortality. Its abundance and its virulence make it a deadly pathogen of significant burden. The primary purpose of this study was to investigate the outcome, at one year, of invasive *Staphylococcus aureus* infection.
METHODS: The study acquired data from three administrative electronic sources as well as a survey from primary care providers. The first data capture was from Vitek I in the microbiology lab at Kingston General Hospital (KGH) and all invasive, first-time isolates of *Staphylococcus aureus*

between January 1, 2003, and April 30, 2004, were enrolled. The Patient Care System (PCS) provided data on outcome, comorbidity, surgical intervention and place of acquisition. KGH pharmacy provided data regarding adequacy of antistaphylococcal therapy. Survival information for each patient was acquired either from the primary care provider or PCS.
RESULTS: In total, 344 isolates were examined. Exclusions included: 122 – point of care was not KGH; 9 – under the age of eighteen; 22 – inadequate antibiotic data. In the end, 191 isolates were analyzed. The 1-year survival for these patients was 85%. A Kaplan-Meier and Cox regression survival analysis was performed. Factors contributing to survival outcome included - female gender (Relative Risk [RR] 2.23; 95% Confidence Interval [CI] 1.14–4.25), increasing age (RR 1.05; 95% CI 1.03–1.08), co-morbidity (RR 1.07; 95% CI 1.01–1.13), hospital acquisition (RR 2.24; 95% CI 1.11–4.55) and length of stay (RR 0.97; 95% CI 0.95–0.99). Interestingly, resistance to methicillin was not a significant factor.
CONCLUSION: Survival at 1 year from invasive *Staphylococcus aureus* was found to be 85% better than other studies. Methicillin resistance, although possibly contributing to increased cost, did not affect survival. Unlike other studies, those acquiring disease in hospital were more likely to die. The finding of worse outcome among women deserves further study.

SP.11
PEDIATRIC INFECTIVE ENDOCARDITIS – A 19-YEAR REVIEW IN NORTHERN ALBERTA

A ALSHAMMARY*, JL ROBINSON, M HERVAS-MALO
University of Alberta, Edmonton, Alberta

BACKGROUND: Advances in cardiovascular surgery and increasing use of long-term intravascular catheters (IVC) may have altered the epidemiology of pediatric IE. The goal of this study was to determine the current risk factors, pathogens and outcome of IE.
METHODS: A chart review was completed in children younger than 17 years of age at the SCH between 1985 and 2003. Cases were included if they fit the Duke criteria for definite IE or the following criteria for possible IE: (a) CHD, fever, and ≥2 blood cultures (BC) with viridans streptococci (VGS); (b) CHD and bacteremia with coagulase-negative staphylococci (CONS) that did not resolve with the removal of vascular catheter; (c) fever, persistent CONS bacteremia and immunologic or vascular phenomena; or (d) CHD and ≥2 BC with any organisms where another etiology is unlikely.
RESULTS: There were 31 cases of definite IE (10 based on pathologic criteria and 21 on clinical criteria) and 9 cases of possible IE in children aged 2 weeks to 16 years. Nineteen cases were nosocomial. Twenty-eight cases had previously known CHD (20 VSD ± other cardiac lesions, 2 PS, 2 bicuspid AV, 2 Co.Aorta, 1 MVP and 1 TGA), 2 had previously undiagnosed CHD (MVP and VSD), 4 had no apparent risk factors and the other 6 had infected cystic hygroma with an IVC, Crohn's disease with an IVC, cerebral palsy with recurrent pneumococcal bacteremia, burn, excoriated skin and NEC. Trans-thoracic echocardiogram (TTE) showed vegetations in 22 cases and transesophageal echocardiogram (TEE) in another 7/8 cases. Cases involved native valves (6 MV, 6 TV, 4 AV, 2 PV), VSD patches (4) and MV homograft (1). Etiologic agent was *S aureus* (16), VGS (6), CONS (3), other streptococci (10), *Enterobacter cloacae* (1), *Stenotrophomonas maltophilia* (1) and culture-negative (3). Surgery was required in 11 (27.5 %) cases (5 valve replacement, 4 valve repair and 2 vegetation removal). Two patients died of IE. Major complications included congestive heart failure (21), definite embolic events (9), possible embolic events (7), endocardial abscess (3) and mycotic aneurysm (1). There were no recurrences of IE.
CONCLUSION: CHD remains the primary risk factor for pediatric IE. TEE was more sensitive than TTE for diagnosis of IE. Embolic events are a common complication.

SP.12
CHOLESTYRAMINE FOR ORAL SUSPENSION USP CAN MASK *CLOSTRIDIUM DIFFICILE* TOXINS IN STOOL WHEN ASSAYED BY TRIAGE *C DIFFICILE* PANEL AND CYTOTOXIN ASSAY

MR MURRAY*[1], MJ ALFA[1], D SITTER[2], J GUENTHER[2], P PANG[2]
[1]University of Manitoba; [2]Saint-Boniface Hospital and Research Centre, Winnipeg, Manitoba

OBJECTIVE: *Clostridium difficile*-associated disease (CDAD) is the most common cause of nosocomial acquired diarrhea. Rapid diagnosis and initiation of therapy is key in the prevention of spread of *C difficile*. Two tests currently used are Triage *C difficile* Panel and the cytotoxin assay. Cholestyramine for oral suspension USP is an absorbant administered orally and which may non-specifically bind bacterial toxins in the gastrointestinal tract. The objective of our research was to determine if cholestyramine treatment of patients may interfere with the Triage or the cytotoxin assay.
METHODS: Clinical stool samples were screened by Triage for *C difficile* toxin A antigen and glutamate dehydrogenase (GD) antigen. Clinical samples were selected for this project if they were positive for both antigens and containing a sufficient volume for analysis. Samples were analyzed within 24 hours. Stools were split and aliquots were analyzed by Triage and cytotoxin assay after incubation at 37°C for 2, 4 and 18 hours either with or without cholestyramine. An additional aliquot was immediately assayed by Triage and the cytotoxin assay to give a baseline.
RESULTS: 4/6 stools were negative for toxin A antigen and GD antigen when assayed by Triage *C difficile* panel after incubation with cholestyramine. 2/4 stools negative by Triage were also negative when a cytotoxin assay was run after incubation with cholestyramine. Two samples were negative for Triage after incubation without cholestyramine. All samples which were not incubated were positive by both assay systems.
CONCLUSIONS: Cholestyramine can mask the detection of toxigenic *C difficile* by two assay systems currently in use, Triage *C difficile* panel and cytotoxin assay. In patients suspected of having CDAD who are on cholestyramine, we recommend that if the Triage and/or cytotoxin tests are negative, then culture is necessary to determine if toxigenic *C difficile* is the cause of the patient's diarrhea.

SP.13
TWO CASES OF PYOGENIC LIVER ABSCESS CAUSED BY HYPERMUCOVISCOUS *KLEBSIELLA PNEUMONIAE*

JK LEE[3], S CHAMPAGNE[1], DL ROSCOE*[2], CS WANG[3], R CHAN[1], W BOWIE[2]
[1]Providence Health Care; [2]Vancouver General Hospital; [3]University of British Columbia, Vancouver, British Columbia

OBJECTIVE: *Klebsiella pneumoniae* (Kp) is now the predominant causative agent of pyogenic liver abscesses (PLA) in Taiwan, Korea and Indonesia, and is associated with metastatic foci such as meningitis and endophthalmitis. We report two Canadian cases of PLA caused by hypermucoviscous Kp.
METHODS: Both patients are Asian immigrants with diabetes mellitus, a risk factor for this infectious syndrome. Case one is a 70-year-old man who presented with shortness of breath, dysuria, abdominal pain, elevated liver transaminases and a 10 cm × 9 cm liver abscess. The patient responded well to abscess drainage, and therapy with piperacillin-tazobactam (five weeks) followed by ciprofloxacin and metronidazole (4 weeks). CT scan of the head was negative for emboli to the brain. Case two is 65-year-old woman presenting in septic shock requiring ICU care. She was found to have multiple liver abscesses but no other hepatobiliary or GI tract disease. She responded well to ciprofloxacin and metronidazole and is currently on therapy. There is no clinical evidence of other foci of infection.
RESULTS: Blood and liver aspirate cultures from both patients were positive for Kp. Hypermucoviscosity was demonstrated by a positive string test, defined by the formation of a viscous string from a colony on solid agar greater than 0.5 cm in length when stretched by an inoculation loop. Isolates were resistant to ampicillin but susceptible to other antibiotics. Molecular characteristics are currently under investigation for markers of tissue-invasive strains previously reported by Ma et al

(JID 2005;192:117-28). Isolates with particular molecular characteristics (*magA*-positive) produce a mucoviscous exopolysaccharide web, rendering the bacteria resistant to phagocytosis by polymorphonuclear leukocytes and serum killing.
CONCLUSION: This emerging tissue-invasive strain of Kp has arrived in Canada. Patients with PLA due to Kp should be investigated for possible other concurrent infectious foci.

SP.14
DEVELOPMENT OF A HEALTH-RELATED QUALITY OF LIFE QUESTIONNAIRE FOR PATIENTS WITH EXTREMITY SOFT TISSUE INFECTIONS

AJ STORCK*, KB LAUPLAND, RR READ, MW MAH, JM GIL, D NEVETT
University of Calgary, Peter Lougheed Centre, Calgary, Alberta

BACKGROUND: Past clinical trials of antimicrobial treatment in soft tissue infections have focused on non-standardized clinical and physiological variables, and have not considered the subjective experience of patients. The objective of this study was to develop a health-related quality of life questionnaire (HRQL) for patients with extremity soft tissue infections (ESTI).
METHODS: The design of this study followed published guidelines used to develop HRQL indicators in other diseases. Standardized methods were used to develop a list of items that might adversely impact the quality of life of patients with ESTI. A questionnaire containing these items was administered to a group of patients to determine their relative frequency and importance. Items for inclusion in the final questionnaire were selected by considering both their frequency and importance. The final questionnaire was pre-tested on a group of patients for clarity and acceptability
RESULTS: A list of 49 items that adversely impact the quality of life of patients with ESTI was generated. A questionnaire containing these items was administered to 103 patients with ESTI. Areas of quality of life impairment included physical symptoms related to their infection, problems performing their activities of daily living, impairment of their emotional functioning, and difficulties in their social interactions. Based on these results, a final questionnaire containing the most important items was prepared and a quantitative index, the ESTI-Score, was developed.
CONCLUSIONS: The *ESTI-Score* is a novel HRQL instrument and future study is warranted to prospectively determine its validity and responsiveness before use as an outcome measure in clinical trials.

SP.15
STAPHYLOCOCCUS EPIDERMIDIS BIOFILMS ARE FORMED DURING PLATELET STORAGE

CA GRECO*, SM RAMIREZ-ARCOS
Canadian Blood Services, Ottawa, Ontario

OBJECTIVE: *Staphylococcus epidermidis* is one of the leading causes of nosocomial septicaemias. The virulence of this bacterium resides in its ability to form biofilms on plastic biomedical implants. Since *S epidermidis* grows slowly in platelets in comparison to other bacteria, its detection by currently used culture methods presents a formidable challenge. Our objective is to investigate whether the slow growth of this species is due to a propensity to form biofilms during platelet storage.
METHODS: *S epidermidis* strains ATCC 35984 and O-47 were used as positive controls and ATCC 12228 was used as a negative control for biofilm formation, respectively. Cultures of these strains were prepared in tryptic soy broth (TSB) containing 0.5% glucose and in outdated platelets supplemented with glucose and/or TSB. Qualitative assays were established in 6-well tissue culture plates and in platelet bags at 22±2°C, with and without agitation. After 5 days, biofilms were quantified by staining adherent cells with 0.3% crystal violet.
RESULTS: Obvious biofilm-positive growth was observed in TSB in both culture plates and platelet bags. Biofilm formation was also substantial in the platelet sample counterparts and became more pronounced with nutrient supplementation. Interestingly, microscopic analysis revealed an intimate association between platelets and bacterial cells, which will be examined more closely using cell-type specific fluorescent markers. Future gene-expression studies will help to identify any gene products involved in the growth characteristics observed herein.
CONCLUSION: *S epidermidis* biofilm-positive strains display the ability to colonize plastic surfaces in the presence of platelets and appear to interact with platelets themselves. Our results have a significant impact in transfusion medicine, as they will help to change the view of *S epidermidis* as an innocuous organism. We are now aware of the potential of this species to express virulence factors in stored platelets and to cause severe or fatal post-transfusion reactions, which may only be recognized in retrospect.

SP.16
SELECTION OF DEREPRESSED MUTANTS AMONG CHROMOSOMAL AMPC BETA-LACTAMASE-PRODUCING ORGANISMS ON BETA-LACTAM THERAPY

POUTANEN S[1], MACDONALD KS[2], MATUKAS LM*[3], FULLER J[4]
[1]Toronto Med Lab; [2] Mount Sinai Hospital; [3] University of Toronto, Toronto, Ontario; [4] University of Alberta Hospitals, Edmonton, Alberta

OBJECTIVES: Derepressed mutants among chromosomal AmpC (chr-AmpC) beta-lactamase-producing gram-negative bacilli (gnb) can develop during therapy with third-generation cephalosporins (3G ceph). Clinical Laboratory Standards Institute (CLSI) suggests testing repeat isolates from patients on 3G ceph. Recently, we identified a patient with a 3G ceph and piperacillin/tazobactam (pip/taz)-susceptible *Enterobacter* bacteremia who was treated with pip/taz; 12 days into treatment, a repeat blood culture was positive for *Enterobacter* resistant to pip/taz and 3G cephs. The purpose of this study was to describe and determine the prevalence of treatment failures due to selection of stably derepressed chr-AmpC beta-lactamase mutants isolated from sterile sites.
METHODS: Using our clinical laboratory microbiology database, all patients from four teaching hospitals for whom sterile site specimens were positive for 3G ceph susceptible chr-AmpC gnb were identified from January 1, 2000, to March 30, 2005. All such patients who had subsequent cultures positive for 3G ceph nonsusceptible isolates within 90 days (d) were selected for chart review.
RESULTS: Twelve patients, including our index case, had subsequent cultures positive for 3G ceph non-susceptible isolates. Nine patients were infected with *Enterobacter* species, and 3 with *Citrobacter* species. All patients had received pip/taz, 3G ceph alone or in combination with a different class of antibiotic. Resistance was documented on average 18 d (range: 4 d to 79 d) after the first susceptible isolate. At least one patient died due to their infection.
CONCLUSIONS: In vivo selection of depressed mutants among chr-AmpC gnb can occur on pip/taz or 3G ceph combination treatment. Clinicians should avoid using beta-lactam/beta-lactamase inhibitors as well as 3G ceph alone or in combination to treat serious infections due to chr-ampC organisms. CLSI should consider revising its warning to include the risk of resistance emerging on beta-lactam antibiotics other than 3G ceph.

SP.17
SCREENING REFUGEE CHILDREN NEW TO CALGARY FOR INFECTIOUS DISEASES AND RELATED CO-MORBIDITIES

LK MACKENZIE*[1,2], SM KUHN, CP PIM[1,2]
[1]University of Calgary; [2] Calgary Refugee Health Program, Calgary, Alberta

OBJECTIVE: Children new to Canada are not consistently screened for infectious diseases despite being at high risk depending on geographic origin, migration history and exposure risk. The objective of this study was to use baseline screening tests (stool, blood and urine) with the population of refugee children seen at the Calgary Refugee Health Program to identify the presence of or susceptibility to select infectious diseases and other potentially important co-morbid conditions.
METHODS: A retrospective chart review was performed of all children (≤15 years old) seen since implementation of the electronic health

record system in January 2005. Demographic data (including country of origin, age and gender) was collected along with results for HIV, Hep B and C serologies, CBC, ferritin, varicella IgG (if ≥10 years old) and stool ova and parasites were reviewed.

RESULTS: A high burden of disease was found in this subpopulation of children, including various gastrointestinal parasites, chronic hepatitis B infection and iron deficiency anemia. Data collection is ongoing and final analysis of results will be available for presentation at the conference.

CONCLUSION: No standardized guidelines exist for recommendations of health screening in immigrants and refugees. Based on the high prevalence of intestinal parasites in our population, it is prudent to do screening tests, including stool exams in children new to Canada. To maximize health and minimize disease in this vulnerable population, screening should be performed shortly after arrival to identify infectious diseases that may potentially compromise the child's well-being or have public health significance. Further work needs to be done to evaluate the interaction of nutritional deficiencies and infectious diseases in this population.

SP.18
METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTION IN A GENERAL SYSTEMS INTENSIVE CARE UNIT: EPIDEMIOLOGY, ANTIMICROBIAL SUSCEPTIBILITY, AND OUTCOMES

W SLIGL*[1], G TAYLOR[1], N GIBNEY[2], R RENNIE[3], L CHUI[4]
[1]Division of Infectious Diseases, University of Alberta; [2]Division of Critical Care, University of Alberta; [3]Medical Microbiology, University of Alberta Hospital; [4]Provincial Laboratory of Public Health, Edmonton, Alberta

OBJECTIVE: Methicillin-resistant *Staphylococcus aureus* (MRSA) infection in intensive care units has increased dramatically in recent years, and is associated with increased morbidity, mortality, and cost of care. Our aim is to describe the epidemiology, susceptibility patterns, and outcomes of MRSA infection in a critically-ill population.

METHODS: We conducted a retrospective cohort analysis of patients infected or colonized with MRSA in our adult General Systems Intensive Care Unit (GSICU) from January 1, 1997, to August 15, 2005. Patients were identified through our Infection Control Database. Clinical records were reviewed, and all isolates were examined by pulsed-field gel electrophoresis (PFGE).

RESULTS: Forty-six cases of MRSA were identified and further subclassified as infection (78.3%) or colonization (21.7%). Infection rates were 3.1 per 1000 admissions and 5.2 per 10,000 patient days. Patient characteristics are described. For infected cases, the most common admitting diagnoses included respiratory failure (41.7%) and sepsis/septic shock (36.1%). The source of infection was identified as hospital-acquired in 58.3% cases (10 unit-acquired) with a median time to infection of 11 days. The most common sites of infection were respiratory tract, skin, and blood. No cases of necrotizing pneumonia were identified. Median lengths of stay were 13 days in the unit and 27 days in hospital. Overall mortality was 55.6%. Time to appropriate antimicrobial treatment was delayed (>24 hours) in 80.5% of patients. Antibiograms demonstrated the following susceptibilities: clindamycin 7%, TMP/SMX 87%, and vancomycin 100%. PFGE results will be presented.

CONCLUSION: MRSA remains an uncommon infection in our GSICU, resulting in delays in instituting appropriate antimicrobial therapy. Rapid screening techniques could shorten this delay. To date, we have not seen any cases of necrotizing pneumonia. Horizontal transmission between patients in the unit is not the main mode of acquisition.

SP.19
GROUP B STREPTOCOCCAL INFECTIONS IN CHILDREN BEYOND 3 MONTHS OF AGE: A HOSPITAL-BASED 5-YEAR RETROSPECTIVE ANALYSIS

M BARTON*, S RICHARDSON, A MATLOW, N SADDIQI, N ZAFAR, L FORD-JONES
Hospital for Sick Children, Toronto, Ontario

OBJECTIVE: To describe the demographic and clinical spectrum of disease caused by Group B beta-hemolytic *Streptococcus* (GBS) in patients beyond 3 months of age at a tertiary care institution.

METHODS: A 5-year hospital-based retrospective chart review of paediatric cases of GBS infection admitted from January 2000 to December 2004 was conducted. Microbiological records were used to identify cases. Isolates obtained from normally sterile body fluids of children aged 3 months to 19 years were selected from laboratory records and then cross-checked with admission records. Significant bacteriuria obtained from >1 mid-stream or catheter specimens of urine were included. Patients with clinical symptoms of UTI who received treatment for GBS were included. A detailed chart review was conducted and demographic, clinic and laboratory data were analyzed using SPSS version 11.

