P4-S3.04 TREPONEMA PALLIDUM MOLECULAR TYPING IN CHINA

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Background Syphilis is resurgent in China, but there have been limited data on molecular epidemiology. A novel *Treponema Pallidum* (*T pallidum*) typing method that uses tp0548 gene in addition to arp and tpr E, G, J genes with greater discriminatory power has recently been developed. This study aimed to analyse *T pallidum* strain types across geographical areas in China using this novel method.

Methods From 2008 to 2010, genital specimens, including those from chancres, condyloma lata and mucosal patches, were collected from patients with clinically suspected primary or secondary syphilis in STI clinics in East China (Nanjing), South China (Guangzhou, Jiangmen and Fuzhou), Southwest China (Nanning and Chengdu), North China (Tianjin), and Northeast China (Harbin). All specimens were first amplified by PCR of polA gene to screen for positive DNA, followed by analysis of arp, tpr E,G, J and tp0548 genes for complete strain type. A χ^2 test was used to compare the distribution of strain types across the 5 geographical areas.

Results Typeable *T pallidum* DNA was detected in 184 of 401 specimens, and 27 strain types were identified. Overall, 3 to 20 repeats (except 4, 11, and 19 repeats) and 25 repeats were found for the 60-bp arp gene. This was the first time 9 and 25 repeats for the arp gene have been detected. For the RFLP pattern of the tpr E, G, J genes, a, d, h, j and l were identified. For the sequence pattern of the tp0548 gene, c, e and f were identified. The distribution of strain types was significantly different across the geographical areas of China (χ^2=29.2, p=0.008), but type 14d/f was most predominant within each geographical area (approximately 40% constituent ratio in each area). Taking all geographical areas into account, types 15d/f, 13d/f, 16d/f, and 14a/f were the next most common in descending order see Abstract P4-S3.04 figure 1.

Conclusions There is substantial genetic diversity of *T pallidum* in China. However, the overall predominance of a single 14d/f strain type may imply a potential link in sexual transmission of syphilis across geographical areas.

P4-S3.05 CLINICAL TITRES AND STABILITY OF *TRICHOMONAS VAGINALIS* RNA IN URINE SPECIMENS

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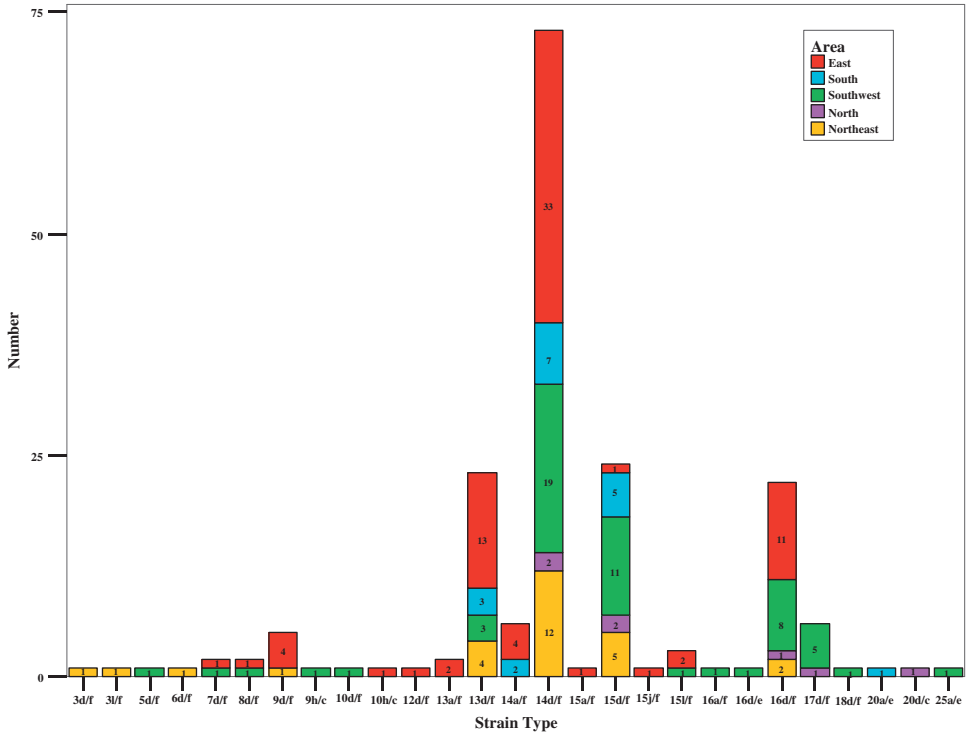
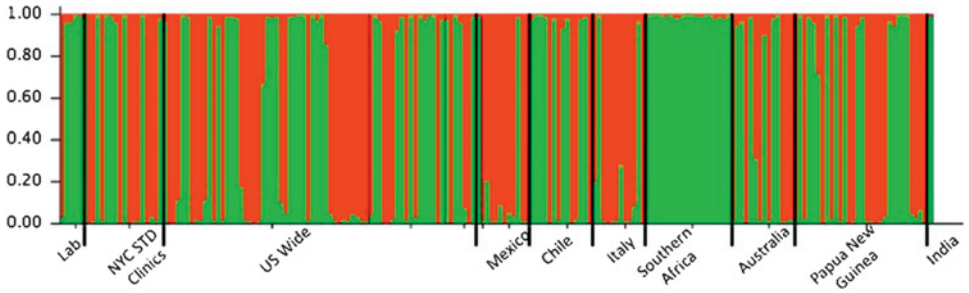
Background This study estimated the range of concentrations of *Trichomonas vaginalis* (TV) present in naturally infected female urine specimens and evaluated the ability of the APTIMA *Trichomonas vaginalis* (ATV, Gen-Probe Incorporated) Assay to detect a clinically-relevant amount of TV cells spiked into TV-negative male and female urine samples and stored at various temperatures.

