P4-S1.04 11C-CHOLINE SMALL ANIMAL PET IN EXPERIMENTAL CHLAMYDIA MURIDARUM INFECTION

doi:10.1136/sextrans-2011-050108.506

¹A Marangoni, ²C Nanni, ²C Quarta, ¹R Aldini, ¹M Donati, ¹P Nardini, ¹C Foschi, ²S Fanti, ¹R Cevenini. ¹University of Bologna, Bologna, Italy; ²Azienda Ospedaliero-Universitaria di Bologna, Policlinico S.Orsola-Malpighi, Italy

Objectives Genital tract infection of female mice with *Chlamydia muridarum* closely mimics *C trachomatis* acute genital tract infection in women. Aim of this study was to assess the predictivity of 11C-Choline Small Animal PET for the identification of reactive animals and their follow-up at several times after vaginal challenge.

Methods Chlamydiae were grown in LLC-MK2 cells, cultivated in Eagle's MEM and elementary bodies were purified from cells by sucrose-gradients density centrifugation. Animals used in this study were 10 female Balb/c mice, 6–8 weeks old. All the animals received 2.5 mg of medroxyprogesterone acetate intramuscular nine and 2 days prior the infection. Nine mice were infected by placing 106 IFUs of *C muridarum* into the vaginal vault. Infection was induced under Ketamine anaesthesia. As control, one animal was challenged with SPG. At 5, 10 and 20 days after challenge mice underwent a 11C-Choline PET, as follows: each animal was anaesthetised with Sevoflurane 3–5% oxygen 1 l/min and was injected with approximately 20 MBq of 11C-Choline, via the tail vein. The residual dose was measured to verify the effective dose injected. The animal was subsequently placed on the scanner bed in the prone position, after an uptake time of 5 min. Images were acquired with a small animal PET tomograph (GE, eXplore Vista DR) for a total acquisition time of 20 min. As the axial field of view was 4 cm, one bed position was sufficient to cover the whole body. Images were reconstructed iteratively (OSEM 2D) and read in three planes (axial, sagittal and coronal). The scan was considered positive if areas of increased Choline uptake were present at sites consistent with the site of inflammation. The standard uptake value (SUV) was calculated by measuring the concentration of the labelled tracer in the region of interest and correcting it for mice body weight and injected dose. Infection was monitored by culturing cervical-vaginal secretions 6, 11 and 21 days after infection.

Results At 5 days, the mean vaginal SUV ($\pm$ SD) of the cases was 0.83 ± 0.39 , whereas the control showed a value of 0.278. At 10 and 20 days, SUVs of the cases were 0.94 ± 0.25 and 1.17 ± 0.21 , respectively, whereas SUV of the control was 0.299 and 0.302, respectively. *C muridarum* was isolated from all the infected animals during the study period.

Conclusions These preliminary data indicate that 11C-Choline PET could be a promising technique to in vivo evaluate inflammatory response to Chlamydia infection.

P4-S1.05 POLYMERASE CHAIN REACTION-BASED TYPING OF PEN A GENES EXHIBITING ELEVATED MIC VALUES TO CEPHALOSPORINS IN ISOLATES OF NEISSERIA GONORRHOEAE

doi:10.1136/sextrans-2011-050108.507

¹D Trees, ²M Burroughs, ²S Harris, ²S Johnson. ¹Centers for Disease Control and Prevention, Atlanta, USA; ²CDC, USA

Objective Develop a PCR based assays for rapid analysis of isolates that exhibit elevated MIC values to penicillin and cephalosporins (MICs of 0.015–0.06 μ g/ml to ceftriaxone). These primers were designed to detect mosaic and nonmosaic sequences in the penA gene.

Methods Two sets of primer pairs were generated that allowed the detection of mosaic-type and nonmosaic-type penA gene sequences in isolates of *N gonorrhoeae* that exhibited elevated MIC values to

three cephalosporins. Additional primer sets were generated that detected the presence of an inserted aspartic acid residue in nonmosaic penA genes involved in resistance and to detect base substitutions to distinguish between two different mosaic penA genes. DNA sequences were determined as necessary by standard sequencing or pyrosequencing.