RESULTS: Of 150 GBS-positive isolates obtained from 90 patients, 50 isolates were obtained from 36 patients (40%) ≥3 months and <19 years of age. Median age was 9.5 years (4 months, 18 years) Spectrum of GBS disease included UTI in 25% (n=9), bacteremia/sepsis in 25% (n=9), bone/soft tissue infections in 28% (n=10), pneumonia in 14% (n=5) and other in 8% (n=3). Chronic underlying medical conditions were present in 75% (n=27) patients and included congenital and acquired immuno-suppression in 22% (n= 8), indwelling lines/catheters in 11% (n= 4), neurospinal disease in 8% (n=3), renal disease in 8% (n=3), liver disease in 6% (n=2) and other organ disease in 19% (n=7). All GBS isolates were sensitive to penicillin (MIC <0.03 mg/L). Two of 4 deaths were GBS-related, both due to sepsis, in a 6-month-old with liver disease and a 30-month-old with chronic renal disease.

CONCLUSION: GBS causes disease in children beyond 3 months of age, with three-quarters of the affected population having underlying immunosuppression, chronic organ disease or indwelling lines or catheters. Urinary tract, blood stream and musculoskeletal infections are the main sites of infection.

SP.20
MYOCARDITIS TEMPORALLY ASSOCIATED WITH VACCINES

M BARTON*, T HO, A OPAVSKY, JL RUSSELL, G TAYLOR, Y FINKELSTEIN, S ITO, L FORD-JONES, L BENSON, R GOLD
Hospital for Sick Children, Toronto, Ontario

OBJECTIVE: (1) To report 3 cases of myocarditis occurring in children following receipt of conjugate meningococcal C vaccine (menC vac) and hepatitis B vaccine (HBV) and (2) to review the literature to identify all published case reports describing any case of myocarditis or pericarditis occurring following immunization.

METHODS: A search was conducted using MeSH headings of "myocarditis, vaccines, hypersensitivity, pericarditis and immunizations". Data concerning vaccine type, age group, timing after vaccine, type of cardiac event and outcome were collected. In addition, for our 3 reports, we included biochemical, electrocardiographic, echocardiographic and histological details.

RESULTS: The 3 cases all occurred in adolescents aged 12, 14 and 15 years. The latter two occurred 10 and 3 days following receipt of menC vac, respectively; the 12-year-old girl developed symptoms 7 d after the second HBV. Penicillin exposure preceded presentation by 5 weeks in 1 case. All cases had supportive biochemical, electrocardiographic and echocardiographic features. Eosinophilic infiltrates were present in 2 biopsies. Viral causes were excluded. Resolution was spontaneous in 1 case and followed corticosteroid administration in the other 2.

A total of 34 case reports/series (26 English and 8 foreign languages) were published between 1969 and 2005. Smallpox-related case reports/series accounted for 50% (n=17), influenza 32% (n=11), HBV 6% (n=2), DPT

triple vaccine 6% (n=2), Yellow fever 3% (n=1), Cholera 3% (n=1), rabies 3% (n=1) and anticatarrh vaccine 3% (n=1). Time of onset following vaccine ranged from 1 d to 30 d. No case report/series of adverse cardiac events following administration of meningococcal vaccine was identified.
CONCLUSION: This represents the first report of cases of temporally associated myocarditis following meningococcal vaccine and the third report following HBV, and highlights the need for population-based surveillance of adverse events following vaccination.

SP.21
CHILDHOOD HERPES SIMPLEX ENCEPHALITIS: A 10-YEAR PROSPECTIVE REVIEW

J ELBERS, A BITNUN*, SE RICHARDSON, EL FORD-JONES, R TELLIER, R WALD, M PETRIC, H KOLSKI, H HEURTER, D MACGREGOR
Hospital for Sick Children, Toronto, Ontario

OBJECTIVE: Herpes simplex virus encephalitis (HSE) is regarded as the most common cause of sporadic fatal encephalitis in patients older than 6 months in the western world. The purpose of this study was to review the experience with HSE at the Hospital for Sick Children over the past decade.
METHODS: All patients admitted with acute encephalitis between Jan 1994 and Dec 2003 were prospectively enrolled in an encephalitis registry. Children from this registry were included in the study if they had CSF PCR and/or serologic evidence of HSV infection and at least one of CSF pleocytosis, EEG abnormalities or neuroimaging abnormalities suggestive of HSE.
RESULTS: Sixteen of 296 (5%) fulfilled the criteria for HSE. Their median age was 3.5 years (range 2 months to 14 years), and 9 were male. Twelve of 16 cases (75%) had HSV detected in the CSF by PCR; 2 had negative PCR on day 1, but positive PCR after day 5. The 4 cases with negative CSF PCR demonstrated a ≥4-fold rise in complement fixation titer between acute and convalescent sera. Evidence of coinfection with at least one other potential pathogen was observed in 10 cases (4 cases with *M pneumoniae*, 3 cases with HHV-6 3). Clinical presentation included fever (100%), focal seizures (75%), hemiparesis (31%), and aphasia (19%). All patients were treated with 14 to 21 days of acyclovir. One child had a relapse of HSE after completion of therapy. Long-term sequelae were observed in 50%, including a seizure disorder in 6 (38%) and/or developmental delay in 3 (19%). There were no deaths. A trend of more adverse neurological outcomes was observed in cases with a shorter duration of acyclovir therapy.
CONCLUSION: HSE continues to be associated with poor long-term neurologic outcome despite appropriate therapy. Our results indicate that CSF PCR may be negative early in the course of HSE; therefore, repeat CSF analysis should be considered if HSE is suspected. HSE is an infrequent but important cause of childhood encephalitis.

SP.22
PERFORMANCE OF TECHLAB *C DIFF CHEK* FOR THE DETECTION OF *CLOSTRIDIUM DIFFICILE* IN STOOL SAMPLES

RC REYES*, MA JOHN, DL AYOTTE, A COVACICH, S MILBURN, R LANNIGAN, Z HUSSAIN
London Health Sciences Centre, Victoria Hospita, Department of Microbiology, London, Ontario

OBJECTIVES: *C Diff Chek* (QC-GDH, TechLab, Blacksburg, Virginia), which detects glutamate dehydrogenase (GDH) in stool samples, was evaluated by comparing it to culture and Triage Micro *C difficile* Panel (TR-GDH Biosite Diagnostics, San Diego, California).
METHODS: Stool samples were inoculated onto CCFA Plates (Q-Labs, Quèbec) after alcohol shock and suspected colonies were identified by the MicroScreen *C difficile* latex slide agglutination test (Microgen Bioproducts Ltd, Surrey, UK). TR-GDH and QC-GDH tests were performed according to the manufacturers' instructions. All stool samples positive by culture or positive for GDH by both the kits were considered true positives.
RESULTS: A total of 87/579 stool samples were positive by all three methods. TR-GDH and QC-GDH failed to detect *C difficile* in six culture-positive samples and one culture-positive sample was negative by TR-GDH. Fourteen samples positive by TR-GDH and QC-GDH were negative by culture. QC-GDH gave 9 false-positive results. Twenty-six results of the TR-GDH could not be evaluated due to the discoloration of panels, and were considered negative. Sensitivities of culture, TR-GDH, and QC-GDH were 87, 86 and 94.4%, respectively, and specificities were 100%, 98.5%, and 98%, respectively.
CONCLUSIONS: GDH tests had a faster turn-around time than traditional culture methods. QC-GDH was most sensitive for the detection *C difficile*-positive stools and was easy to use.

SP.23
MULTI-LOCUS SEQUENCE TYPING FOR COMPARISON OF WATER, CHICKEN, RAW MILK AND HUMAN ISOLATES OF *CAMPYLOBACTER JEJUNI* (CJ) IN QUÉBEC

S LEVESQUE*, E FROST, S MICHAUD
Université de Sherbrooke, Sherbrooke, Quèbec

INTRODUCTION: Multi-locus sequence typing (MLST) has emerged as the state-of-the-art method for resolving bacterial population genetics. This technique is ideally suited for studying the epidemiology of highly diverse, weakly clonal bacterial species, such as CJ.
OBJECTIVE and METHODS: To explore the possible sources of transmission of CJ in humans, we compared 243 CJ isolates from humans (127), chickens (55), raw milk (27) and environmental water (34) cultured in Québec between 1998 and 2003, using MLST. Susceptibility profiles to ciprofloxacin, erythromycin and tetracycline were also determined by agar dilution.
RESULTS: Isolates were distributed in 84 sequence types (STs), forming 19 clonal complexes of 2 to 58 isolates each: 50 STs (20% of all CJ isolates) were composed of a single isolate each, and 41 STs were new compared to the existing international MLST database (www.mlst.net). The two largest clonal complexes (ST-21 and ST-45) contained 47% of all isolates and were composed of all type of isolates. On the other hand, 5 smaller complexes (ST-42, ST-48, ST-49, ST-1212 and ST-1219) were composed of human and chicken isolates only, 2 complexes (ST-637 and ST-1226) were strictly composed of water isolates, and one complex (ST-403) was composed of human isolates only. Overall, 38% of human isolates belonged to clonal complexes strongly associated with chicken. Antibiotic resistance was randomly distributed in densely populated clonal complexes. However, resistance to tetracycline was more frequent in complexes strongly associated with human and chicken isolates and antibiotic resistance was particularly infrequent in complexes associated with water isolates.
CONCLUSION: These results suggest that certain clones of CJ have specific ecological niches such as water or chicken, and that other distinct clones circulate in humans, bovines, chickens and environmental water. Additional isolates should be included in the database to gain more insight into the sources and mechanisms of transmission of CJ.

SP.24
MODELLING MICROBIAL SURVIVAL IN BUILD-UP BIOFILM FOR COMPLEX MEDICAL DEVICES

R HOWIE*, M ALFA
Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba

OBJECTIVE: Despite approved guidelines for reprocessing of flexible endoscopes, transmission of infectious agents (e.g. hepatitis C, *Mycobacterium tuberculosis*) can occur due to microbial survival in accumulated organic material that prevents adequate disinfection. The aim of this study was to compare the effect of chemical disinfectants on microbial survival within build-up biofilm versus traditional biofilm.
METHOD: The organisms used included *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Candida albicans* and *Mycobacterium chelonae*. The MBEC pin model facilitated biofilm formation (BFF) studies under the following conditions: traditional biofilm formation (TBF) in high (ATS)

and low nutrient media (sterile tap water) over 30 days; as well as 1 and 3 day cycles of wet/dry build-up biofilm (BBF). The biofilm was challenged with 2.6% Glutaraldehyde or 7% Hydrogen Peroxide (HLD), or 0.5% Hydrogen Peroxide. Survival was evaluated by a quantitative viability assay (cfu counts) and a modified metabolic assay (NBT-BCIP).

RESULTS: In high versus low nutrient media, the organisms achieved approximately 8 to 10 Log_{10} per peg compared to approximately 3 to 4 Log_{10}, respectively. Notably in TBF/low nutrient media, *E faecalis* survived to day 31 and *P aeruginosa* to day 21. Longer TBF or BBF resulted in better organism survival to disinfectant exposure. Survival of Glutaraldehyde HLD occurred by day 6 for *P aeruginosa* in TBS/ATS versus day 15 for *E faecalis*. In 1-day BBF/ATS, both organisms survived HLD by day 6. Overall organisms in BFF survived exposure to Glutaraldehyde earlier and to higher levels than with Hydrogen Peroxide, e.g. breakthrough survival for vegetative organisms in 1-day BBF/ATS was day 6 versus day 12, respectively.

CONCLUSION: Survival to drying and disinfection was unique to BFF conditions (BBF versus TBF) and the organism. Hydrogen peroxide was more effective at killing organisms, especially in a nutrient-depleted environment. BFF (especially BBF) in an organic medium resulted in disinfection protection, most dramatically for Glutaraldehyde, suggesting it may not be the optimal disinfectant for reprocessed endoscopes.

SP.25

COMPLIANCE WITH CONTACT PRECAUTIONS IN AN ACUTE CARE TEACHING HOSPITAL

MR ALKAABI*, AM JOFFE, S WOOLSEY, S WILSON, S LAFFERTY, M CORDOVIZ

[1]University of Alberta; [2]Royal Alexandra Hospital, Edmonton, Alberta

OBJECTIVES: To monitor compliance with contact precautions during an MRSA epidemic, we conducted an observational study among physicians, nurses, medical students and other healthcare workers (HCWs) providing routine care for adult patients.

METHODS: The study was conducted in a tertiary acute care teaching hospital. Observations were performed on 10 hospital inpatient units (5 medical and 5 surgical), housing patients who were colonized or infected with MRSA. Isolation rooms were clearly labelled with contact precaution signs. All rooms were equipped with sinks and soap inside the room and alcohol-based dispensers, gloves, gowns, and disinfectant sprays for cleaning equipment outside the room. We monitored compliance with hand washing, usage of gloves, gowns and masks, and cleaning of patient care equipment after usage and upon removal from the room (infection control precautions [ICPs]).

RESULTS: We observed 455 patient encounters and opportunities for compliance with ICPs among 94 physicians, 199 nurses, 50 medical students, and 114 other HCWs. Overall compliance rate for all HCWs was 67%. Compliance observed among males was significantly less than among females, at 73% vs 80% (P=0.05). The overall compliance rate for hand hygiene was 77%. There was no significant difference in hand hygiene compliance between physicians and nurses (P=0.9). However, nurses were statistically more likely to don gowns, gloves and masks than were physicians. Eighty five per cent of HCWs used alcohol-based hand-rub for hand hygiene rather than soap and water. The rate of cleaning equipment when removed from the patient room by any HCW was only 25%.

DISCUSSION AND CONCLUSION: Significant deficiencies in compliance with infection control precautions for patients isolated and on contact precautions for MRSA were observed and may contribute to ongoing transmission of antibiotic-resistant organisms. In particular, cleaning and disinfection of patient care equipment is clearly lacking.

SP.26

NOSOCOMIAL *SERRATIA* BACTEREMIA IN A LARGE TERTIARY-CARE CENTRE: EPIDEMIOLOGY, ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND OUTCOMES

J JOHNSTONE*[1], G TAYLOR[1], RP RENNIE[2]

[1]University of Alberta; [2]Alberta Provincial Laboratory for Public Health, Edmonton, Alberta

OBJECTIVES: *Serratia* species (spp.) are a known cause of nosocomial blood stream infection (BSI). We sought to describe the epidemiology, antimicrobial susceptibility patterns (AS) and outcomes of patients (pts) with *Serratia* spp. BSI in our institution.

METHODS: Prospectively identified episodes of BSI were reviewed to identify pts with *Serratia* spp. BSI from January 1, 1999, to July 31, 2005. Patient charts were reviewed to obtain risk factors for acquisition, outcome and AS. Pulsed-field gel electrophoresis (PFGE) was performed on consecutive strains after October 2002 to determine relatedness and to retrospectively identify the presence of isolated clusters.

RESULTS: There were 41 *Serratia* spp. BSI in 39 pts in our cohort, 2.86 per 100,000 pt days, 2% of all BSI. Mean pt age was 59 years, and 73% were male. 73% had a central venous catheter, 61% a Foley catheter, and 51% were mechanically ventilated. 54% of pts were in an ICU. 98% were *S marcescens*, and 2% were *S liquefaciens*. BSI sources were both primary (34%) and secondary (66%). AS were as follows: 80% susceptible to ciprofloxacin, 98% imipenem, 98% piperacillin, and 84% tobramycin. Ciprofloxacin and tobramycin susceptibility was inferior to imipenem and piperacillin (P=0.0075 and P=0.029, respectively). 32% of pts died prior to discharge. PFGE analysis of 14 isolates revealed that several suspected clusters were unrelated but identified 2 identical strains in separate pts from one unit separated by 146 days.

CONCLUSIONS: *Serratia* spp. is an uncommon nosocomial BSI in our institution, typically occurring in ICU pts with multiple invasive devices, associated with high in-hospital mortality. Clusters of *Serratia* BSI were rare and self-limited; real-time PFGE analysis would help confirm or refute common source clustering. Imipenem and piperacillin are more effective agents and should be considered as empiric therapy in critically ill patients with gram-negative BSI.

SP.27

COMMUNITY-ACQUIRED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: THE EPIDEMIOLOGY OF AN EMERGING PATHOGEN

TM O'SHEA*, C MAIN, C RUTHERFORD

Hamilton Regional Laboratory Medicine Program, McMaster University, Hamilton, Ontario

OBJECTIVE: Outbreaks of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have been increasingly reported over the past ten years, mostly in the United States. A 2004 report in our community detailed several cases of CA-MRSA in a correctional facility. More recently, infections with CA-MRSA have been seen with increasing frequency in individuals with no apparent link to previous outbreaks. The objective of our research was to characterize the recent outbreak of infections with relation to microbiologic and epidemiologic characteristics.

METHOD: Potential cases of CA-MRSA were identified through active surveillance in regional microbiology laboratories. Suspected cases were forwarded to a central location where they were subjected to pulsed-field gel electrophoresis (PFGE) identification. Patients with confirmed cases of CA-MRSA were then contacted and an epidemiologic survey was conducted.

RESULTS: A total of 29 isolates were identified and examined by PFGE. Of these, 25 were identified as CA-MRSA10, the same strain that was found in the earlier correctional facility outbreak. One isolate was identified as CA-MRSA 7. A further four isolates showed identical patterns on PFGE but could not be matched to any known strain. All isolates were positive for the Panton-Valentine Leukocidin virulence factor. The average age of patients included in this analysis was 32, and there was a marked geographic concentration of cases. The majority of infections were skin

and soft tissue related. There was one reported bacteremia with associated endocarditis and one epidural abscess reported.

CONCLUSION: CA-MRSA is an emerging pathogen that should be considered in the differential diagnosis of skin and soft tissue infections in areas with increasing prevalence of disease. A better understanding of the epidemiology of CA-MRSA is needed to allow prompt initiation of appropriate therapy.

SP.28

CHARACTERISTICS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG PATIENTS PRESENTING WITH SKIN AND SOFT TISSUE INFECTIONS TO THE EMERGENCY DEPARTMENT

GN AL-RAWAHI*[1], S REYNOLDS[2], S PORTER[1], WR BOWIE[2], L KISHI[3], T CHONG[3], PW DOYLE[1]

[1]Department of Pathology and Laboratory Medicine; [2]Department of Medicine, Division of Infectious Diseases, University of British Columbia; [3]Department of Pathology and Laboratory Medicine, Vancouver General Hospital, Vancouver, British Columbia

OBJECTIVES: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is an emerging pathogen described mainly among healthy individuals with little or no previous contact with healthcare facilities. The purpose of this study was to determine the proportion of CA-MRSA, defined by pulsed-field gel electrophoresis (PFGE), in MRSA skin and soft tissue infections presenting to the Emergency Department. We also aimed to determine the laboratory characteristics of CA-MRSA and define the clinical risk factors associated with the acquisition.

METHODS: Retrospectively, we reviewed all frozen MRSA isolates from skin and soft tissue infections from the Emergency Department from June 1, 2001, to May 30, 2005. The isolates were characterized by in vitro examination of antibiotic susceptibilities, *mecA* confirmation and PFGE, as well as staphylococcal cassette chromosome (SCC) *mec* type IVa and Panton-Valentine leukocidin (PVL) gene testings on representative isolates from each PFGE pattern. In addition, the medical records were reviewed to define the risk factors for the acquisition.