Methods Female urine samples collected as part of a prospective, multicenter US clinical trial were tested with the ATV Assay, a nucleic acid amplification test for the diagnosis of TV infection in asymptomatic and symptomatic women. To determine TV cell titres, serial dilutions of TV-positive urine samples were tested and results were compared to results from serial dilutions of a laboratory culture of TV with a known cell titre. To assess the stability of TV cells in urine samples, 10 male and 10 female urine samples from non-infected volunteer donors were spiked with a cultured strain of TV, stored at 4°C, 20°C and 30°C, and tested daily for up to 14 days with the ATV assay.

Results Of 39 randomly selected TV-positive female urine samples, the median titre in unprocessed samples was 311 cells/ml (mean=2040 cells/ml; SD=4765 cells/ml), with a range of 2–28 430 cells/ml. Of these 39, 87.2% (34/39) had a TV cell titre of ≥20 cells/ml in neat urine see Abstract P4-S3.05 figure 1. To assess stability of TV cells in urine, freshly collected male and female urine samples from volunteer donors were spiked with TV to 20 cells/ml (4 cells/reaction in the ATV assay), stored at various temperatures, and then tested with the ATV assay. For samples stored at 4°C, the ATV assay was 100% reactive for both male and female samples after 14 days of storage. For samples stored at



Abstract P4-S3.04 Figure 1 Strain types of *T pallidum* identified in clinical specimens from five geographical areas in China.



Abstract P4-S3.05 Figure 1 Two-phylotype population structure of global *Trichomonas vaginalis* isolates.

20°C, male samples were 100% reactive after 7 days storage, while female samples were 100% reactive after 6 days of storage. For samples stored at 30°C, 100% reactivity was obtained at 2 days storage for male samples and 4 days storage for female samples.

Conclusions This study shows urine samples from women infected with TV have a wide range of cell titres, with an average of ~2000 TV cells/ml. TV can be detected for 14 days when stored refrigerated or for about 1 week at 20°C. The use of sensitive, automated molecular tests such as the ATV assay for testing urine samples should facilitate screening for TV.

P4-S3.06 STUDY ON EARLY INFECTION OF THE LOW GENITAL TRACT OF FEMALE MICE BY DIFFERENT SEROTYPES *UREAPLASMA UREALYTICUM*

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Objective To observe the early inflammatory response by animal model with low genital tract Uu infection for investigating the relationship between the serovars and their pathogenicity.