Results PCR analysis of 28 isolates, exhibiting elevated MIC values to cephalosporins and from different geographic areas, that used these primer sets in combination allowed for the detection of mosaic penA genes in 12 isolates. The remaining isolates possessed nonmosaic genes. Not all of the 12 isolates contained complete mosaic-type sequences with five of the isolates indicating mosaic sequences present in the 5' region of the penA gene with wild type DNA sequence replacing the last 120 bp of the gene. Another isolate contained nonmosaic penA sequences with an aspartic acid insertion (after aa 345) in the 5' region of the gene but mosaic sequences in the 3' end. Finally, mosaic-related sequences were detected by PCR and confirmed by DNA sequencing in the last 106 base pairs of the penA gene of a penicillin sensitive strain isolated prior to 1976.

Conclusion PCR can be used to easily detect the presence of mosaic penA genes in isolates of *N gonorrhoeae*. This study has shown the 3' region of the gene may contain mosaic, partial mosaic and nonmosaic-type sequences and that mosaic-type sequence has been encountered in the 3' region of penA in a sensitive strain. We have also observed isolates with increased MICs to cephalosporins that do not contain mosaic sequences. Finally, isolates that possess mosaic penA genes have been detected in dispersed geographic regions in the US, which is of concern due to the evidence mosaic-type penA alleles are involved in the process of increasing gonococcal MICs to cephalosporins.

P4-S1.06 HIGH-RESOLUTION MULTILOCUS SEQUENCE TYPING (MLST) OF CHLAMYDIA TRACHOMATIS FROM THREE CATCHMENT AREAS IN NORTH AND CENTRAL NORWAY

doi:10.1136/sextrans-2011-050108.508

¹B Herrmann, ¹L Christerson, ²K Gravningen, ¹A Stahlsten, ¹K Ödman, ¹D Nguyen, ¹J Isaksson, ²A S Furberg, ³G S Simonsen. ¹Uppsala University, Uppsala, Sweden; ²University of Tromsø, Tromsø, Norway; ³University Hospital of North Norway, Tromsø, Norway

Background The *C trachomatis* incidence rate in the county of Finnmark in North Norway is twice as high as in the rest of the country. This study genetically characterises the *C trachomatis* strains in Finnmark and compares them with strains from two other counties in Norway.

Methods DNA sequencing of ompA, which encodes the major outer membrane protein MOMP, and a high-resolution multilocus sequence typing (MLST) system, which is based on PCR amplification and DNA sequencing of five highly variable genetic regions (not housekeeping genes as in conventional MLST), were used. In total, 80 specimens from adolescents mainly aged 15–19 were collected in five high schools (n=60) and in the laboratory routine (n=20) in Finnmark (extended county with sparse population in minor municipalities) and compared to 80 laboratory specimens from Tromsø county (capital of North Norway) and 88 laboratory specimens from Trondheim in Central Norway.

Results Sequencing of ompA detected 11 genotypes in these 248 specimens. The MLST analysis displayed 50 sequence types (STs), providing five times higher resolution than ompA. Thirty-one (62%) of the STs were novel. The study revealed overlapping STs between the different counties, but 64% of the STs were unique for specific counties (Abstract P4-S1.06 figure 1). The MLST genetic diversity was lower in Finnmark (0.26 ST per specimen compared to 0.34 in Tromsø and 0.32 in Trondheim, respectively), but the geographical

Abstract P4-S1.06 Figure 1 Distribution of sequence types (n=50) of 248 *C trachomatis* cases in the counties of Finnmark (n=80), Tromsø (n=80), and Trondheim (n=88).

differences were not statistically significant. The common ompA genovar E predominated in Tromsø and Trondheim (55%) whereas genovar G (36%) was most frequent in Finnmark.

Conclusions Our MLST provided a significantly higher differentiation capacity than conventional ompA typing, which has predominated genotyping of *C trachomatis* for decades. Thus high-resolution MLST is a useful tool for molecular epidemiology of chlamydial infections.

P4-S1.07 CHLAMYDIA TRACHOMATIS DNA STABILITY INDEPENDENT OF PRESERVATION TEMPERATURE, TYPE OF MEDIUM EN STORAGE DURATION

doi:10.1136/sextrans-2011-050108.509

¹L van Dommelen, ²F H van Tiel, ³N H Dukers, ²P F Wolffs, ²S B Herengreen, ³C J P A Hoebe. ¹PAMM, Veldhoven, Netherlands; ²Maastricht University Medical Centre, Netherlands; ³South Limburg Public Health Service, Netherlands

Background In almost all diagnostic studies, stored samples are used. This is also the case in *Chlamydia trachomatis* research. The performance of diagnostic assays, such as nucleic acid amplification tests, is dependent on the preservation of *C trachomatis* DNA. However, the influence of different storage conditions on *C trachomatis* DNA has not been thoroughly explored yet. Therefore, we have studied the impact of temperature, type of medium and duration of storage on the preservation of *C trachomatis* DNA.