RESULTS: Of 98 isolates, 94 were available for analysis, out of which 57 (61%) were CMRSA-10 (USA-300), mostly from 2004/2005. All representative isolates in this group had PVL and SCC*mec* IV genes. Their antibiogram showed 100% susceptibility to cotrimoxazole, rifampin and fusidic acid, 98% to tetracycline, 81% to clindamycin, and 4% to ciprofloxacin. Clinical comparison of CMRSA-10 and hospital strains showed 12% versus 73% for recent hospitalisation (P<0.0001), 19% versus 65% for recent antibiotic use (P<0.0001), 35% versus 5% for intravenous drug use (IVDU) (P=0.002), 54% versus 5% for soft tissue abscess (P<0.0001), and 0 versus 38% for post-operative wound infections (P<0.0001).

CONCLUSION: CMRSA-10 is a major pathogen in skin and soft tissue abscesses in our Emergency Department. It has a characteristic susceptibility pattern. In this particular population, it was associated with IVDU, but not with recent antibiotic usage.

SP.29

FUNGEMIA DUE TO *CANDIDA* AND MUCOR IN AN IMMUNOCOMPETENT PATIENT

MD CHRISTIAN*[1], C LEE[2]

[1]Departments of Infectious Diseases and Critical Care; [2]Pathology and Molecular Medicine and Infectious Diseases, McMaster University, Hamilton, Ontario

OBJECTIVES: To describe a rare case of disseminated mucor infection detected in a blood culture and to consider the contribution of broad-spectrum antimicrobials in selecting for resistant organisms.

METHODS: A retrospective chart review was conducted to extract data on this case. To identify previous similar cases, the English literature from 1966 to 2005 was searched via Medline using the following search strings: "mucor and blood culture", "fungemia or septicemia or blood steam infection and mucor", and "mucor and disseminated".

RESULTS: The patient in this case was a 59-year-old woman admitted to hospital with cholecystitis and acute renal failure. Her past history included substance abuse, smoking, depression, closed head injury, peptic ulcer disease, and a brain aneurysm. Failing conservative management, laproscopic surgery was preformed but complicated by rupture of the gallbladder and contamination of the peritoneal cavity. Subsequently, a prolonged admission to the critical care unit ensued, complicated by recurrent infections requiring broad-spectrum antimicrobials (Figure 1). While on pipercillin-tazobactam, ceftazidime, vancomycin and caspofungin, the patient developed a recurrent febrile illness, and blood cultures obtained at the time were positive for *Candida* and mucor. A short time later, life support was withdrawn at the request of the patient's family. The literature review failed to identify any articles primarily describing or dealing with mucor fungemia or detection in blood cultures.

DISCUSSION: Invasive zygomyces infections, such as mucor, have been on the increase recently in the era of broad-spectrum antimicrobials. However, mucor has rarely been isolated in blood cultures. The authenticity of the mucor blood stream infection in this case was demonstrated by the presence of both yeast and mucor in the original gram stain from the blood culture bottle. This case is exemplifies the increasing trend in resistant organisms selected for by the use of broad-spectrum antimicrobials. Three unusual and resistant organisms were cultured from this patient. As the widespread use of broad-spectrum antimicrobials continues, we are likely to see more infections with resistant organisms.

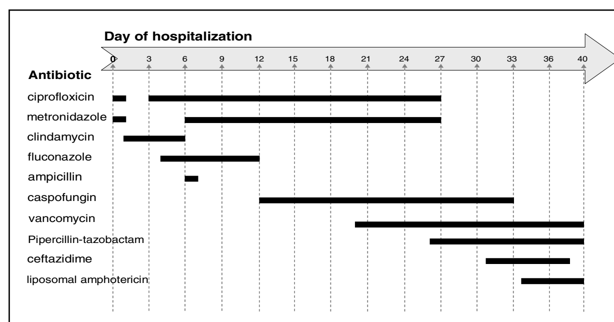

Figure 1) *Summary of antimicrobial treatment*

SP.30

PAEDIATRIC TYPHOID FEVER IN A NON-ENDEMIC CITY: THE IMPORTANCE OF CHILDREN VISITING FRIENDS AND RELATIVES

LJ SAUVE*[1], S KRAJEWSKI[4], SE RICHARDSON[1], F JAMIESON[2], U ALLEN[1], JS KEYSTONE[3], EL FORD-JONES[1]

[1]Hospital for Sick Children; [2]Central Public Health Laboratory, Ontario Ministry of Health and Long-term Care; [3]Centre for Travel and Tropical Medicine, Toronto General Hospital, University of Toronto; [4]University of Toronto, Toronto, Ontario

OBJECTIVES: To examine the demographics, diagnosis, clinical features and antibiotic susceptibility profile of *Salmonella typhi* infection in children hospitalized in a tertiary children's hospital in an ethnically diverse city.

METHODS: A retrospective chart review was performed of all cases of typhoid fever hospitalized in a children's hospital serving the Toronto region from 1985 to 2004. Patients were identified by discharge diagnosis and review of microbiology records.

RESULTS: Forty-seven children had *S typhi* isolated, 40 (85.1%) from the blood, 7 (14.9%) from the stool alone, and 17 (36.2%) from both blood and stool. Eight children had typhoid fever diagnosed clinically without positive cultures. The age range was 8 months to 17 years. Five infections (10.6%) were acquired within Canada, while 69.1% were acquired in the Indian subcontinent, 10.9% in Latin America and 7.3% in Southeast Asia; nearly all of these cases were in children who had visited friends and relatives overseas (VFRs). All of the children reported fever. Gastrointestinal symptoms were the predominant clinical presentation. From 1985 to 1989, there were no antibiotic-resistant isolates, but during the last 5 years of the study, only 54% were fully susceptible to antibiotics, with four (31%) strains resistant to ampicillin and two (15%) resistant to

ciprofloxacin. Most children were treated for two weeks, starting on intravenous therapy and stepping down to oral therapy, with one relapse. Eighteen (39%) children were started on antibiotics only after *S typhi* was isolated.

CONCLUSIONS: In a diverse city with many immigrants, unimmunized child VFRs account for most of the typhoid fever seen in our centre. Diagnosis may be enhanced by performing stool cultures and multiple blood cultures on all patients with prolonged fever and a significant travel history. VFRs should be targeted for strategies to prevent typhoid fever.

SP.31

CHARACTERIZATION OF A *vanD* GENE CLUSTER IN A *CLOSTRIDIUM* SPECIES ISOLATED FROM THE NORMAL FLORA OF HUMAN BOWEL

M-C DOMINGO*, A HULETSKY, R GIROUX, FJ PICARD, MG BERGERON

Centre de Recherche en Infectiologie de l'Université Laval, Sainte-Foy, Quèbec

OBJECTIVE: We have previously shown that anaerobic bacteria of the human bowel may serve as a reservoir of *vanB, vanD, and vanG* genes. During a hospital surveillance program to detect VRE carriers, 71 of 162 fecal specimens were *vanD*-positive with the PCR assay while showing no culturable vancomycin-resistant enterococci. A *vanD*-positive bacteria was isolated from one of the 71 specimens (specimen ERV-110) and characterized. The *vanD* cluster from this strain was also characterized.

METHODS: Broth and agar subcultures in anaerobic chamber of specimen ERV-110 using Brain Heart Infusion supplemented with vitamin K, haemin, L-cystine, lactate, pyruvate, vancomycin and aztreonam was used to isolate the *van*D-positive anaerobic bacteria. Identification of the strain was performed by sequencing and analyzing its 16S rRNA genes. PCR primers targeting *vanRD, vanSD, vanYD, vanHD, vanD*, and *vanXD* were used to amplify and/or sequence the complete *vanD* cluster of this anaerobic bacterium. PCR primers specific to *intD* encoding a putative integrase-like protein associated with the *vanD* cluster in *E faecium* were also tested.

RESULTS: An anaerobic Gram-positive bacillus was isolated and a comparison of its 16S rRNA gene sequence with databases revealed a potentially novel species that was most similar (94% identity) to *Clostridium indolis*. The complete DNA sequence of the *vanD* cluster was most similar (98.2% identity) to that of *E faecium* BM4339 containing the *vanD1* allele. An *intD* gene with 99% identity with that of this *E faecium* strain was found to be associated with the *vanD* gene cluster of this *Clostridium* species.

CONCLUSIONS: These results describe for the first time a *Clostridium* species harbouring the *vanD* gene cluster. The presence of *vanD*-containing anaerobic bacteria in the human bowel flora suggests that these bacteria may serve as a reservoir for vancomycin-resistant *vanD* genes.

SP.32

EVALUATION OF THE PREVALENCE OF FOOD- AND WATERBORNE PARASITES IN ENVIRONMENTAL AND CLINICAL SPECIMENS IN HANOI, VIETNAM

LMN HOANG*[1], T NGUYEN[2], P CAM[2], M FYFE[3], J ISAAC-RENTON[3], C ONG[3]

[1]University of British Columbia Department of Pathology and Laboratory Medicine, Vancouver, British Columbia; [2]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam; [3]BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: *Cyclospora cayetanensis*, an emerging coccidian parasite, has caused a number of food-borne outbreaks in BC and, recently, one in Ontario. There currently is limited knowledge on their biology and available diagnostic tools are primitive. The purpose of our study is to (1) apply epidemiological surveillance methods to determine specific exposure and risk factors associated with *Giardia, Cryptosporidium* and *Cyclospora* in Hanoi, Vietnam; (2) determine the prevalence of *Cryptosporidium* and *Cyclospora* in both environmental and clinical specimens in Hanoi, Vietnam; (3) identify novel genomic targets for molecular epidemiology studies.

METHODS: Patients with diarrhea admitted to 3 general hospitals (St. Paul, Viet-French and Bach Mai) and one HIV rehabilitation centre (Bavi) were interviewed using a standard questionnaire addressing potential risk factors for cyclosporiasis and cryptosporidiosis. These hospitals, spanning across Hanoi, cover the pediatric, foreigners, adult HIV and HIV-positive populations. Their stools were collected and screened for *Cryptosporidium, Giardia* and *Cyclospora* by conventional methods. Irrigation water and fresh herbs from the farms and markets were collected weekly and examined by both conventional and molecular methods for *Cyclospora* oocysts.

RESULTS: Of the environmental specimens collected, 24% of specimens collected from the markets were positive for *Cyclospora* oocysts and 10% of specimens from farms were positive. All clinical specimens collected to date were negative for *Cyclospora* by microscopy. *Giardia* (12%) and *Cryptosporidium* (22%) were detected by immunofluorescence from pediatric patients. Analysis of questionnaire for risk factors are pending. Results from molecular testing are pending.

CONCLUSION: Preliminary data show the presence of *Cyclospora* oocysts in environmental herbs and irrigation water from both markets and farms in Hanoi. *Cryptosporidium* oocysts and Giardiain trophozoites were detectable from pediatric patients admitted to hospital with diarrhea. *Cyclospora* is not an identifiable cause of diarrhea amongst hospitalized patients in Hanoi.

SP.33

ATYPICAL MYCOBACTERIAL OSTEOMYELITIS IN A 17-YEAR-OLD PATIENT WITH AN INTERFERON-GAMMA RECEPTOR DEFICIENCY

M MACFARLANE CONRAD*[1], B BORTOLUSSI[2], A ISSEKUTZ[2]

[1]Dalhousie University; [2]IWK Health Centre, Halifax, Nova Scotia

BACKGROUND: *Mycobacterium avium intracellulare* (MAI) rarely causes life-threatening infection in immunocompetent patients. Investigation of an underlying defect in macrophage (Mo) function should be considered when a diagnosis of MAI is confirmed. Interferon gamma (IFN-gamma) is required for efficient activation of Mo; thus, a deficiency in IFN-gamma receptor 1 (IFN-gamma R1) expression on the surface of Mo can lead to incomplete killing of this organism.

OBJECTIVES: To describe an adolescent girl with weight loss, chronic bone pain, fever, and disseminated bony and soft tissue lesions, as well as the outcome of investigations.

PRESENTATION: A 17-year-old girl presented with a four-month history of fever, malaise, night sweats, amenorrhoea, anorexia, nausea, and weight loss. She complained of pain in the left arm and leg, left flank, and lower back. A bone scan revealed multiple hot spots in the ribs, pelvis, skull, and long bones.

INVESTIGATIONS AND TREATMENT: Imaging studies revealed multiple lesions in several ribs and vertebrae, the pelvis, the left humerus, and in the spleen. Bone marrow biopsies were non-specific. Cultures were negative. A PPD test was administered and reported negative. Destruction and collapse of T3/T4 vertebrae occurred, leading to extreme back pain and a 45-degree kyphosis. Underlying malignancy was investigated. However, a second PPD was strongly positive and aspirates of a rib lesion showed acid-fast bacilli. MAI was subsequently identified using 16S RNA gene sequencing. She was diagnosed with disseminated MAI with multifocal osteomyelitis. Because of MAI as a cause, immune deficiency was suspected, and an investigation for HIV and cytokine defects in IFN-gamma, TNF and IL-12 were undertaken. Genetic testing revealed an IFN-gamma R1 partial depletion. With antimicrobial treatment and IFN-gamma (50 $\mu g/m^2$ s/c), the patient recovered slowly, and her kyphosis was partially reduced. She has resumed her daily activities.

CONCLUSION: Although clinical features may mimic malignancy, multifocal osteomyelitis with unrecognised immunodeficiency due to an IFN-gamma R1 partial depletion should be considered.

SP.34
IMPLEMENTATION OF A CITY-WIDE URINARY TRANSPORT SYSTEM FOR URINE SAMPLES: A LABORATORY-BASED COST-BENEFIT ANALYSIS

RC REYES*, R LANNIGAN, MA JOHN, L STOAKES, Z HUSSAIN
London Health Sciences Centre, Victoria Hospita, Department of Microbiology, London, Ontario

OBJECTIVES: Urine cultures comprise the largest single category of specimens tested by most medical microbiology laboratories. Delays in transport and processing may result in false-positive urine culture results. The objectives of this study were to analyze the impact of the implementation of a new transport system on culture results and to perform a laboratory-based cost-benefit analysis.
METHODS: The new urine transport system consists of a sterile evacuated glass tube containing a preservative. All urine specimens were transported to the Department of Microbiology for processing. Colony counts were determined and interpreted. Urine culture data were gathered from the Cerner Millennium Hospital information system. Data were gathered for 2281 urine samples from 3 sites (one inpatient and two outpatient services) prior to the implementation of the urine transport system and 2547 urine samples after implementation. Each time period included samples gathered in summer and winter months.
RESULTS: There was a significant (P=0.04) decrease in the proportion of positive samples using the preservative-containing urine transport method (16%) versus no preservative (19%). There was no significant difference on culture results between the winter and summer months before (P=0.77) or after (P=0.44) implementation of the new transport system.
CONCLUSIONS: A new urine transport system was implemented for the London, Ontario hospitals. Although the numbers of positive cultures decreased significantly, there was no overall cost savings from the laboratory perspective.

SP.35
INFLUENZA A VIRUS ANALYSIS OF GENOTYPE DISTRIBUTION, 2003-2005

R RAHMANIAN*[2], A MAK[1], V LEI[2], D LAWRENCE[1], M KRAJDEN[1], RC BRUNHAM[1], DM SKOWRONSKI[1], Y LI[3], T BOOTH[3], S-H GOH[1], M PETRIC[1]
[1]British Columbia Centre for Disease Control; [2]University of British Columbia, Vancouver, British Columbia; [3]Canadian Centre for Human and Animal Health, Winnipeg, Manitoba

OBJECTIVE: Influenza A viruses cause yearly epidemics, in part due to their ability to overcome immunity from previous infections through acquisition of mutations leading to antigenic drift, which is characterized phenotypically by HAI or neutralization tests, both of which depend on availability of reference antibodies. We examined influenza isolates for evidence of genetic drift through genotype determination.
METHODS: Amino acid sequences encoded by genes 4 (HA), 6 (NA), 7 (M) and 8 (NS) from 80 influenza A isolates collected between November 2003 and March 2005 were analyzed to determine the extent to which the viruses mutated within epidemic periods and between epidemics.
RESULTS: Nucleotide and amino acid sequences were stable throughout the epidemic periods but experienced substantial changes in the inter-epidemic period. Major changes occurred in the HA gene in 5 to 7 amino acids and in the NA gene in 11 to 13 amino acids; changes of 5 amino acids occurred in the M and NS genes. In the HA gene, changes occurred in sites known to be epitopes that determine the hemagglutination inhibition reactivity and these were shown to be associated with a change of strain from A/Fujian-like to A/California-like viruses.
CONCLUSION: Our findings indicate that genotype determination promises to be a rapid approach for detecting new strains of influenza A viruses in a population.

SP.36
THE CHANGING EPIDEMIOLOGY OF HEPATITIS A IN BRITISH COLUMBIA: UTILIZING HEALTH AUTHORITY FOLLOW-UP DATA TO INFORM POLICY AND PRACTICE

SL POLLOCK*[1], A SHEIKHOLESLAMI[2], B EDGAR[2], ST DAVID[2], M KRAJDEN[2], JA BUXTON[1]
[1]Department of Health Care and Epidemiology, University of British Columbia; [2]British Columbia Centre for Disease Control, Vancouver, British Columbia

OBJECTIVES: Individuals at high risk for hepatitis A virus (HAV) infection include men who have sex with men (MSM), household contacts of HAV-infected individuals, and travellers to endemic countries. In BC, HAV cases are followed-up by their local health authorities (HA) but not shared provincially. The objectives were to review the processes of HAV case follow-up, to characterize the epidemiology associated with HAV infection, and to make recommendations to enhance HAV follow-up and prevention strategies.
METHODS: This was a retrospective review using non-nominal records of HAV follow-up from health authorities (1997/98 to 2004). Descriptive analyses were conducted to ascertain burden of disease and epidemiologic trends.
RESULTS: Four out of five HA participated; follow-up data varied by collection method and completeness. There were 985 cases of HAV: 124 (12.6%) self-identified as MSM (86% from one HA). Cases of HAV peaked in the MSM and IDU groups in 1998 (77 and 34, respectively). In 2004, there were no cases identified in either risk group. Travel to another country during the incubation period was identified in 245 cases (25%) and has remained constant from 19 in 1997 to 22 in 2004. At least one-third of cases with known ethnicity were from endemic countries.
CONCLUSION: Trends show a decrease in HAV rates following targeted vaccine policies at MSM and IDU groups. Travellers now represent a significant proportion of the known disease burden. Efforts should be directed at encouraging HAV vaccine for travellers, including those originally from endemic countries. Data collection methods and follow-up procedures should be standardized to inform HAV vaccination strategies at the provincial and national levels.

POSTER PRESENTATIONS
Friday, March 17, 2006

P2.01
USE OF THE TECAN MINIPREP 75 FOR PREAMPLIFICATION PROCESSING FOR THE ROCHE COBAS AMPLICOR *CHLAMYDIA TRACHOMATIS* (CT) PCR TEST

T RAHIM, C SHAW*, J ISAAC-RENTON
BCCDC Laboratory Services, Vancouver, British Columbia

OBJECTIVE: Repetitive pipetting steps required for manual processing of specimens for the Roche Cobas Amplicor CT PCR test may result in repetitive strain injury when large volumes of specimens are processed. The objective of this study was to compare results obtained when specimens were processed manually with those obtained when specimens were processed using the Tecan MiniPrep 75.
METHODS: Urine specimens from 800 patients were vortexed, an aliquot withdrawn and the urine container placed on the MiniPrep for processing. Double-headed swabs were used to take cervical swabs from 1615 patients. The double-headed swabs were split into 2 swabs and each placed in a separate dry specimen tube for transport to the laboratory. Lysis buffer (0.5 mL) was added to one of the swabs and the swab processed manually (Method 1). Saline (1.0 mL) was added to the second swab, the swab was vortexed and placed on the MiniPrep for processing (Method 2). Seeding and dilution studies using the Roche CT PCR positive control were also conducted to determine the lowest number of copies of positive control that could be detected by the 2 methods used for processing swabs.