Methods Fifty 8–10-week-old, female BALB/c mice were randomly divided into five groups, namely, control group, serovar 1 group, serovar 3 group, serovar 4 group, and serovar 8 group, and then treated respectively with vaginal inoculation of liquid medium and Uu inoculae of serovars 1, 3, 4 and 8 after subcutaneous injection of oestradiol benzoate. The vulval symptoms and vaginal and cervical swabs were observed weekly after Uu inoculation. Four weeks later, all mice were sacrificed and their cervixes and vaginae were removed for histopathology.

Results There was a significant difference in the inflammation severity of cervical mucosa ($p<0.001$–0.05) except vaginal mucosa among all groups. The inflammation severity of cervical mucosa in serovar 4 and 8 infection was superior to that in serovar 1 infection ($p<0.01$–0.05), but there was no statistical difference among other groups ($p>0.05$).

Conclusions The results suggest that Uu could colonise and infect the cervical columnar epithelium other than vaginal squamous epithelium. Uu-induced inflammation response is different among its serovars. Serovars 4 and 8 are more virulent compared with biovar 1 such as serovar 1.

P4-S3.07 POPULATION GENOMICS OF *TRICHOMONAS VAGINALIS* REVEALS A GLOBALLY DISTRIBUTED TWO-PHYLOTYPE POPULATION STRUCTURE

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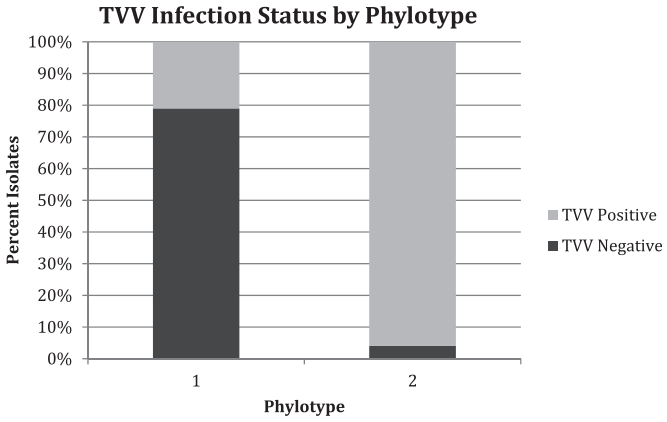
Objective *Trichomonas vaginalis*, the causative agent of human trichomoniasis, is the most prevalent non-viral sexually transmitted infection and has been associated with increased risk of HIV transmission, making detection and treatment a global health priority. In this study, we evaluate the population genomics of globally distributed clinical isolates to characterise genetic diversity and identify population structure.

Methods We use a panel of 21 microsatellite and three single copy gene markers to evaluate the population genomics of 18 clinical isolates collected from female patients attending New York City STD clinics in 2008, as well as 177 extant isolates collected from the USA, Mexico, Chile, Italy, Southern Africa, Papua New Guinea, and Australia. We use a panel of population genetic tools including Arlequin 3.11 and FSTAT to calculate expected heterozygosity (HE) and population differentiation (FST) statistics. To infer population structure, we use STRUCTURE 2.2, Network 4.516, and SeaView 4.2.4. We test for significance in clinical and demographic variables (χ^2 and t-tests) using JMP Genomics 5.0.

Results We detect significant genetic diversity within the species (HE=0.66) that is observed across global regions (range 0.52–0.67), and find that a two-phylotype population structure is maintained globally with few instances in population differentiation defined by geographical origin. This two-phylotype structure is further supported by minimum spanning networks, hierarchical clustering and phylogenies inferred from three single copy genes. These two phylotypes appear at nearly equal frequencies globally. Defining characteristics of the phylotypes include significantly different prevalence of *T vaginalis* virus infection (3% in phylotype 1 and 73% of phylotype 2 ($p<0.0001$, χ^2)) see Abstract P4-S3.07 figure 1 and a significantly higher minimum inhibitory concentration of metronidazole in phylotype 1 ($p=0.0024$, χ^2 Wilcoxon Rank Sums).

Conclusions We detect clear population structure and high levels of genetic diversity from global *T vaginalis* isolates. This preliminary data



Abstract P4-S3.07 Figure 1 TVV infection status by phylotype.