Methods Phosphate buffered saline (PBS), 2-sucrose-phosphate medium (2-SP), COBAS Amplicor medium and urine samples were spiked with *C trachomatis* serovar D elementary bodies and stored at room temperature, 4°C, -20°C and -80°C. DNA was isolated using the Qiagen DNA mini kit (Qiagen GmbH, Hilden, Germany). Samples were tested in triplicate with a TaqMan PCR at day 0, 1, 7, 14 and 30. Furthermore, clinical *C trachomatis* positive urine samples were collected and pooled, and stored and tested in triplicate, at the same temperatures and time intervals. This same procedure was executed with pooled *C trachomatis* positive swab samples.

Results *C trachomatis* was detected in all samples, irrespective of the different storage temperature conditions, different types of medium

and the different durations of storage. Regarding storage time, the cycle threshold (ct) overall did not increase, in fact it tended to decrease within 30 days. If there was an increase in ct over time, this occurred in <10% of the experiments, it did not outreach three (corresponding to a decline in load of 1 log). This mainly occurred in the spiked COBAS samples which were frozen.

Conclusions All samples tested positive for *C trachomatis* in our experiments, with on average no decrease in cycle threshold over time. We can therefore conclude that PBS, 2-SP, COBAS Amplicor medium and urine are all excellent media to preserve *C trachomatis* DNA for a longer period of time, independent of storage temperature.

P4-S1.08 PERFORMANCE COMPARISON OF NEISSERIA GONORRHOEA (NG) SEQUENCE TYPING METHODS NG-MAST AND NG-MLVA ON A DEFINED PANEL OF ISOLATES

doi:10.1136/sextrans-2011-050108.510

¹R Heymans, ²D Golparian, ¹S Bruisten, ²M Unemo. ¹Health Service of Amsterdam (GGD), Amsterdam, Netherlands; ²Örebro University Hospital, Sweden

Background The increase in *N gonorrhoeae* (NG) prevalence since the mid-1990s, increasing antibiotic resistance, and the absence of an effective vaccine, demand more efficient national and international surveillance of the NG epidemiology. Accordingly, objective, robust and discriminatory genotyping is a crucial tool. The performance characteristics of the internationally frequently used NG multi-antigen sequence typing (NG-MAST) and the recently developed multi-locus variable-number tandem repeat analysis (NG-MLVA), were compared.

Methods From 2002 to 2003, NG isolates (n=330) were obtained from multiple anatomical locations from the same patient (n=118) and from self reported sexual contacts (54 couples). These isolates, were genotyped using NG-MAST, by sequencing of more polymorphic segments of porB and tpbB, and by NG-MLVA, which examines polymorphisms in five different tandem repeat loci.

Results From the 118 patients with multiple isolates per visit, 97 (82.2%) had identical NG-MAST STs. Of these 97, 68 patients (57.6%) had isolates with identical MLVA types, 22 patients (18.6%) had single-locus variants (SLVs), six patients (5.1%) had double-locus variants (DLVs), and one patient (0.8%) had a multilocus variant. Of the isolates of 13 patients (11.0%) with non-identical NG-MAST STs, nine patients had identical MLVA types, three patients had SLVs, and one patient had a DLV. For the isolates of the remaining eight patients (6.8%), both typing methods identified divergent genotypes. So for 76 patients (64%) fully concordant clustering was obtained. Considering a SLV as an identical MLVA type the concordance between the two techniques increased to 83%. From 44 of the 54 couples (81.5%), the isolates of the patient and the contact had identical NG-MAST STs, while there were 34 MLVA couples (63.0%) with identical MLVA types, seven couples (13.0%) with SLVs, and three couples (5.6%) with DLVs. The isolates of 6 couples (11.1%) had identical MLVA types with different NG-MAST STs. In the isolates of four couples (7.4%) both typing methods identified divergent genotypes. So for 45 couples (83%) the two typing techniques were concordant.

Conclusions For efficient international surveillance of gonorrhoea transmission, objective, highly discriminatory, but stable genotyping is essential. Both NG-MAST and NG-MLVA displayed a high resolution and are sequence-based portable genotyping methods. They can either be used singly or in combination, depending on epidemiological questions.