RESULTS: Of 61 positive urine specimens, 60 were positive using the MiniPrep and 61 were positive by manual processing. Swabs from 69 patients were positive; 63 by the MiniPrep and 66 by the manual method. The lowest number of copies of positive control detected in 3 replicate tests was 107 and 430 per specimen for Methods 1 and 2, respectively. This represented a copy number of 5.37 in the final reaction mixture for both methods.
CONCLUSIONS: Use of the MiniPrep is an acceptable method for processing specimens for the Roche Cobas Amplicor CT PCR test.

P2.02
CAN REDUCED CIPROFLOXACIN (CP) SUSCEPTIBILITY BE USED TO PREDICT THE PRESENCE OF POINT MUTATIONS IN THE QUINOLONE RESISTANT DETERMINING REGION (QRDR) OF *STREPTOCOCCUS PNEUMONIAE* (SP)

M DESJARDINS*[1], SB HARVEY[2], C SEETERAM[2], C GUIBORD[2], B TOYE[2]
[1]The Ottawa Hospital, Ottawa; [2]The Ottawa Hospital Research Institute, Ottawa, Ontario

BACKGROUND: Prior exposure to fluoroquinolones (FQ) has been shown to increase the risk of infection with FQ resistant Sp, likely related to selection of strains with first step mutations in the QRDR of the *par*C gene. Laboratory detection of isolates with first step mutations using phenotypic methods is currently difficult. We evaluated the use of Cp disk diffusion (DD) testing and MIC determination by E-test as a marker for first step mutations in the *par*C gene.
METHODS: Sp isolates recovered from blood and respiratory specimens were tested for Cp susceptibility by DD as per CLSI M2-A8. Cp MICs for isolates with zones of inhibition ≤21 mm were determined by ET. PCR of the *par*C and *gyr*A QRDR and sequencing of the amplified product was performed using published methods. *Par*C gene sequences were compared with FQ susceptible strains and the presence of mutations was correlated with the Cp susceptibility.
RESULTS: From June 2004 to July 2005, 196 Sp isolates were screened for reduced Cp susceptibility by DD. Of these, 20 were identified with a zone of inhibition ≤21 mm (17±5 mm) by DD with Cp MIC_{50} of 2 mg/L. For 5 of the 20 isolates, the Cp MIC was ≥4 mg/L. Sequencing of the *par*C gene of these 5 isolates identified a first step mutation in 2 isolates at amino acid position 83 and 137, and a 2 step mutation in the *par*C (position 79) and *gyr*A (position 37) genes in a third isolate. For the remaining 2 isolates, no mutations were identified in one and for the remaining isolate a mutation in position 107 was identified in *par*C but was not associated with a change in amino acid sequence.
CONCLUSION: Screening Sp by DD followed by MIC determination for isolates with reduced Cp susceptibility may be a useful approach for identifying isolates at increased risk of FQ resistance. Testing of larger populations of Sp isolates will be required.

P2.03
COMPARATIVE STUDY OF URINE CULTURE RESULTS AS PREDICTED BY URINE DIPSTICK

A BELHAJ*, L WILCOX, S AL-JOHANI, CH LEE
McMaster University; Hamilton, Ontario

INTRODUCTION: Urine microscopy and culture are some of the most common tests ordered. In both inpatient and outpatient settings. 29,000 urine samples are submitted for culture each year in our region, representing a significant portion of the laboratory workload. Current laboratory practice is to screen urine samples submitted for culture by using reagent dipstick as a tool to reduce the number of urine cultures processed in the microbiology laboratory. Reports of sensitivities and specificities of urine dipstick testing as compared to urine cultures as the gold standard have shown variable results, with the sensitivity resulting between 50% and 70%. In our setting, if the urine sample requested for culture screens negative for protein, leukocytes and nitrite by dipstick, then no further testing is performed, unless the specimen is from a pregnant woman, collected via cystoscopy, renal patients or a child younger than 3 years old.
OBJECTIVES: To evaluate the sensitivity of the current standard of urine testing (urine dipstick for protein, nitrites or leukocytes) as predictive of positive urine culture results.
STUDY DESIGN, METHODS AND ANALYSIS: Design: Prospective, randomized comparative study. Setting: St Joseph's Healthcare laboratories. Sample size: We will start with 420 urine samples that give negative dipstick at the initial screening.
METHODS: After excluding specimens from pregnant women, children younger than 3 years old, nephrology patients and cystoscopy-collected, a total of 920 urine specimens were evaluated over a two-month period. 500 urine specimens were processed as per laboratory protocol based on the dipstick results. 420 specimens were cultured and examined after 24 h of incubation regardless of the dipstick results.
RESULTS:

No growth	No significant growth	Mixed growth	Growth	Total
184	82	121	58	429

CONCLUSION: The sensitivity of dipstick based on our study was 79.3%. Chart review will be done to determine clinical significance of the negative dipstick results but positive culture results.

P2.04
EVALUATION OF LEUKOCYTE ESTERASE AND NITRITE DIPSTICK TESTS FOR SCREENING OF URINE SPECIMENS FOR CULTURE

T ASH*, E NAGLE, GB HORSMAN, PN LEVETT
Provincial Laboratory, Saskatchewan Health, Regina, Saskatchewan

OBJECTIVES: To evaluate the use of dipstick tests for detection of leukocyte esterase (LE) and nitrites (Nit) in urine specimens as a predictor of significant bacteriuria.
METHODS: Urine specimens from clinics throughout Saskatchewan were received in the Provincial Laboratory after transport at ambient temperature. Urine specimen containers contained boric acid as a bacteriostatic agent. The presence of LE and nit were detected using a commercial dipstick assay.
RESULTS: 968 urine specimens were tested, of which 268 (28%) gave positive dipstick results for either LE or Nit. Significant bacteriuria was detected in 405 (42%) of urines. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of detection of either LE or Nit or both were 46%, 87%, 70% and 71%, respectively. For LE alone, the sensitivity, specificity, PPV and NPV were 28%, 88%, 63% and 63%, respectively. For Nit alone, the sensitivity, specificity, PPV and NPV were 28%, 96%, 85% and 65%, respectively.
DISCUSSION: Many of the specimens studied had been in transit for several days, leading to potential sources of error as leukocytes degraded and lysed and bacterial metabolism may have affected nitrite levels. The specificity of Nit detection was high, leading to a high PPV, but sensitivity was unacceptably low. The sensitivity of detection of LE and Nit alone or in combination was too low for these to be useful screening tests in this population.

P2.05
A PILOT EVALUATION OF OXOID RAPID METHYL-A-D-GALACTOPYRANOSIDASE (R-MGP) TO DIFFERENTIATE RAPID-XYLOSE POSITIVE (R-XYL-POS) VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM* (VR-EFE) FROM *E GALLINARUM* (EGAL)

N KREISWIRTH*[1], D YAMAMURA[2], P RALTA[3], O IMAS[1], M LUM[1], C LAROQUE[1], S POUTENAN[1], F JAMIESON[3], A MCGEER[1], D LOW[1], B WILLEY[1]
[1]Toronto Medical Labs and Mount Sinai Hospital, Toronto; [2]MDS Laboratories, Toronto; [3]Central Public Health Laboratories, Etobicoke, Ontario

OBJECTIVES: EFE and *E faecalis* (EFC) are typically R-XYL-neg while Egal and *E casseliflavus* (Ecas) are typically R-XYL-pos. Among >100 known cases in a multi-centre 2005 Southern Ontario monoclonal *van*A

VR-EFE outbreak (PF type "EG"), two R-XYL-pos variants of the same PF clone were identified. As R-XYL-pos is used by many labs as a front-line test to rule out VRE, the identification of R-XY-pos variants is of great concern. This study evaluated Oxoid's newly formulated semisolid R-MGP as an alternate rule-out test to R-XYL (Prolab) and Oxoid's standard overnight MGP (o/n-MGP).
METHODS: 65 enterococci of various species and resistance profiles were tested including the 2-XYL-pos VR-EFE, 22 additional VR-EFE of which 20 were PF type "EG" from different patients, 35 consecutive Egal, 2 consecutive Ecas, 3 VS-EFC and 1 ea VS-EFE and VR-EFC. Each R-MGP, R-XYL and o/n-MGP test was inoculated with a bulging 1 µL loop of organism, incubated at 35°C, and read blinded by 4 technologists. The R-MGP was read from 1 h every 30 min to 5 h and o/n; the R-XYL was read after 1.5 h, 2 h and 2.5 h (30 min longer than recommended); and the o/n-MGP was read from 1.5 h every 30 min to 5 h and o/n. Yellow was pos in all tests.
RESULTS: All 25 EFE and the 4 EFC were neg by R-MGP even after o/n incubation. The 2 Ecas and 33 of 35 Egal were R-MGP pos at 1 h, and the remaining 2 were pos at 2.5 h. For the R-XYL, 5 of 24 VR-EFE were pos: 2 at the recommended 2 h and 3 at 2.5 h. 31 of 35 Egal and both Ecas were pos by 1.5 h; all remaining isolates were R-XYL-neg after 2.5 h when tests were discarded. All EFE and EFC were neg after o/n incubation in o/n-MGP. Both Ecas were pos in the o/n-MGP at 2 h, whereas of the 35 Egal, 2 were pos at 2.5 h, 1 ea at 4 h and 4.5 h, but 30 were pos only after o/n incubation and 1 isolate remained negative.
CONCLUSIONS: The R-MGP proved to be a viable alternative to the R-XYL in view of the advent of R-XYL-pos VRE, the prevalence of which remains unknown. Further evaluation of R-MGP is required.

P2.06
COMPARATIVE EVALUATION OF STREPTEX AND MED-STREP TEST KITS FOR LANCEFIELD GROUPING OF BETA-HEMOLYTIC STREPTOCOCCI

S FOSTER-TOURANGEAU*, M KEMP, N SNIDER, J KEISIG, C SCHAMBER, K CRAIG
Dynacare Kasper Medical Laboratories, Edmonton, Alberta

OBJECTIVE: Beta-hemolytic streptococci are frequently encountered pathogens in the clinical laboratory. The rapid identification of these organisms into Lancefield groups is important for patient treatment. The objective of this study was to compare two commercially available kits for accuracy, ease of use and potential cost savings.
METHODS: A total of 240 strains of beta-hemolytic streptococci (Groups A, B, C, F and G) were included in the study. All strains were isolated from routine samples submitted to the laboratory for bacterial culture. Each isolate was first tested by the Streptex acid extraction method (Remel, USA), then with the Med-Strep acid extraction reagents (Med-Ox, Ottawa, Ontario) and group specific latex suspensions. Testing was performed in accordance to the manufacturer's instructions.
RESULTS: Of the 240 strains tested, 85 isolates tested latex positive for Group A streptococci, 50 were latex positive for Group B, 37 isolates were group C, 3 were group F, 63 were group G and 2 beta-hemolytic isolates failed to group with either kit. We found agreement of results between the two kits on every isolate tested. Sensitivity and specificity were identical.
CONCLUSION: Both kits were easy to use and provided rapid results. The Med-Strep extraction reagents can be purchased in standard 50 mL bottles or in 400 mL bottles for high-volume testing. Advantages to larger reagent bottles include less environmental waste and decreased frequency of new lot quality controlled testing. We also found that switching to this method resulted in substantial cost savings for our laboratory.

P2.07
THE EFFECT OF SILCONIZED TUBES ON THE RAPID PLASMA REAGIN TEST FOR SYPHILIS

YY SIMPSON*[1], M KWOK[1], Q WONG[1], L KNOWLES[3], H JONES[3], MM MORSHED[1], J ISAAC-RENTON[1,2]
[1]Laboratory Services, BC Centre for Disease Control; [2]Department of Pathology and Laboratory Medicine, University of British Columbia; [3]Division of Sexual Transmitted Disease/AIDS Control, BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: As part of an initiative to improve safety in the laboratory and reduce the risk of blood and body fluid exposure to staff due to tube breakage, the laboratory investigated the effect of serum collected in siliconized plastic tubes compared with samples collected in the standard glass vacutainer tube without additives on the Rapid Plasma Reagin (RPR) test, a standard screening test for syphilis. Various agglutination tests have been documented as being adversely effected by silicone. One such example is the Cryptococcal Latex agglutination test. The RPR test is based on the principle of flocculation and has the potential to be adversely effected by silicone, although no conclusive documentation could be found.
METHODS: 306 patients' blood samples were collected in plastic vacutainer tubes coated with silicone (Becton-Dickinson #367815) at the STD Control Clinic BCCDC at the same time as a blood sample in a glass vacutainer tube, without additives was collected. After clotting, both blood collection tubes were immediately centrifuged and tested by the RPR method in the laboratory. 132 tubes were stored for a further five days at 4°C to simulate a longer transport time before being retested by RPR.
RESULTS: The RPR sensitivity and specificity in samples collected in siliconized plastic tubes was 100% compared with samples collected in glass tubes without additives. Neither the sensitivity nor the specificity was affected when tubes were stored for longer periods.
CONCLUSIONS: No adverse effect caused by siliconized tubes was noted in this evaluation. The siliconized plastic tubes did not lead to false-positive RPR results nor were any false-negative results observed. Therefore, plastic siliconized tubes may be used for RPR testing when safety is a concern.

P2.08
EXTERNAL VALIDATION OF BEDSIDE PREDICTION-SCORING MODEL FOR LATE-ONSET NEONATAL SEPSIS (LNS)

C OKASHAROEN*[1], C HUI[2], J CAIRNIE[1], A MORRIS[3], H KIRPALANI[1]
[1]McMaster University, Hamilton; [2]Children's Hospital of Eastern Ontario, Ottawa; [3]Hamilton General Hospital, Hamilton, Ontario

OBJECTIVE: The accurate diagnosis of late-onset neonatal sepsis (LNS) is difficult. We have developed a simple clinical prediction score for LNS which performs well (Okascharoen. J Perinatol 2005), and does not use any expensive added tests. The objective of this study was to validate this clinical prediction score for LNS in an independent patient population.
METHODS: The data was prospectively collected in neonates who were in the NICU who were suspected for sepsis. The study was performed at a level III NICU from March 2003 to May 2004. LNS was defined as a positive blood or CSF culture.
RESULTS: One hundred five neonates were assessed for sepsis. Demographics were as follows [mean (SD)]: Gestational age (GA) 29 (3) weeks; BW 1232 (620) g; and there were 39 female infants (37%). Fifteen infants (14%) had UVC in situ, and 28 infants (27%) had other central lines. Thirty-five (33%) neonates had LNS (35 positive blood culture; 2 positive CSF). No significant differences in GA, BW, sex and central line utilization were found between LNS and no LNS group. The pathogens were CNS (30), *Staphylococcus aureus* (1) *Enterobactor* species (3) and *Bacillus cereus* (1). The diagnostic performance is presented in the table below.

Diagnostic performance of prediction score

Score	Sensitivity	Specificity	Likelihood ratio +	Likelihood ratio –
≤3	0.97	0.39	1.7	0.07
≥8	0.20	0.98	10	0.8

The area under Receiver Operating Characteristic curve =0.78

Ninety-four infants (90%) received empirical antibiotics; sixty-two infants (66%) were later classified as no LNS.

CONCLUSION: This simple clinical prediction score developed for LNS performs similarly well in a different patient population. Adoption of the clinical prediction score may reduce use of antibiotics.

P2.09
A CASE-CONTROL OF ANTIBIOTIC CHOICE AND CLINICAL OUTCOMES IN 166 PATIENTS WITH *STREPTOCOCCUS PNEUMONIAE* BACTEREMIA

KR FORWARD*, R MELANO, R DAVIDSON
Queen Elizabeth II HSC, Halifax, Nova Scotia

OBJECTIVES: *Streptococcus pneumoniae* (SP) bacteremia is associated with considerable morbidity. Resistance and ineffective empirical treatment may contribute to poor outcomes. We conducted a case-control study to examine initial and subsequence antibiotic treatment, as well as the impact of antimicrobial resistance.

METHOD: Cases (83) and controls (83) were patients with pneumococcal bacteremia seen at tertiary care centres in 2002 and 2003. Cases included patients with strains resistant to penicillins and/or macrolides and/or fluoroquinolones. Controls had fully susceptible SP strains. Demographic information, antimicrobial usage, antimicrobial susceptibility and clinical outcomes were evaluated. Susceptibility tests were supplemented by genotypic and phenotypic characterization.

RESULTS: Of the 166 patients studied, 123 were adults and 43 were children. There were no significant differences in comorbidities between cases and controls. Nine cases vs 11 controls had recently been hospitalized. Otitis media and sinusitis were the most common underlying conditions in children (9/23 and 9/20), pneumonia was most common in adults (44/60 and 45/63). Most patients received multiple antibiotics; cases received an average of 2.5 antibiotic classes vs 2.3 for controls. Children generally received fewer antibiotics and more commonly received beta lactams alone. None of the children died. Twelve adults with resistant SP died, 15 died among adult with susceptible SP. Three and eight deaths, respectively, were directly attributed to SP infection.

CONCLUSION: Concurrent use of more than one antibiotic class for SP infections is widespread, especially in adults. Perhaps because of this, antibiotic-resistant SP bacteremia was not associated with more treatment failures than controls. Only 3/83 patients died as a direct consequence of resistant SP infection.

P2.10
DIFFERENTIATING VARIOUS LYME DISEASE-ASSOCIATED *BORRELIA* SPECIES BY REAL-TIME PCR DETECTION USING *OSPA* GENE

D JORGENSEN*[1], MK LEE[1], K FERNANDO[1], Q WONG [1], MG MORSHED[2]
[1]BC Centre for Disease Control; [2]University of British Columbia, Vancouver, British Columbia

INTRODUCTION: A number of *Borrelia* species, including the *Borrelia burgdorferi* sensu lato complex can cause Lyme Disease. Therefore, it is important to be able to differentiate these species. An attempt has been made to differentiate various Lyme Disease associated *Borrelia* species by real-time PCR detection method using *ospA* gene. Through the use of hybridization probes, which have different binding affinities to each species' *ospA* gene sequence, they can be differentiated by melting curve analysis.

METHODS: A total of 11 different species were used in this study, including *Borrelia burgdorferi, B garinii, B afzelii, B japonica, B bissettii, B turdi, B tanukii, B coriaceae, B anserina, B parkeri* and *B hermsii*. This real-time PCR was developed on the Roche LightCycler 2.0, and uses Roche LightCycler FastStart DNA Master Hybridization Probe kits. The PCR requires the use of a forward primer containing an internal LightCycler Red 640 molecule attached at its 3′ end, and is asymmetric with the LightCycler Red 640 probe being at a concentration 2.5 times more than the flourescein probe. Differentiation between the various species was obtained using Melting Curve analysis.

RESULTS: Of the 11 species tested, the 7 Lyme Disease-causing species were differentiated by unique melting curves. A standard curve was used to determine the PCR efficiency and error, which were 1.873 and 0.0114. As well the detection limit was determined to be 1-10 spirochetes/mL. The specificity of the primers and probes were determined by testing *B parkeri, B coriaceae, B anserina* and *B hermsii*, as they were not expected to amplify since they are transmitted by Ornithodoros ticks.

CONCLUSIONS: This method is a rapid and accurate way to determine the presence of various *Borrelia* species in the environment and has a potential for testing clinical samples.

P2.11
METHOD DEVELOPMENT FOR DETECTING TICK-BORNE RELAPSING FEVER *BORRELIA HERMSII* BY REAL-TIME PCR ASSAY

MK LEE*[1], D JORGENSEN[1], K FERNANDO[1], Q WONG[1], MG MORSHED[1,2]
[1]BC Centre for Disease Control, Vancouver; [2]University of British Columbia, Vancouver, British Columbia

OBJECTIVE: In British Columbia, *Borrelia hermsii* is the primary causative agent for tick borne relapsing fever found in *Ornithodoros hermsii* ticks. Currently, relapsing fever spirochetes are cultured by injecting patient blood in mice interperitoneally. Subsequently, the isolate are confirmed by immunofluoresence and GlpQ (Western blot) assays. Therefore, the objective of our study was to develop a real-time PCR method to identify and confirm the presence of tick-borne relapsing fever causal agent *Borrelia hermsii* without animal testing.

METHODS: *B hermsii* DNA was extracted using Qiagen Tissue Kit. PCR was performed on Roche LightCycler2.0 platform with Roche SYBR Green chemistry. Primers were designed in integral outer membrane protein regions (amplicon size: 379 bp) based on alignment of known sequences from GenBank. Varieties of Borrelia strains had been tested for the specificity. Serial dilutions of *B hermsii* DNA were used for the evaluation of sensitivity. Melting curve analysis was served as for differentiation and confirmation. Patient's isolates previously confirmed by IFA and GlpQ (Western blot) were used to evaluate this method.

RESULTS: *Borrelia hermsii* could be identified by its melting temperature of 79.5°C in melting curve analysis. Detection limits of this study reached to 1-10 spirochetes with amplification efficiency 1.80. All isolates previously confirmed by serological methods were positive by this method as well.

CONCLUSION: The sensitivity and specificity indicate that this new real-time PCR is an efficient method for identifying *Borrelia hermsii*. This method can be conducted in 1-day period and will also allow us to avoid animal testing. It could serve as an excellent tool for both diagnostic tests and epidemiological studies.

P2.12
EVALUATION OF COMMERCIAL HANTAVIRUS IGG AND IGM ELISA

F SIDAWAY*[1], D CASWELL[1], D DICK[2], PN LEVETT[1], G HORSMAN[1]
[1]Provincial Laboratory, Regina, Saskatchewan; [2]National Microbiology Laboratory, Winnipeg, Manitoba

OBJECTIVE: Comparison of commercial hantavirus IgG and IgM ELISA test kits for detection of hantavirus antibody in human serum with reference laboratory in-house ELISA assays.

METHODS: A reference panel of 8 sera known to have IgG and IgM antibody to hantavirus, and 58 patient sera submitted for hantavirus serology, were analysed using hantavirus-specific IgM and IgG ELISA assays (Focus Diagnostics, Cypress, USA). The 58 patient sera were also tested at the National Microbiology Laboratory (NML) in parallel using the CDC ELISA protocol. Statistical analysis was done using EP Evaluator.

RESULTS: Overall agreement between the commercial and reference IgM results was 93.1% (100% positive agreement and 91.4% negative agreement). Overall agreement between the commercial and reference IgG results was 97.2% (positive agreement 100% and negative agreement of 96.5%).
CONCLUSION: Focus hantavirus IgG and IgM test kits provide a good alternative for screening of human serum. The Focus test product showed a marginal positive bias for detection of hantavirus-specific IgG and IgM antibody in our laboratory.

P2.13
REAL-TIME PCR DETECTION OF BACTERIAL PATHOGENS CAUSING MENINGITIS IN CLINICAL SPECIMENS

A CAOBARIANYK*, RR MCDONALD, NA CROZIER, KL MONTGOMERY, T HANSEN, N A ANTONISHYN, PN LEVETT, GB HORSMAN
Provincial Laboratory, Regina, Saskatchewan

OBJECTIVE: Bacterial meningitis is a serious infection that can be fatal. To reduce the risk of death or permanent neurological damage, rapid detection and identification of the organism is vital for effective treatment. It was the objective of this research to develop a highly sensitive and specific real-time PCR assay for simultaneous detection of three major pathogens causing bacterial meningitis: *N meningitidis, H influenzae* and *S pneumoniae*.
METHODS: Real-time TaqMan PCR primer/probe sets were designed or chosen from the literature to detect these organisms of interest. The outer membrane protein gene P6 was chosen as a target for *H influenzae*. Primers and probes were designed to detect genes encoding pneumolysin (*ply*), and 2-keto-3-deoxygluconate kinase (kdgK) in *S pneumoniae*. Detection of the autolysin gene (*lyt*A) was performed as previously published. Analytical sensitivities were determined using spiked whole blood and CSF. Specificity was assessed against a panel of other bacterial species.
RESULTS: The *H influenzae* assay was able to detect 29 cfu/mL and did not cross-react with the other bacterial templates. Sensitivities of the *ply* and *kdg*K assays were 2 and 16 cfu/mL, respectively, for *S pneumoniae*. All three *S pneumoniae* targets were successful at detecting 100 different clinical isolates of *S pneumoniae*. Cross-reactions with other alpha-hemolytic streptococci were observed for all three targets, with ply being the least specific. For example, both *ply* and *kdg*K cross-reacted with *S sanguinis*.
CONCLUSION: A two-target approach is required to confirm *S pneumoniae* and to rule out cross-reaction. In practice, a positive *S pneumoniae* call requires amplification of both *kdg*K and *lyt*A. As a result, a sensitive and specific method for the detection of *H influenzae* and *S pneumoniae* has been developed to run simultaneously with an existing diagnostic assay for *N meningitidis*.

P2.14
INVESTIGATION OF A BACTERIAL CONJUNCTIVITIS OUTBREAK CAUSED BY NON-SEROTYPABLE *STREPTOCOCCUS PNEUMONIAE*

KL MONTGOMERY*[1], RR MCDONALD[1], Z ABBAS[2], M HENNINK[2], NA ANTONISHYN[1], PN LEVETT[1], GB HORSMAN[1]
[1]Provincial Laboratory; [2]Regina Qu'Appelle Health Region, Regina, Saskatchewan

OBJECTIVE: Nonserotypable (NT) *Streptococcus pneumoniae* is a major cause of bacterial conjunctivitis. Symptoms include red or itchy eyes, pain, eye-lid edema and discharge. In the spring of 2005, an outbreak of conjunctivitis was identified in the Regina Qu'Appelle Health Region (RQHR). Objectives of this study were to confirm the cause of the outbreak and to trace and identify cases related to the outbreak using molecular subtyping.
METHODS: The RQHR was contacted by a local pharmacist reporting abnormally high sales of nonprescription over the counter eye medication. From March 9, 2005 to April 14, 2005, samples were collected and cultured. All isolates were analysed and typed by fbAFLP. fbAFLP fingerprint scoring and cluster analysis was performed using GeneScan v.3.5 and BioNumerics v.3.5 software, respectively. Representative isolates were serotyped by the National Centre for Streptococcus. Eight NT isolates that were not part of the outbreak were also added for comparison.
RESULTS: 47 cases of conjunctivitis were reported in adults and children. *S pneumoniae* was isolated from 31 cases and fingerprints for 29 isolates were obtained. Two isolates did not generate fbAFLP fingerprints. The fbAFLP data produced two major clusters, the larger containing 26 isolates, and the other containing three isolates. Representative isolates from each major cluster were NT. The eight isolates unrelated by time and location did not cluster with the outbreak isolates.
DISCUSSION: NT *S pneumoniae* was confirmed to be the major cause of this outbreak involving a rural community school. Also, NT isolates not related to the outbreak were distinguished from the major outbreak clusters using fbAFLP. fbAFLP identified at least two clusters of *S pneumoniae* isolates involved with the outbreak, as supported by epidemiological investigation.

P2.15
A PROSPECTIVE TEN-YEAR REVIEW OF ACUTE CHILDHOOD ENCEPHALITIS

A BITNUN*[1], E FORD-JONES[1], D MACGREGOR[2], R TELLIER[3], H HEURTER[1], M PETRIC[4], S RICHARDSON[3]
[1]Division of Infectious Diseases; [2]Division of Neurology, [3]Department of Pediatric Laboratory Medicine, Hospital for Sick Children, University of Toronto, Toronto, Ontario; [4]British Columbia Centre for Disease Control, Vancouver, British Columbia

BACKGROUND: The identification of pathogens in acute childhood encephalitis (ACE) remains suboptimal; in most reports, no cause is identified in 60% to 70% of cases. In this report, we describe 10-year results of an ongoing prospective encephalitis registry at the Hospital for Sick Children (HSC), Toronto, Ontario.
METHODS: All children with ACE diagnosed between January 1996 and December 2005 underwent extensive microbiological investigation for evidence of acute infection with putative viral and bacterial pathogens. Encephalitis was defined by the presence of encephalopathy and ≥2 of the following: fever (≥38.0°C), seizure(s), focal neurological findings, pleocytosis (>5 WBCs/μL), EEG or neuroimaging findings compatible with encephalitis.
RESULTS: A total of 264 children were admitted to HSC with a diagnosis of ACE during the study period. The mean age was 7.3±4.9 years; 52% were male. Potential etiological agents were found in 172 cases (65%); of these, a single potential cause was detected in 106 (62%). The most frequently identified pathogens were *Mycoplasma pneumoniae* (31%), HHV-6 (12%), influenza A or B (10%), HSV-1 or HSV-2 (8%), enteroviruses (7%), HHV-7 (6%), EBV (7%), VZV (5%) parainfluenza viruses (5%), adenoviruses (3%) and *Bartonella henselae* (2%). A clear seasonal predilection was apparent for two organisms: influenza viruses (92%, December to May) and enteroviruses (61%, July to October). Microbiological evidence of *M pneumoniae*-herpes virus and *M pneumoniae*-respiratory virus coinfections were observed in 32 (12%) and 13 (5%), respectively. Mixed herpesvirus infections (eg, HSV and EBV) were seen in 13 (5%). Among the six fatalities a possible cause was identified in four: measles (1) and EBV (3).
CONCLUSIONS: An ongoing registry with standard microbiological investigations enhances identification of potential etiological agents in ACE. The identification of more than one potential pathogen in 25% of cases may indicate the importance of coinfection in the pathogenesis of ACE.

P2.16
MOLECULAR TYPING OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* USING THE *espZ* GENE ENCODED IN THE LOCUS OF ENTEROCYTE EFFACEMENT

DM TRACZ*, AK ANDRYSIAK, CG CLARK, S TYSON, A SEVERINI, L-K NG, MW GILMOUR
National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba

OBJECTIVE: Shiga toxin-producing *Escherichia coli* (STEC) result in frequent cases of sporadic and outbreak-associated enteric bacterial disease in

humans. The *esp*Z gene encoded in the locus of enterocyte effacement (LEE) is known to be hypervariable among *E coli* serotypes. Our goal was to develop an allelic discrimination assay at the *esp*Z locus for the detection and subtyping of STEC.
METHODS: Sequencing of *esp*Z from a panel of STEC serotypes (including O26:H11, O121:H19, O111:NM, O145:NM, O165:H25, O121:NM, O157:NM, O157:H7 and O5:NM) led to the identification of polymorphisms in *esp*Z that correlated to specific serotypes. Light Upon eXtension (LUX) fluorogenic primers were designed to target these serotype-specific regions in *esp*Z. Real-time PCR was performed using template prepared from a panel of *E coli* clinical isolates on a Cepheid Smartcycler. For liquid microsphere suspension assays, *esp*Z PCR products were hybridized to xMAP microspheres coupled to serotype-specific oligonucleotide probes and processed through a Luminex Liquichip station.
RESULTS: Real-time PCR primer sets had 100% specificity for each of four targeted *esp*Z lineages, which also correlated with intimin type. Characterization of STEC isolates was further supported by *stx*1/2 LUX real-time PCR. Luminex xMAP microsphere technology was also successful in identifying serotype-specific sequences in *esp*Z PCR products and provided rapid allelic discrimination requiring only a single PCR reaction.
DISCUSSION: Genetic variation at the *esp*Z locus is an excellent target for the detection and subtyping of STEC and provides a means to initiate molecular-based serotyping in *E coli*.

P2.17
IDENTIFICATION OF *BACILLUS THURINGIENSIS* AMONG *BACILLUS CEREUS* GROUP ISOLATES ASSOCIATED WITH BRITISH COLUMBIA FOOD POISONING EVENTS

L MCINTYRE*[1], L DEWINTER[2], K BERNARD[2], B AUK[1], J FUNG[1], J ISAAC-RENTON[1], D BENIAC[2], B NG[2], D WIEBE[2], T BURDZ[2]
[1]BC Centre for Disease Control, Vancouver, British Columbia; [2]National Microbiology Laboratory, Winnipeg, Manitoba

OBJECTIVES: In February 2005 a food-borne illness implicated *B thuringiensis* (Bt), which is not normally differentiated from the known food pathogen, *B cereus*, using conventional laboratory methods. A retrospective study of *B cereus* group (Bcg) isolates implicated in food poisoning events in British Columbia (BC) were characterized with the purpose of differentiating among members of the *B cereus* group.
METHODS: 180 Bcg isolates relating to approximately 50 food poisoning events in BC from 1991 to present were assayed by 2 different laboratories. Isolates were tested by PCR for the presence of *cry* 1 and 2 (insecticidal crystal protein found only in Bt), NRPS (the nonribosomal peptide synthetase that produces cereulide, the emetic toxin), and Nhe/HBL (gene families associated with enterotoxin and diarrhea). Phenotypic tests included biochemical profiling, EM examination for crystals and commercial enterotoxin kit assays.
RESULTS: *Cry* genes were identified in 4.4% of isolates and were identified as Bt; 4% of isolates demonstrated a rhizoidal colony, resulting in an identification of *B cereus* var *mycoides*. 16% of isolates carried the genes responsible for cereulide production. For enterotoxin gene families, 21% of isolates had either the 3 genes necessary to code for the tripartite toxin proteins Nhe or HBL (nonhemolytic enterotoxin or Hemolysin BL) and 14.5% of isolates contained all 6 genes.
DISCUSSION: This collaborative investigation correlates the phenotypic and genotypic profile of Bcg isolates with epidemiological data collected during the food poisoning incidents. It is noteworthy that 4.4% of Bcg isolates implicated in food poisoning events were identified as Bt. Further investigation is required into whether Bt can cause food poisoning as evidence in the literature is not conclusive. This study suggests that more rigorous testing to discern among taxa in the Bcg may be required for isolates from foods.

P2.18
HCV F PROTEIN AS A POTENTIAL DIAGNOSTIC MARKER: ANALYSIS OF REACTIVITY WITH INNO-LIA HCV AB III INDETERMINATE CLINICAL SERUM SAMPLES

T CUTTS*, C OSIOWY, R HE
Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba

OBJECTIVE: Chronic hepatitis C virus (HCV) infection is one of the leading infectious diseases in the world. Accurate detection of HCV antibody requires a complex algorithm involving enzyme immunoassay screening followed by a confirmatory test. The INNO–LIA HCV Ab III confirmatory assay is an immunoblot-based test with an improved ability to detect weakly reactive samples; however, indeterminate results can occur if only a single probe line is reactive. The present study investigated the reactivity of INNO–LIA HCV Ab III indeterminate serum samples with in vitro expressed HCV alternate reading frame (F) protein, as a potential diagnostic marker.
METHODS: The HCV F protein (genotype 1a) was expressed both as a 6XHIS fusion protein and as a GST fusion protein in bacterial expression systems and was purified. Following SDS-PAGE electrophoresis, purified F protein was transferred to PVDF membrane and probed with 8 clinical serum samples that were determined to be indeterminate by the INNO–LIA HCV Ab III test.
RESULTS: In vitro expressed HCV F protein migrated on SDS-PAGE gels at the expected size of approximately 17 kDa. Protein specificity was confirmed by reaction with anti-GST antibody and mass spectrometry analysis. Following western blot analysis with purified HCV F protein, 2/8 (25%) clinical samples tested positive for the presence of antibody to the F protein.
CONCLUSION: Antibody to HCV F protein is present in a small percentage of individuals testing indeterminate by the INNO-LIA HCV Ab III test. The HCV F protein may supplement current confirmatory HCV protein markers to facilitate the accurate detection of antibodies to HCV.

P2.19
SPONTANEOUS CLEARANCE OF HEPATITIS C VIRUS AMONG ABORIGINALS IN BRITISH COLUMBIA

WD HILL*[1], G BUTT[1], P MELANSON[3], M CROSBY[3], C MAHONEY[3], C PANESSA[1], M ALVAREZ[2], M KRAJDEN[1]
[1]BC Centre for Disease Control, Vancouver; [2]University of British Columbia, Vancouver; [3]North Island Liver Service, Campbell River, British Columbia

OBJECTIVE: Spontaneous clearance of hepatitis C virus (HCV), defined as anti-HCV reactive but HCV-RNA negative, has been postulated to be due to different host and viral factors. The objective of this study was to investigate differences in the rates of spontaneous clearance between Aboriginal and nonAboriginal individuals assessed at one of four integrated hepatitis prevention and care project sites in British Columbia.
METHODS: Administrative databases were used to collect data on demographics, comorbid diseases, transmission mode, interventions, clinical status and therapy outcomes. Differences in spontaneous clearance were determined by assigning the clients into Aboriginal and nonAboriginal groups based upon self-reported ethnicity and comparing HCV RNA results upon initial intake into the clinics. Clients who were anti-HCV reactive but were HCV RNA-negative upon initial intake were considered to have spontaneously cleared their infection.
RESULTS: Intake HCV RNA results were available for 111 Aboriginal clients (age range 17 to 64 yrs; mean 45 yrs) and 986 nonAboriginal clients (age range 20 to 82 yrs; mean 49 yrs). A significant difference ($P<0.001$) was observed in spontaneous clearance between the Aboriginal (20/111 [18%]) vs nonAboriginal (79/907 [9%]). Post hoc comparisons revealed that a significantly greater number of Aboriginal women than men spontaneously cleared the virus.
CONCLUSIONS: This study revealed a significant association between Aboriginal ethnicity and spontaneous HCV clearance. These results highlight the need for further research to investigate the specific host factors

that suggest Aboriginal populations, especially females, are possibly more likely to spontaneously clear HCV infections.

P2.20
TREATMENT OUTCOMES OF A PUBLIC HEALTH/ PHYSICIAN COLLABORATIVE PRACTICE MODEL FOR THE PREVENTION AND TREATMENT OF HCV

WD HILL*, G BUTT, M KRAJDEN
BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: To determine if interferon/ribavirin treatment outcomes for HCV in locally coordinated, integrated prevention and care pilot projects match those of randomized control trials.
METHODS: Four integrated prevention and care projects in rural and small urban centres in BC provided comprehensive health promotion/prevention, care and treatment through a public health nursing/physician collaborative practice model. A customized database tracked demographics, comorbid diseases, transmission mode, interventions, duration of health care events and the provider, clinical status and therapy outcomes. Treatment outcome data was analyzed and compared with published data from randomized control trials.
RESULTS: From January 2002 to November 2005, a total of 359 clients initiated combination interferon/ribavirin treatment (Rebetron [20%]/ Pegetron [80%]). As of December 1, 2005, treatment outcome data was available on 205 patients (98 [48%] genotype 1; 107 [52%] genotype 2, 3). Demographics: Age range: 26 to 72 yrs, mean 49.5; 72% male. Employment status on referral: 57% employed full or part time, 23% unemployed, 14% on disability, 6% unknown. Transmission modes: 73% infected through IDU, 16% from blood products received before 1992. Comorbidities: 7% cirrhosis or ESLD, 9% diabetes, 2% HIV coinfection. Time from referral to assessment averaged 5.4 weeks. Outcomes: SVR 47/98 [48%] genotype 1, 79/107 [74%] genotype 2/3.
CONCLUSIONS: A public health/physician collaborative practice service delivery model produces treatment outcomes comparable with clinical trials and effectively uses health care resources to improve access, reach and capacity for HCV prevention and treatment.

P2.21
COMPARISON OF BIORAD MRSA*SELECT* CHROMOGENIC AGAR AND MAST MASTASCREEN MRSA BROTH FOR RAPID DETECTION OF MRSA FROM PATIENTS IN A HIGH PREVALENCE SETTING

N AFACAN[1], C BROSNIKOFF*[1], R RENNIE[1], D HARBERG[2], C MIHALCHEON[2], O OLSON[2], L ROSWELL[2], R SAND[2], M SMYL[2]
[1]Medical Microbiology Laboratory, University of Alberta Hospitals, Edmonton; [2]East Central Regional Health Authority, Camrose, Wainwright and Vegreville, Alberta

OBJECTIVE: The purpose of this study was to compare the sensitivity and specificity of chromogenic agar and a selective broth medium for the presumptive detection of MRSA. The study was undertaken as a point prevalence screening in an institution known to have MRSA-positive patients in both the acute and long-term care sections of the facility.
METHODS: MRSA*Select* (MR) agar (Bio-Rad, Marnes la Coquette, France) is a selective, chromogenic media for presumptive identification of MRSA. MAST MASTASCREEN MRSA ID (MT) Broth (MAST Diagnostics, UK) is a nutrient broth incorporating mannitol and trehalose. A disc containing phenol red, aztreonam, cefoxitin and colistin is added to the broth a minimum of 30 min before inoculation. 235 clinical swabs were collected from 111 acute and long-term care patients. Swabs were taken from the nose (109), groin (111), G tube (2) and wounds (11); two urine specimens were also collected. Quality control was performed according to manufacturers' package insert. Specimens were plated directly onto the MR agar after inoculating the MT broth. 142 swabs were inoculated to both media and 91 swabs were inoculated onto MR plates only. Both media were incubated at 35°C in ambient air. MR plates were read at 24 h and the MT broths were read at 18 h – 24 h.
RESULTS: Pink colonies on the MR plates were subcultured for further workup. MT tubes in which the pH indicator turned yellow (a presumptive positive result for MRSA) were also subcultured. For this evaluation, any tubes demonstrating an orange colour change were also subcultured for further workup. The MR plates demonstrated sensitivity and specificity of 88% and 97%, respectively. When an orange or yellow colour change was considered positive in the MT broth, the sensitivity was 68% and specificity 85%. When the package insert was followed and only a yellow colour change was positive the sensitivity and specificity were 62% and 95%, respectively. 18 patients were found to be MRSA positive demonstrating seven different pulse field fingerprints.
CONCLUSIONS: There were fewer false positive samples on MR agar. The MT broth was specific, but there were more false positives that required extensive workup to rule out MRSA. MT broth may require modifications including pH adjustment to improve its value for presumptive identification of MRSA. MR agar appears to be an excellent alternative to conventional methods to identify MRSA patients, especially in a high prevalence setting.

P2.22
HAND WASHING COMPLIANCE IN NICU: MORE QUESTION THAN ANSWER

K CLARK*[1], B CZERNIAWSKI[2], G INGLIS[2], S HO[2], C OKASCHAROEN[2], S BLATZ[2], AM SMITH[2], M LOEB[1], H KIRPALANI[3]
[1]Infection Control, HHS-MUMC; [2]Pediatrics, McMaster; [3]Clinical Epidemiology, McMaster University, Hamilton, Ontario

OBJECTIVE: Hand washing remains key in prevention of nosocomial infection, yet it achieves poor compliance. The study objective was to observe the rates of hand washing in a level III NICU and record rates of missed opportunities (MO) of hand washing. MO was defined as failure to decontaminate hands using appropriate techniques (handwashing with soap or antiseptics) before/after direct or indirect patient care.
METHODS: Observations were performed over a 4-month period (March to July, 2005) by trained observers. Twenty-eight observation periods were allowed for a total of 46.7 h of data collection. Direct patient care (DC) was defined as care involving any contact with patient or body fluid (including IV line insertion/manipulation). Indirect patient care (IC) was defined as care that contact patients equipments/environment (eg, clipboard, chart, computer, monitors or housekeeping).
RESULTS: In total, observations numbered 1927 for registered nurses (RN); 148 acute care nurse practitioner (ACNP); 95 for allied health (AH); 262 for family/visitor; 272 for support services; 154 for respiratory therapists (RRT); 17 for technicians; 166 for physicians (MD); and 37 for others. The overall total observations were 6520, of which 1837 (29%) were associated with DC and 4683 (71%) were associated with IC. More than 50% of MO for DC were observed in MD, RN, RRT and AH. More than 68% of MO for IC were observed in all disciplines.
CONCLUSIONS: Poor compliance of hand-washing practice was observed in all care givers, irrespective of subspecialty. Multi-faceted interventions are needed to improve compliance of handwashing.

P2.23
LABORATORY-BASED SURVEILLANCE OF *SALMONELLA TYPHI* INFECTIONS IN CANADA FROM 2000 TO 2004

LK NG*, WHB DEMCZUK, H TABOR, R AHMED
Public Health Agency of Canada, Winnipeg, Manitoba

OBJECTIVE: The severity of disease associated with *S typhi* remains a significant public health concern worldwide. Multiple drug resistance and decreased susceptibility to fluoroquinolones is of particular concern. This study describes the surveillance of *S typhi* by phage typing, pulsed-field gel electrophoresis (PFGE) and antimicrobial resistance (AMR) testing in Canada during 2000 to 2004.
METHODS: All strains were submitted by the provincial public health laboratories or enteric reference centres (PPHLs) of Canada. AMR was determined by disc diffusion on isolates collected during 2000 to 2002 or broth microdilution (using the Sensititre ARIS Automated Microbiology System) for isolates collected during 2003 and 2004. Phage typing and PFGE were performed using standard methods.

RESULTS: Of 557 strains, 52.2% (n=291) were from blood, 29.9% (n=167) from stool and 17.8% (n=99) were from other sources. Annual incidence rose from 83 in 2000 (0.28 cases/100,000 population) to 129 (0.40/100,000 population) in 2004. Phage type (PT) E1 was predominant accounting for 39.1% (n=216) of all isolates, followed by PT A with 7.2% (n=40), PT E9 and PT Untypeable each with 6.1% (n=34), UVS (I+IV) with 4.7% (n=26). Resistance to more than 4 antimicrobials was observed in 85 strains (15.7%), 111 (20.5%) were resistant to 1 to 4 agents and 63.8% (n=345) were susceptible to all. Of 252 isolates tested during 2003 and 2004, no isolates were resistant to ciprofloxacin; however, 59.5% (n=150) had susceptibilities of 0.06 to 0.5 μg/mL and 50.4% (n=127) were resistant to nalidixic acid. PFGE analysis on 32 isolates resulted in 17 distinct *Xba*I and 5 *Bln*I banding patterns.
CONCLUSIONS: The incidence of *S typhi* remains relatively low in Canada. Multidrug-resistant phenotypes and resistance to nalidixic acid, a marker for decreased susceptibility to fluoroquinolones, are of particular concern. To guide prevention and control activities, continued national surveillance and targeted research studies will monitor trends, detect emerging issues and investigate risk factors and burden of illness.

P2.24
SCREENING TO PREVENT NOSOCOMIAL SPREAD OF ANTIBIOTIC-RESISTANT ORGANISMS

RM WIENS*, LM BUCHANAN-CHELL, JC DURAND, TA KIRKLAND, GD TAYLOR
University of Alberta Hospital, Edmonton, Alberta

OBJECTIVE: Success in controlling nosocomial transmission of antibiotic-resistant organisms (AROs) including methicillin-resistant *Staphylococcus aureus* (MRSA) is dependent on the application of barrier precautions for patients infected or colonized with the organism and rigorous active surveillance cultures to identify colonized patients. The objective of our project was to determine a practical and cost-effective method to identify patients colonized with MRSA to minimize the potential reservoir of this microorganism within our facility.
METHODS: A telephone survey of several tertiary care facilities in Canada and acute care facilities in Capital Health Region, Edmonton, was conducted to identify screening practices for AROs in similar facilities. In addition, cases of MRSA in our hospital from January to September, 2005 were identified. The percentage of community-acquired and nosocomial infections was then calculated by admissions to different services, including transfers within our own facility. Using this information, we drafted three options for screening patients for MRSA. A cost analysis for each option was also performed. This information was submitted to the Infection Control Committee for their consideration.
RESULTS: The Infection Control Committee approved the option to conduct routine prevalence screening for MRSA in services where nosocomial rates were highest. Prevalence screening will be performed for a six-month period at which time the percentage of cases of nosocomial MRSA by admission by service will be reevaluated.
CONCLUSION: Identifying potential reservoirs for AROs within a health care facility is imperative to control nosocomial transmission. Achieving this goal in a practical and cost-effective method is important for both the facility and our patients.

P2.25
ANTIMICROBIAL RESISTANCE IN *S PNEUMONIAE* (SPN) ISOLATES FROM BACTEREMIC VS RESPIRATORY TRACT INFECTIONS IN CANADA

M DECORBY*[1], B WESHNOWESKI[2], R VASHISHT[1], K KARLOWSKY[1], M EDEL[2], GG ZHANEL[1], DJ HOBAN[2]
[1]University of Manitoba; [2]Health Sciences Centre, Winnipeg, Manitoba

OBJECTIVES: Antibiotic resistance in SPN is continuing to evolve in Canada and worldwide. This study examined the resistance rates of isolates collected over several years from bacteremic and respiratory tract specimens to determine the differences in resistance rates for various antibiotics.
METHODS: 25 medical centres representing 9 out of 10 provinces submitted isolates of SPN to the coordinating reference laboratory (Health Sciences Centre, Winnipeg, Manitoba). Respiratory and bacteremic isolates of SPN were collected as part of an ongoing national surveillance study from 1997 to 2004 and 2002 to 2004, respectively. Susceptibility testing was performed using CLSI-approved broth microdilution methods.
RESULTS: For bacteremic SPN, the percentage of penicillin intermediate (Pen I, MIC 0.12-1 μg/mL) and resistant (Pen R, MIC ≥2 μg/mL) strains from 2002, 2003 and 2004 was 9.4%, 9.3%, 10.4% and 5.1%, 6.2%, 6.6%, respectively. The prevalence of clarithromycin (Clari R, MIC ≥1 μg/mL) and ciprofloxacin (Cip R, MIC ≥4 μg/mL) resistance in bacteremic SPN for the past 3 years was 7.6%, 11.6%, 9.8% and 1.7%, 0.4%, 1.0%, respectively. Over 7 years, the percentage of Pen I strains among respiratory SPN ranged from 13.1% to 17.3%, and Pen R strains from 2.1% to 9.8%. Clarithromycin resistance increased from 8.1% in 1997 to 1998 to 13.5% in 2004, and ciprofloxacin resistance increased from 0.6% to 3.7%.
DISCUSSION: Overall, *S pneumoniae* isolated from bacteremic infections showed lower resistance rates to penicillin, clarithromycin and ciprofloxacin as compared with respiratory tract isolates.

P2.26
EVALUATION OF A MOLECULAR ASSAY FOR RAPID IDENTIFICATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) FROM CLINICAL ISOLATES

L TURNBULL*, J FULLER, S SHOKOPLES, R RENNIE
Medical Microbiology, University of Alberta Hospitals, Edmonton, Alberta

OBJECTIVE: Rapid MRSA identification and detection is essential for the implementation of appropriate therapy and infection control measures. However, routine identification of MRSA can often take several days to confirm. We evaluated the Genotype *Staphylococcus* assay (Hain Lifescience, Germany), which provides selected species, *mecA* and Panton-Valentine leukocidin (PVL) gene identification, in comparison with conventional methods.
METHODS: Twelve clinical isolates of *S aureus* were tested, including 2 methicillin-susceptible *S aureus* (MSSA) and 10 MRSA, of which 3 were PVL positive and 1 was PVL negative according to conventional PCR. Conventional methods consisted of routine identification tests, Kirby-Bauer susceptibility testing and PCR for *mecA* and PVL genes. The GenoType *Staphylococcus* assay involved multiplex DNA amplification and reverse hybridization of amplified product on an immobilized DNA strip-blot containing probes for 6 staphylococcal species, *mecA* and PVL genes.
RESULTS: The GenoType *Staphylococcus* assay produced correct species and *mecA* identification for all 12 isolates (100%), as well as PVL identification in 4 characterized strains. The costs per specimen were comparable for each test method; however, the turn-around time of the GenoType assay was 4 h in comparison to 48 h for conventional testing.
CONCLUSION: Based on this pilot study, the GenoType *Staphylococcus* assay can be used as a rapid, cost-effective, and reliable identification method for MRSA. Continued evaluation is required to clearly define a role for this novel assay in the prevention and control of MRSA.

P2.27
VALIDATION OF DISC DIFFUSION (DD) FOR DETECTION OF LOW-LEVEL MUPIROCIN RESISTANCE (LL-MUP-R) IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

BM WILLEY*, N KREISWIRTH, S HO, C LAROCQUE, M LUM, K PIKE, C PORTER, P LO, A MCGEER, S POUTENAN
Toronto Medical Laboratories and Mount Sinai Hospital, Toronto, Ontario

OBJECTIVES: Mup-R MRSA has reached approximately 22% in the greater Toronto area (GTA) due to the circulation of two epidemic clones: CMRSA-2 (LL-Mup-R) and CMRSA-9 (high-level Mup-R).

Where Mup eradication therapy is common, routine testing is recommended. Proposed non-CLSI DD breakpoints suggest Mup-R can be predicted by DD <14 mm. This study was set up to determine the ability of the more cost effective DD method to detect LL-Mup-R in MRSA.

METHODS: 123 MRSA with known Mup MIC (tested by E-Test and/or CLSI broth microdilution) were selected from 1125 new 2005 cases, to include 62 Mup-S (MIC range: <0.12 mg/L to 2 mg/L), 61 LL-Mup-R (MIC range: 4 mg/L to 64 mg/L). DD was performed as per CLSI methods using Oxoid 5 µg mupirocin discs on Oxoid MH agar. Zone diameters were read by 5 technologists blinded to MIC values and to each other's readings for a total of 620 independent reads. Scatter plot analysis comparing DD zones to MIC values was used to compare the ability of published non-CLSI DD breakpoint criteria to predict LL-Mup-R.

RESULTS: Of the 61 LL-Mup-R MRSA, 59 (95%) had zones <14 mm (range 6 mm to 13 mm) but 3 outliers (5%), with MIC of 4 mg/L, 8 mg/L and 16 mg/L, had multiple reads from the original testing and 5 independent repeats with zones ≥14 mm (range, 14 mm to 18 mm). All 62 Mup-S MRSA had zones of ≥14 mm (17 mm to 34 mm), with all ≥19 mm except for 2 reads from a single strain, with MIC of 1 mg/L, that were 17 mm and 18 mm. Repeats of the Mup-S outlier resulted in zones of 18 mm to 21mm.

CONCLUSIONS: If DD is to be used as a cost-effective screening method that aims to capture 100% of MRSA with LL-Mup-R in current circulation in the GTA, the most sensitive breakpoint that incurs the least very major errors would be <19 mm for resistance and ≥19 mm for susceptibility. Further testing using E-test or broth microdilution would be required for MRSA with zones <19 mm to confirm resistance and to distinguish between low-level Mup-R and the high-level Mup-R that is associated with the *mupA* resistance determinant.

P2.28

PHENOTYPIC CHARACTERISTICS AND ANTIFUNGAL SUSCEPTIBILITY OF *CANDIDA DUBLINIENSIS* (CD) ISOLATES FROM CLINICAL SPECIMENS IN SASKATCHEWAN

H CONGLY*

Provincial Laboratory, Regina, Saskatchewan

OBJECTIVE: To study the phenotypic features and antifungal susceptibility of CD isolates from HIV-negative patients in Saskatchewan.

METHODS: 20 CD isolates were used for study. All isolates were identified with the AUXACOLOR 2 (BioRad) yeast system. Phenotypic assays included growth at 45°C, colony colours on BBL CHROMagar Candida (CB) and Paris, France CHROMagar Candida (CF), chlamydospore morphology and production of esterase activity. Susceptibility testing for amphotericin B (AMB), 5-flucytosine (5-FC), ketoconazole (KET), itraconazole (ITRA) and fluconazole (FLU) using the NCCLS broth microdilution method was performed at the National Center for Mycology in Edmonton, Alberta.

RESULTS: All CD isolates showed growth at 45°C. On CB 95% of isolates were light green and 5% were dark green. 80% of isolates were light green and 20% were dark green on CF. Chlamydospore morphologies were 55% singlets and doublets (SD); 30% singlets, doublets and triplets (SDT) and 15% singlets. Esterase activity was present in 60% and absent in 40% of isolates. The MIC ranges were 0.5 mg/L to 1 mg/L, ≤0.06 mg/L to 1 mg/L, ≤0.015 mg/L to 0.12 mg/L, 0.06 mg/L to 1 mg/L and ≤0.06 mg/L to 1 mg/L for AMB, 5-FC, KET, ITRA and FLU, respectively.

CONCLUSION: In this study, all CD isolates displayed growth at an elevated temperature (45°C). The majority of isolates produced light green colonies on CB and CF. Chlamydospore SD formation was the most common morphology. Lack of esterase activity correlated with chlamydospore SD and SDT formation. All isolates were susceptible to 5-FC and FLU. One isolate was resistant to ITRA.

P2.29

COMPARISON OF RPMI AGAR WITH 2% GLUCOSE (RPG) AND MUELLER-HINTON AGAR WITH METHYLENE BLUE AND 2% GLUCOSE (MBE) FOR FLUCONAZOLE (FC) SUSCEPTIBILITY TESTING OF YEAST BY E-TEST (ET) AND DISK DIFFUSION (DD)

M DESJARDINS*[1], E CAMERON[2], C SEETERAM[2], C GUIBORD[2]

[1]The Ottawa Hospital; [2]The Ottawa Hospital Research Institute, Ottawa, Ontario

BACKGROUND: With increasing incidence of invasive fungal infections and use of empiric antifungal therapy, in vitro susceptibility testing of yeast isolates may be helpful to guide therapy. We compared the performance of ET and DD using RPG and MBE for FC susceptibility testing of clinical yeast isolates. Yeast one (YO) microtitre plates were also evaluated and compared with ET and DD.

METHODS: A total of 37 isolates (14 *C albicans*, 9 *C parapsilosis*, 7 *C glabrata*, 4 *C krusei* and 3 *C tropicalis*) were tested against FC by ET and DD on RPG and MBE according to CLSI M2-A8 and M27-A. Plates were incubated at 35°C in ambient air for 48 h. YO microtitre plates were inoculated and incubated according to manufacturer's recommendations. Interpretations for FC MICs by ET and YO were based on CLSI recommendations (M27-A2). For DD, zones of inhibition were interpreted as follows: ≤14 mm susceptible, 15 mm to 18 mm susceptible dose dependent (SDD), ≥19 mm resistant. Quality control with appropriate strains was performed with each run.

RESULTS: All *C albicans* and *C tropicalis* isolates were susceptible and all *C krusei* isolates were resistant to FC by all methods. Both RPG and MBE agar produced similar results for ET or DD testing. Trailing end points and regrowth on RPG agar was significant and made interpretation difficult compared to MBE. Discrepancies between RPG and MBE were found for 2 of 5 *C glabrata* isolates. Both were SDD on RPG but susceptible on MBE by ET. For *C parapsilosis*, there were 3 discrepant isolates: 2 were SDD by ET but resistant by DD, and 1 isolate was susceptible by ET and resistant by DD. Although there was good interpretative agreement between YO and ET, YO plates failed to support growth of *C glabrata*.

CONCLUSION: Overall there was good agreement between all susceptibility methods on either RPG or MBE. However, we found MBE to be superior because of difficulties interpreting MIC or zones of inhibitions on RPG agar. DD testing on MBE correlated well with ET and can provide a cost-effective and reliable approach for yeast susceptibility testing.

P2.30

EXPERIENCE WITH SENSITITRE YEASTONE COLORIMETRIC ANTIFUNGAL PANELS FOR SUSCEPTIBILITY TESTING OF INVASIVE *CANDIDA* SPP IN A LARGE CANADIAN HEALTHCARE REGION

S ELSAYED*, D CHURCH, D GREGSON, K LAUPLAND

University of Calgary, Calgary, Alberta

BACKGROUND: *Candida* spp are important causes of invasive fungal infections in medically compromised patients. Due to rising rates of resistance to azoles, newer agents have played a greater role in empiric treatment. Although susceptibility testing is important for guiding therapy, lack of commercial methods has hampered their utility. We report our experience with commercial microdilution panels for susceptibility testing of invasive Candida spp isolates in a regional healthcare system.

METHODS: A single centralized laboratory provides virtually all acute care diagnostic microbiology services in our health region, including hospitals, nursing homes and doctor's offices. Nonduplicate blood culture isolates of *Candida* spp recovered from patients in our region during the 5-year period Jan 2000 to Dec 2004 underwent susceptibility testing using Sensititre YeastOne Y05 colorimetric broth microdilution panels (Trek DS, USA). Isolates were tested against amphotericin B (AMB), 5-fluorocytosine (5FC), fluconazole (FLU), itraconazole (ITR), voriconazole (VOR) and caspofungin (CAS) according to the manufacturer's instructions.

RESULTS: A total of 178 bloodstream isolates of *Candida* spp were tested, including 96, 40, 14, 13, 10 and 5 strains, respectively, of *C albicans*, *C glabrata*,

C tropicalis, *C parapsilosis*, *C krusei* and other *Candida* sp. Testing was easy to perform, with minimal technologist effort, and results were interpretable within 24 h, in most cases. Colorimetric endpoint MICs for all antifungals were readily determined although skipped wells were occasionally seen with voriconazole. The MIC_{90} ranges for AMB, 5FC, FLU, ITR, VOR and CAS were (in mcg/ml) 0.25–0.5, 0.03–64, 0.5–64, 0.06–2, 0.008–1 and 0.06–1, respectively, and compared favourably with those of existing (Etest) methods.
CONCLUSIONS: Our experience with YeastOne Y05 panels suggests they are suitable for routine implementation in any diagnostic microbiology lab.

P2.31
ATTITUDES OF HEALTH CARE WORKERS (HCW) TOWARDS ANNUAL INFLUENZA VACCINATION: A PROSPECTIVE STUDY

A MUKHERJI*[1], S COMPTON[2]
[1]Dalhousie University and AHSC; [2]University of New Brunswick, Saint John, New Brunswick

BACKGROUND: HCWs are at high risk of influenza infection and are reservoirs for transmitting infection to coworkers and pts. The literature supports the role of HCW in reducing transmission and recommends annual vaccination. Underimmunization of HCWs is the key cause of outbreaks in HC settings. Immunization rates of HCW at our regional hospital are low (36%), and reflect a national underimmunization trends.
METHODS: Survey (6/04 to 7/04), voluntary participation, convenient sample 20% full-time RNs, employed 2 or more years in oncology/ICU/ER. Structured and open-ended questions (OEQ).
RESULTS: 50 female nurses participated, age 23 to 46 yrs and 32.1% with at least one chronic illness (HTN/DM). When asked why vaccination is recommended (OEQ), all were aware of the major rationales, ie, reduce transmission/personal protection. 22% reported receiving vaccine in the past two yrs and all did so, for protection of self and pts/coworkers. Among those (78%) who did not receive vaccine in past 2 yrs, 100% reported (OEQ): 1) *Vaccine safety*: fear of getting sick, prior self reported adverse effect, financial loss due to lost work-time from being "sick from the flu shot", fear of vaccine components, harm to fetus, long-term uncertain effect on immune system and belief vaccine flu strains were oncogenic; 2) *Vaccine efficacy*: doubt vaccine prevented flu, uncertainty about the right circulating strain being covered and belief prevention strategies, such as hand washing, healthy diet and exercise were more efficacious; 3) *Lack of access*: No problems; 4) *Personal risk*: Perceived themselves healthy with sufficient immunity by "working with sick patients". Fear of needles, allergy to vaccine, being advised against vaccine and media were not reported to impact their beliefs.
COMMENTS: Post survey, we conducted evidence-based education sessions to address the concerns of the nurses. Despite this, immunization demand remained low and was 53% in 2004. We believe peer role modelling and social marketing strategies are needed to enhance personal incentive of the individual HCW to seek vaccination.

P2.32
EPIDEMIOLOGY OF FEBRILE NEUTROPENIA IN CANADA AND PROVINCES: HOSPITALIZATION-BASED PREVALENCE

MC MEILLEUR*, V BERBERIAN
Merck Frosst Canada Ltd, Montreal, Quèbec

OBJECTIVES: To determine the prevalence of hospital-based fungal infections associated with febrile neutropenia (FN) in Canada and to forecast province-specific febrile neutropenia cases with three-year horizon.
METHODS: Using 2001 Canadian hospital discharge data, provincial diagnoses of neutropenia, invasive candidiasis (IC) and invasive aspergillosis (IA) were identified according to international classification of disease coding (neutropenia: ICD-9, ICD-10 codes 288.0, D70-D70.8; IC: ICD-9, ICD-10 codes 1124, 1125, 1128, 11281, 11283, 11284, 11285, 11289, B370, B371, B376, B377, B3780, B3781, B3782, B3783, B3788, B379; IA: ICD-9, ICD-10 codes 117.3, B44-B44.9). As ICD-9 and ICD-10 codes do not report FN cases, literature-based figures were used to identify the share of neutropenic patients that will go on to develop FN. National and provincial population estimates were used to estimate disease prevalence rates and to forecast future disease cases for the 2005 to 2007 period. A subsample analysis of the occurrence of IC and IA was performed among patients with neutropenia as the diagnosis most responsible for a recorded hospitalization stay.
RESULTS: In 2001, it was assessed that 0.055% of Canadians were diagnosed with neutropenia. Provinces demonstrate important variability in neutropenia prevalence rate (0.013% to 0.084%). Based on provincial population estimates and observed prevalence rates, it is expected that close to 18,000 Canadian will be diagnosed annually with neutropenia. More than 7000 patients are expected to experience FN annually in Canada. Among patients with neutropenia as the diagnosis most responsible for a recorded hospitalization stay, prevalence of IC was 1.226% and prevalence of IA of 0.149%, respectively, 68 and 50 times higher than that found in the total Canadian population.
DISCUSSION: FN is a frequent complication among cancer patients undergoing chemotherapy. In light of the Canadian prevalence of the disease and of associated fungal infections, antifungal therapy options adequately addressing the specific needs of this patient population should be available in all Canadian provinces.

P2.33
PHARMACOECONOMIC EVALUATION OF THE TREATMENT OF FEBRILE NEUTROPENIA IN CANADA

MC MEILLEUR*, V BERBERIAN
Merck Frosst Canada Ltd, Montreal, Quèbec

INTRODUCTION AND OBJECTIVES: Pharmacological treatment options for fungal infections have important health and financial consequences for the patient and the health care system. The objective of this analysis is to determine the pharmacoeconomic implications of antifungal treatments in febrile neutropenia (FN) cases from a Canadian perspective.
METHODS: Using clinical outcomes data for all antifungal agents with indication for the empirical treatment of FN in Canada, Canadian drug costs, and estimated burden of treatment-induced nephrotoxicity, the mean cost of treating one episode of febrile neutropenia was assessed for each available therapeutic option. Canadian Institute for Health Information hospital morbidity databases were queried to establish annual FN prevalence and estimate potential savings that could be incurred under different utilization scenarios.
RESULTS: When assessing only direct costs, caspofungin acetate is the most costly agent. When assessing both direct and indirect costs, caspofungin acetate is least expensive agent. Therapy with conventional amphotericin B can cost up to 134% more than with caspofungin.

Summarized results

Mean treatment costs (2003 CDN$)	Caspofungin	Conventional amphotericin B	Liposomal amphotericin B
Direct drug cost	$5,847	$497	$8,446 to $10,250
Indirect cost of nephrotoxicity	$1,245	$16,135	$5,506 to $8,953
Total treatment cost	$7,092	$16,632	$13,952 to $19,203

It is estimated that 7425 Canadians will experience FN annually. Based on occurrence of FN and current utilization of antifungal agents, treatment of all FN cases with caspofungin acetate could generate important savings to the Canadian health care system annually.
DISCUSSION: Due to the significant secondary clinical outcomes of antifungal treatments, important cost differences exist among options for treating FN patients. Compared with cost containment efforts based on direct drug costs, consideration of total costs including patient health outcomes can provide important savings.

P2.34
A PROSPECTIVE EVALUATION OF INFLUENZA VACCINATION AND BURDEN OF ILLNESS IN CHILDCARE CENTRES DURING RESPIRATORY VIRAL SEASON 2004-2005

NM LE SAUX*[1], N BARROWMAN[1,2], D SALISBURY[3], I GABOURY[1,2]
[1]Children's Hospital of Eastern Ontario; [2]Chalmer's Research Group; [3]City of Ottawa Public Health, Ottawa, Ontario

OBJECTIVES: To determine the rate of influenza immunizations and burden of the respiratory illness for children younger than 5 years of age in childcare in the 5th year of the universal influenza immunization program.
METHODS: Parents from 11 city-sponsored childcare centres provided immunization and illness information before and during 8 weeks of the influenza season in 2004 to 2005.
RESULTS: 386 children and 90 childcare workers were surveyed weekly for 8 weeks. 49 (54.4%) of the childcare workers received influenza vaccine. Age was not recorded for 2 children and they were excluded from the analysis.

	Number (%) of children		
	6–17 m, n=20	18–35 m, n=139	36–60 m, n=225
Parents flu vaccine	4 (20%)	52 (37.4%)	105 (46.7%)
Child flu vaccine			
1 dose	2 (10%)	43 (30.9%)	85 (37.8%)
2 doses	0	13 (9.4%)	13 (5.8%)
Average days missed (95% CI)	N/A	4 (2-5.2)	2 (1.6-2.4)
Average days URT symptoms (95% CI)	N/A	14.8 (10–19)	6 (4–8)
% who started antimicrobials	8 (40.0%)	43 (31.0%)	36 (16.0%)

During the 40-day observation period, children 18 to 36 months of age had 1.2 visits/ child to doctors and children 3 to 5 years had approximately 0.4 visits/per child.
CONCLUSION: Influenza immunization rates for preschool children in city-sponsored childcare centres are below the proposed target of 90%. Other measures need to be explored to optimize influenza vaccine uptake in this group.

P2.35
USE OF STANDARD PRECAUTIONS BY MEDICAL LABORATORY TECHNOLOGISTS: A CANADIAN SURVEY

CL MAIN*[1], M JIANG[2], K DAVIS[2], M LOEB[1]
[1]Hamilton Regional Laboratory Medicine Program; [2]McMaster University, Hamilton, Ontario

OBJECTIVES: A pilot study, presented at CACMID in 2004, identified several safety concerns for microbiology laboratory workers. To determine the magnitude of this problem we conducted a national survey of medical laboratory safety practices.
METHODS: A 37 item questionnaire was sent to 1918 medical technologists who are members of the Canadian Society of Medical Laboratory Sciences (CSMLS).
RESULTS: 1341 surveys (70%) were returned. Respondents were from all laboratory disciplines and all provinces. 92% reported working with blood or blood products, 79% on a daily basis. 57% reported having had a needlestick injury at some point in their career and 19% admitted to not reporting all needlestick injuries to their workplace. The most common reason for not reporting was "I did not feel this was a high-risk injury" (43%). Splashes to the eyes or mouth with blood or bodily fluids were less common (28%); however, they were more likely to go unreported (47%). Glove use for every potential blood exposure was 38%. Common reasons for not wearing gloves included interference with job skills (40%), risk of infection is small (31%) and forgetting to wear them (23%). Most respondents work with other bodily fluids (82%) with 59% of these wear gloves for all exposures. Consistent use of goggles or face shields was low when handling blood (12%) or bodily fluids (14%) outside of a biological safety cabinet. Most respondents reported consistent use of sharps containers; however, 12% reported recapping contaminated needles. Hepatitis vaccination was completed by 85%. 13% of responders have been immunized against *Neisseria meningitidis*. Safety training was provided to 55% before beginning work in their current laboratory and 54% receive safety training on an annual basis.
DISCUSSION: This study confirms that there is significant room for improvement in standard precaution use by medical technologists. The results of this study may be used to target safety deficits and provide additional training.

P2.36
COMPARATIVE EVALUATION OF TWO COMMERCIAL NUCLEIC ACID EXTRACTION SYSTEMS FOR CLINICAL DIAGNOSTIC SPECIMENS

GD APPLEYARD*, S WONG, V KHURANA, T STOWE, J FOX
Provincial Laboratory for Public Health, Calgary, Alberta

OBJECTIVE: Rapid and sensitive nucleic acid extraction of clinical specimens is critically important for diagnostic laboratories and versatile automation is becoming a key feature of the equipment needed to process specimens. The objective of the study was to compare the capabilities of two commercial nucleic acid extraction systems, Nuclisens easyMAG (EM) and NucliSens Extractor (NE), using different types of clinically-derived and experimentally-derived samples.
METHODS: Nucleic acid was extracted by both the EM and NE instruments from known positive and negative clinical specimens to compare results: Nucleic acid was extracted from additional negative samples to seek possible cross-contamination problems and false negatives. Quality of nucleic acids produced was evaluated by spectrophotometry. Invalid-run rate, and "down time" was monitored.
RESULTS: Detection limits of each of 8 viral pathogen assays (influenza A and B, parainfluenza, respiratory syncytial virus, adenovirus, human metapneumovirus, enterovirus and norovirus) were similar between the two extractors. Agreement rate was very good, though differences were noted in the extraction of some test kit-specific internal controls. In six months of observation, no contamination events could be attributed to either extractor. The rate of invalid-runs attributable to extraction failures was very low. Small differences in instrument reliability were noted.
CONCLUSION: Both instruments were capable of extracting high quality nucleic acids from a wide variety of clinical specimens (stool, BALs, swabs, serum, plasma, etc). The EM instrument requires less pre-extraction specimen handling thus simplifying molecular procedures for diagnostic and epidemiological studies. The EM instrument can handle more than twice the number of specimens in a day, benefiting the laboratory's capacity to handle both an increasing daily workload, as well as unanticipated surges in testing.

P2.37
EVALUATION OF STARPLEX MULTITRANS MEDIA FOR MAINTAINING VIABILITY OF CLINICAL SAMPLES

R WHEELER*, D HATHAWAY, T HAYES, D JASKOT, J KRAHN, A TIROLESE, R LANNIGAN
London Laboratory Services Group, London, Ontario

OBJECTIVE: In the wake of laboratory centralization and service regionalization, the integrity of transport media(s), an integral part of diagnostic services, has been put to the test. The Starplex S160 transport media was evaluated for its ability to maintain virus viability under various extremes including room temperature storage and expired product conditions.
METHOD: To mimic the conditions that may occur during routine clinical sample submission, viral transport media was spiked with laboratory quality control stock viruses and tested continuously for viability. Various stock virus control material was tested under a range of conditions to establish the efficacy of this transport media. Routine cell line quality control protocol was followed with the exception of spiking transport media with equivalent viral loads and testing for viability over time. Transport media was evaluated at a storage temperature of 4°C and room temperature, as well as in date (long expiry) versus expired media.
RESULTS: Testing was conducted over a period of 34 days with the last inoculation of tissue cultures occurring 30 calendar days after creating the spiked QC material. Our results indicated that the expired media was very

comparable with the in-date lot for keeping the viruses viable with the viral recovery diminishing only slightly, starting at the 14 day mark. A comparison of room temperature to 4°C storage showed identical results for viral recovery until day 27 and only slight percentage decreases for HSV and parainfluenza at that time. As suspected the more fastidious viruses like cytomegalovirus became completely negative first, by day 25, while some conditions for parainfluenza (expired media at room temperature) also became irretrievable before the last day of inoculation. The other viruses HSV and Echo 2 retained a degree of positivity until the experiment was final.

CONCLUSIONS: Sample quality plays an integral part of the diagnostic process in patient care. After proper collection techniques have been implemented, transport media then becomes the backbone of sample integrity. Today, this is becoming increasingly important with the trend to amalgamate smaller lab services into more citywide or regional operations, affecting transportation times as an end result. The Starplex Multitrans Media is able to maintain virus viability over long periods of time and at a range of temperatures.

P2.38
EVALUATION OF COPAN UNIVERSAL TRANSPORT MEDIUM

T STOWE*, K FONSECA
Provincial Laboratory of Public Health, Calgary, Alberta

OBJECTIVE: Remel M5 Transport Media (M5) is stored in a refrigerator until ready for use, which poses a storage problem for the user and the laboratory. It also poses a distribution problem for the laboratory. Copan Diagnostics Inc manufactures a Universal Transport Media (UTM) that can be stored at room temperature for up to one year. The objective of this study was to compare the recovery of clinical viral isolates at various times and temperatures of storage using UTM and M5.

METHODS: The following viruses were grown in tissue cultures, harvested and titrated to determine titre: influenza A/New Caledonia, influenza A/California, influenza B/Shanghai, parainfluenza types 1, 2, 3, respiratory syncytial virus, adenovirus type 1, herpes simplex type 1 and echovirus type 9. Using a flocked swab, three concentrations of each virus were inoculated to two tubes each of UTM and M5. One tube of each medium was stored in the refrigerator and at room temperature for the duration of the sampling. Samples were taken at 1 h, 24 h and again at 96 h, post inoculation of the transport media. Samples for adenovirus, herpes virus and echovirus were inoculated into tube cultures and examined for cytopathic effects for 5 days. The other respiratory viruses were inoculated into plates containing a mixed cell culture. These cultures were examined after 24 h of incubation using a polyclonal fluorescent stain.

RESULTS: All viruses, at the highest concentrations, were recovered after 96 h storage from either media at all temperatures of storage. Samples were also detectable after 96 h from the midconcentrations of the following viruses: influenza A/New Caledonia, influenza B/Shanghai, all three parainfluenza viruses and the adenovirus. The herpes simplex virus was detected at the mid concentration of virus only in M5 at 96 h.

CONCLUSION: Copan's UTM was comparable with Remel's M5 in the recovery of the clinical viral isolates. The temperature of storage, post inoculation, did not affect the recovery of virus from either media. Storing the UTM at room temperature before use is a definite advantage for the user and the laboratory.

P2.39
A CASE OF ACQUIRED RIFAMPIN RESISTANCE IN *MYCOBACTERIUM BOVIS* BACILLUS CALMETTE-GUERIN INDUCED CYSTITIS: NECESSITY FOR TREATMENT GUIDELINES

J N WOLFE[1], M K SHARMA*[1], KS BLACKWOOD-ANTONATION[1], VJ COOK[2]
[1]National Reference Centre for Mycobacteriology, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba; [2]TB Control, BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: We present a case of acquired rifampin resistance in a *Mycobacterium bovis* BCG isolate.

BACKGROUND: We were presented with a case of presumed BCG cystitis in an elderly female patient (with no HIV risk factors) following direct intravesical BCG instillation treatment for papillary transitional cell carcinoma. The patient received discontinuous antimicrobial therapy due to health concerns and rifampin monotherapy was given for a total of three months during the course of treatment.

METHODS: To identify and further speciate this organism, the partial 16S, *gyrB*, major polymorphic tandem repeat (MPTRa) gene sequencing and spoligotyping were performed on the isolate. First-line antimicrobial susceptibility testing was performed in accordance with BACTEC460 methodology and NCCLS guidelines for *M tuberculosis* complex. The partial *rpoB* gene was sequenced to confirm molecular resistance to rifampin and rifabutin.

RESULTS: The organism was isolated from urine samples of the patient and was identified as *Mycobacterium bovis* BCG. Antimicrobial susceptibility testing results indicated that the isolate was sensitive to isoniazid and ethambutol and resistant to pyrazinamide and rifampin. Sequencing of the *rpoB* gene detected a known mutation responsible for conferring high levels of resistance to both rifampin and rifabutin (Ser531Tyr).

DISCUSSION: To our knowledge, this is the first reported case of *M bovis* BCG disease in a non-HIV subject and a second reported case of *M bovis* BCG where the organism had acquired drug resistance to rifampin. This case demonstrates the necessity to re-evaluate appropriate guidelines for effective treatment of BCG disease.

P2.40
IDENTIFICATION OF AN UNKNOWN BACTERIAL PATHOGEN IN A RESPIRATORY ILLNESS OUTBREAK USING BROAD-RANGE 16S RDNA CLONAL ANALYSIS

W MEI*[1], C DE LIMA[1], C HARLTON-STREZOV[1], P TANG[3], F JAMIESON[1], DE LOW[1,2], G BROUKHANSKI[1]
[1]Ontario Central Public Health Laboratory, Toronto; [2]Department of Microbiology, Mount Sinai Hospital, Toronto, Ontario; [3]University of California San Francisco, San Francisco, California

BACKGROUND: The laboratory diagnosis of an unknown respiratory pathogen can be difficult using traditional methods such as culture, DFA and PCR. As an alternative, we employed a simple and rapid genomics-based method to identify bacterial species present in clinical specimens.

METHODS: Clinical specimens were subjected to broad-range 16S rDNA PCR. Resultant amplicons were cloned into suicide vectors and transformed into *E coli*. A subset of the transformants were sequenced.

RESULTS: All of 26 specimens were positive for bacterial 16S rDNA. 60 transformants from 6 specimens were sequenced. A variety of commensal bacteria were identified, but 4 of 7 sequences from one lung specimen matched to a respiratory pathogen, *Legionella pneumophila*. An alignment of the sequences showed 100% nucleotide identity to one another as well as to a NCBI reference strain. These results were confirmed by culture, serology and specific PCR for *L pneumophila*.

CONCLUSION: We demonstrate the use of an unbiased, culture-independent, genomics-based method to identify a potential pathogen within 72 h. This method could be performed in parallel to culture and other diagnostic modalities and could be useful for noncultivatable organisms due to the fastidious nature of the organism or previous antibiotic treatment. With recent advances in cloning and sequencing methodologies, this and other genomics-based strategies should be within the reach of all laboratories.

P2.41
RAPID DETECTION OF NOROVIRUS BASED ON AN AUTOMATED EXTRACTION PROTOCOL AND A REAL-TIME MULTIPLEXED SINGLE-STEP RT-PCR

NA CROZIER*, NA ANTONISHYN, RR MCDONALD, PN LEVETT, GB HORSMAN
Saskatchewan Health, Provincial Laboratory, Regina, Saskatchewan

OBJECTIVE: Molecular diagnosis of norovirus infection can be a complex multistep process, which requires significant user intervention and expertise, and is not amendable to automation without extensive validation and

optimization. The objective was to develop and evaluate a real-time multiplexed RT-PCR assay with automated sample preparation that requires only a single step for reverse transcription, amplification and detection while maintaining or exceeding the clinical sensitivity of conventional PCR for broad spectrum norovirus detection.
METHODS: 46 known positive samples and four known negative samples were used to assess the clinical sensitivity of a two-step versus single-step multiplex real-time R-PCR protocol. A dilution series of norovirus RNA was used to assess the analytical sensitivity of a newly developed real-time multiplexed single-step R-PCR protocol. The clinical sensitivity of the novel TaqMan protocol in conjunction with automated sample preparation using a MagaZorb RNA kit and a KingFisher ML instrument was measured against a validated extraction protocol and conventional R-PCR. One hundred samples were tested using both methods with discordant results resolved by reciprocal testing of the extractions by the other method.
RESULTS: The analytical sensitivity of the real-time assay was reduced by 0.5 logs, as measured with the dilution series, due to the automated RNA extraction protocol. However, the real-time R-PCR single step procedure showed no reduction in the number of positives detected. The novel approach presented here, using clinical specimens, found 14 more positives than manual RNA extractions followed by conventional RT-PCR and agarose gel electrophoresis.
CONCLUSIONS: A semiautomated and simplified molecular diagnostic protocol for the rapid detection of norovirus has been achieved. PCR inhibitors are present in human fecal specimens and have been shown to cause a significant problem for norovirus detection by RT-PCR, such that cleaner RNA preparations translate into better clinical sensitivity.

P2.42
DISPARITY IN NOROVIRUS STRAIN RELATEDNESS WITH SEQUENCES GENERATED FROM POLYMERASE AND CAPSID REGIONS

A MCNABB*, R CHEN, B AUK, L MCINTYRE, J ISAAC RENTON
Laboratory Services, BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: To ascertain the epidemiological relatedness of norovirus strains that caused gastroenteritis outbreaks in British Columbia from 2001 to 2004 by comparing partial polymerase and capsid gene sequences respectively.
METHODS: Amplicons generated by RT-PCR from the partial polymerase B and capsid C regions from norovirus positive samples were sequenced and compared against a heuristic library of catalogued sequevars to determine strain relatedness.
RESULTS: 110 polymerase sequevars and 193 capsid sequevars were detected among the approximately 460 samples examined. Of the 110 polymerase sequevars, only 80 were discriminated by capsid sequences, with as many as 7 polymerase sequevars contained within a single capsid sequevar. Temporal shift in capsid sequence was detected in a prevalent polymerase sequevar (BCCDC 02-007-GII.4 Farmington Hills strain) during a prolonged outbreak within the province during 2002 and 2003.
CONCLUSIONS: Strain relatedness as defined by partial polymerase and capsid sequences vary, with capsid sequencing more discriminatory and temporally less stable. The recently described phenomenon of norovirus chimeras or recombinants were quite common (21 instances based on capsid sequences) in our population, indicating that sequencing of both gene regions is required to obtain complete epidemiological relatedness of Norovirus sequevars within our population.

P2.43
PREVALENCE OF GENOTYPE AND LAMIVUDINE RESISTANCE IN HEPATITIS B VIRUSES IN BRITISH COLUMBIA FROM 2000 TO 2005

A MCNABB*, R CHEN, G MCNABB, M KRAJDEN, J ISAAC RENTON
Laboratory Services, BC Centre for Disease Control, Vancouver, British Columbia

OBJECTIVE: To determine genotype and lamivudine resistance prevalence in selected HBV isolates in British Columbia.
METHODS: Between January 2000 and September 2005, 254 plasma samples were tested for detectable HBV DNA by an in-house nested PCR assay targeting a region of the DNA polymerase gene. All samples with detectable HBV DNA had the amplicon sequenced to determine both virus genotype by comparing the sample's sequence to a database of HBV genotypes downloaded from GenBank and its Lamivudine resistance status determined by detecting the presence of a YVDD or YIDD mutation within the YMDD domain of the DNA polymerase gene.
RESULTS: HBV DNA was detected in 201/254 samples. 2 samples had HBV DNA detected but could not be sequenced. Of the 199 sequenced specimens, 21 (10.5%) were Genotype A, 51 (25.6%) B, 115 (57.8%) C, 9 (4.5%) D, 1 (0.5%) E and 2 (1%) G. Lamivudine resistance was detected in 69 samples (34.6%) with equal distribution between YIDD and YVDD mutations (35 versus 34). The percentage of samples showing detectable lamivudine resistance varied with genotype. Samples demonstrating an YVDD mutation were more likely to have a replication compensatory mutation at codon 180 (100% versus 40%).
CONCLUSIONS: HBV genotype C was the most prevalent genotype detected, followed by genotypes B and A, reflecting BC's large Asian population. Lamivudine resistance was common in our study group (34.6%), with YIDD and YMDD mutations equally distributed. A compensatory replication mutation at codon 180 was more commonly associated with an YVDD mutation.

P2.44
INVESTIGATION OF A MUMPS OUTBREAK IN NOVA SCOTIA USING MOLECULAR METHODS

TF HATCHETTE*[1], JJ PETTIPAS[1], G WATSON-CREED[2], A SAUNDERS [2], KR FORWARD[1]
[1]QE II Health Science Centre; [2]Nova Scotia Department of Health, Halifax, Nova Scotia

OBJECTIVE: An outbreak of mumps was identified in Nova Scotia between August and December 2005. This provided us with the opportunity to evaluate and compare conventional culture and molecular methods for the diagnosis of mumps and subsequently to characterize isolates phylogenetically to facilitate outbreak investigations.
METHODS: Throat and urine specimens were collected from patients identified during the course of the investigation. They were submitted for tissue culture using primary CMK and Vero cells and subjected to a heminested RT-PCR specific for the SH gene. Sensitivity of the PCR assay was assessed by comparing the TCID50 with a dilution series characterized by RT-PCR. Amplicons were sequenced and compared with reference strains from GenBank. Phylogenetic analysis of the patient and reference isolates was done using MegAlign (DNAStar).
RESULTS: During the outbreak period throat and urine specimens from 62 patients were submitted for mumps culture. There were 10 culture confirmed cases of mumps during the outbreak period; however, specimens from the first three cases were unavailable for RT-PCR. Five patients had only throat cultures. All of these specimens were positive for mumps by RT-PCR. Two cases had positive urine and negative throat cultures. Both had mumps identified in the throat swab by RT-PCR. The R-PCR was 100- to 1000-fold more sensitive than culture.
There were two separate epidemiologically linked clusters (3 and 5 cases, respectively). Although there was no clear epidemiological link between these two clusters, phylogenetic analysis suggested they are all related and belonged to genotype G5. Two other cases without clear epidemiological links belonged to different subgroups (G1 and G2).
CONCLUSION: RT-PCR specific for the SH gene is more sensitive than culture and provided sequence data which enabled public health investigators to differentiate sporadic cases of mumps infection from outbreak related cases.

P2.45
RAPID PAN-RESPIRATORY PATHOGEN SCREENING OF NASOPHARYNGEAL WASHES BY GENACO RESPIRATORY INFECTION PANELS I AND II

J BRUNSTEIN*, E THOMAS
[1]CWHCBC; [2]CTAG, Vancouver, British Columbia

OBJECTIVE: Multiple respiratory pathogens of widely varying clinical and public health significance present with nearly identical symptoms. Rapid identification of the causative agent(s) is critical to effective patient care. We report here on a pilot validation of two newly available highly multiplexed molecular assays which employ liquid-phase bead-based array (xMap/Luminex) technology for the rapid determination of a wide spectrum of common respiratory pathogens in routine clinical nasopharyngeal wash specimens.
METHODS: Nucleic acids were extracted from patient nasopharyngeal wash specimens obtained and previously classified by DFA methods through our laboratory's VIRAP program. Aliquots of each extract were analyzed through the use of the Genaco Respiratory Panels I and II. A total of 21 common respiratory pathogens were screened for in each specimen by this method.
RESULTS: For 15 out of 16 specimens representing two each previously identified as positive for influenza A, influenza B, RSV A, RSV B, human metapneumovirus, PIV 1, PIV 3 and adenovirus, there was agreement between results of the Genaco panels and our prior classification. In the one discordant case, additional testing confirmed the results of the Genaco panels. As the Genaco panels we employed did not include a PIV 2 detector, we also tested two known PIV 2 positive specimens to examine cross reactivity and false positive rates for the assay. These samples showed no signal above those in blank background controls. Interestingly, approximately 20% of the specimens showed evidence for coinfections which were of a clinically relevant and plausible nature.
CONCLUSION: Overall, we found the Genaco panels to be 100% accurate in our test with no evidence for false positives or negatives. The broad spectrum of pathogens simultaneously detected by this assay lends itself to use in clinical situations where rapid discrimination between multiple pathogens with similar presentation is essential to effective infection control and patient management.

P2.46
COMPARATIVE STUDY OF DIRECT FLUORESCENT ANTIBODY AND SHELL-VIAL CULTURE FOR DETECTION OF INFLUENZA A AND B IN RESPIRATORY SPECIMENS DURING THE OUTBREAKS OF INFLUENZA

A BELHAJ*, S CASTRICIANO, M GRIFFITHS-TURNER, R STEVENSON, CH LEE
McMaster University, Hamilton, Ontario

INTRODUCTION: Influenza viruses are orthomyxoviruses of three types A, B and C. The current laboratory method of diagnosis in our virology laboratory is to perform direct florescent antibody (DFA) testing and if DFA is negative then to do shell-vial culture.
OBJECTIVES: To determine the test performance characteristics of DFA for detection of influenza A and B.
METHODS: During December 2004 and April 2005, there were two influenza-related respiratory outbreaks at our tertiary 400-bed hospital. This coincided with other health care facilities and community-related influenza outbreaks. We evaluated 108 patients who had laboratory confirmed diagnosis of influenza A or B by DFA or shell-vial culture.

Age	<1	1-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	>90
n	6	14	2	4	2	12	7	9	23	25	4

Gender ratio (females:males) 66:52. Influenza A:Influenza B ratio 78:30. All 108 specimens were tested by DFA followed by culture.
RESULTS: Total number of DFA negative but culture positive 19/108 (20.52%) DFA: sensitivity 82.4%; specificity 100%. Vaccinated patients who presented with fever 29 out of 48 (60%) and 36/60 (60%) nonvaccinated had fever. DFA negative but culture positive in vaccinated patients with no fever 1 out of 9
CONCLUSIONS: DFA has sensitivity of 82.4% and specificity of 100% compared to shell vial culture If DFA is positive, it is not necessary to confirm the result with culture; however, given sensitivity of 82.4%, negative DFA should be confirmed by culture.

P2.47
COMPLETE GENOMIC ANALYSIS OF 18 VARICELLA-ZOSTER VIRUS ISOLATES: INSIGHT INTO MECHANISM OF ATTENUATION AND GENOTYPING STRATEGIES

GA PETERS[1], SD TYLER[1], C GROSE[3], A SEVERINI[1], MJ GRAY[1], R GARCEAU[4], V TCHEREPANOV[5], C UPTON[5], GA TIPPLES*[1]
[1]National Microbiology Laboratory; [2]University of Manitoba, Winnipeg, Manitoba; [3]University of Iowa, Iowa City, Iowa, USA; [4]L'Hôpital Régional Dr GL Dumont, Moncton, New Brunswick; [5]University of Victoria, Victoria, British Columbia

OBJECTIVES: Long considered one of the most genetically stable of the herpes viruses, varicella-zoster virus (VZV) has been found to be less immutable than previously thought. To better understand the mechanism of attenuation of the vaccine VZV strain(s), and to establish a useful genotyping system, we have completely sequenced 13 wild-type VZV strains and have analyzed them along with 5 VZV strains reported in GenBank.
METHODS: The 13 strains we sequenced were all North American in origin with 3 of the isolates representing early (5), mid (22) and late (72) passages in cell culture of the same strain (VZV-32). Two of our sequenced strains are the MSP and BC glycoprotein E mutant strains. These were compared with the 5 other complete genomes currently in GenBank (Dumas, pOka, vOka, Varilrix, Varivax).
RESULTS: Polymorphisms were identified in numerous locations throughout the genomes but all strains retained a high degree of identity (approximately 99.8%) and all resemble the Dumas prototype, approximately 125,000 bp in size. As with the Japanese Oka strains, the North American reiteration regions were found to be variable within a given strain. We examined polymorphisms that developed in VZV-32 as it was passaged in cell culture. Surprisingly 13 polymorphisms accumulated in IE62 between the early and late passages, 4 noncoding and 9 coding. Among the most intriguing were S628G, R958G and I1260V, which were shared with vOka. Phylogenetic analyses using the whole genomes, 5 glycoproteins plus IE62, and the ORI region gave similar clustering into 4 distinct clades.
CONCLUSIONS: Full genome sequencing is the definitive method by which to define genomic differences amongst the VZV wild-type and vaccine strains. The similarities in the IE62 S628G, R958G and I1260V polymorphisms and their correlation with vaccine strains suggest a role in attenuation. Clade-specific polymorphisms and regions for genotyping utility have been identified.

P2.48
COMPARISON OF THE ARTUS EBV PCR KIT WITH THE LIGHTCYCLER – EBV QUANTIFICATION KIT (ROCHE) FOR QUANTITATION OF EBV IN WHOLE BLOOD SAMPLES

AK PETRICH*[1], S CASTRICIANO[1], G MOUSSA[2], T MAZZULLI[2]
[1]St Joseph's Healthcare, Hamilton; [2]Mount Sinai Hospital, Toronto, Ontario

OBJECTIVES: 1. To evaluate the performance of the artus EBV PCR kit with the LightCycler – EBV Quantification Kit (Roche) for Quantitation of EBV in Whole Blood. 2. To compare two DNA extraction methods for EBV quantitation using the artus EBV PCR kit. 3. To examine the reproducibility of the artus EBV PCR kit with Qiagen extracted DNA.
METHODS: One hundred four whole blood samples sent for EBV quantitation were tested using the QIAamp mini DNA kit for extraction (200 μL) followed by real-time amplification with the artus EBV PCR kit according to manufacturer's specification. The remainder of the whole blood samples was frozen at –80°C. An aliquot of the thawed blood was extracted using the miniMag (bioMérieux) method and tested with both the artus EBV

PCR kit with the LightCycler – EBV Quantification Kit. To evaluate reproducibility, the Qiagen extracted sample was tested a second time at a second site using the artus EBV PCR kit.
RESULTS: Fifty-one samples extracted by miniMag were positive by both the Artus EBV PCR kit and the LightCycler – EBV Quantification Kit. An additional 17 samples were positive in the Roche assay only. Five of these were positive only in the Roche assay and on retesting were found to be negative, suggesting contamination. When the Qiagen and miniMag extracted DNA was tested by the artus EBV assay, the Qiagen extracted material had 78 positive samples and the miniMag had 56. 76 of the 78 positive confirmed positive. The Qiagen extracted material was tested at two different sites using the Artus kit and showed excellent reproducibility with 76 samples positive and 28 samples negative at both sites. The remaining 4 samples were low-level positives with 72 EBV copies/mL to 1739 EBV copies/mL.
CONCLUSIONS: The artus EBV PCR kit and the LightCycler – EBV Quantification Kit are both reliable methods for the quantitation of EBV in whole blood. The Qiagen spin columns appear optimal for extraction of whole blood for EBV quantitative evaluation using the artus assay.

P2.49
COMPARATIVE MOLECULAR PCR-RFLP STUDY OF NATIVE HERPES SIMPLEX VIRUS TYPE 1 (HSV-1) WITH KOS STRAIN

N ABOLHASSANI*[1], M SADEGHI ZADEH[2]
[1]Islamic Azad University, Saveh, Iran; [2]Tarbiat Modares University, Tehran, Iran

OBJECTIVES: Herpes simplex virus (HSV) genome has been fully sequenced and consists of two covalently linked components, designated as L (long) and S (short). Genome analysis by restriction enzymes is used to differentiate types 1 and 2 of the virus and even strains of each type. The HSV gene layout for UL was found to be very similar to that for the corresponding part of the genome of varicella-zoster virus (VZV). As recent research on several DNA fragments covering open reading frames (ORFs) of 1 to 37 shows mutations in VZV strains, we used UL29 gene which is one of seven essential genes for DNA replication to find mutations or a genetic marker in HSV-1. In addition, previous studies using PCR sequencing technique have shown that the thymidine kinase (TK) gene of HSV 1 is polymorphic. In this study, thymidine kinase gene and UL29 gene of HSV 1 were selected. Both genes were analyzed with restriction endonucleases to compare Iranian strains of HSV 1 with KOS foreign strain.
METHODS: Three isolates of HSV 1 as well as standard strain of KOS were propagated in Vero cells. Initially, a pair of specific primers for each gene was designed to amplify UL29 and TK genes of these isolates. Subsequently, PCR products of these genes were digested separately with 5 restriction enzymes and analyzed by polyacrylamide gel electrophoresis.
RESULTS: Results indicate that the patterns of restriction endonuclease digestion of UL29 and TK genes of the 3 isolates show no differences when compared to KOS strain.
DISCUSSION: Probably the genotype of Iranian isolates are the same as KOS genotype and both genotypes may be derived from a common ancestor.