P1.011 WHOLE GENOME SEQUENCE OF THE TREPONEMA PALLIDUM SSP. ENDEMICUM, STRAIN BOSNIA A

doi:10.1136/sextrans-2013-051184.0232

[1]B Staudova, [1]M Strouhal, [1]M Zobanikova, [1]D Cejkova, [2]L Giacani, [2]A Centurion-Lara, [3]L L Fulton, [3]L Chen, [3]G M Weinstock, [1]**D Smajs**. *[1]Masaryk University Brno, Brno, Czech Republic; [2]University of Washington, Seattle, WA, United States; [3]Washington University in St. Louis, Saint Louis, MO, United States*

Background *Treponema pallidum* ssp. *endemicum* (TEN) is the causative agent of endemic syphilis (bejel). The TEN Bosnia A strain was isolated in 1950 from a patient's penile lesion in northeastern Bosnia.

Methods To define genetic differences between TEN Bosnia A and other pathogenic treponemes including the agents of syphilis (*T. pallidum* ssp. *pallidum*, TPA) and yaws (*T. pallidum* ssp. *pertenue*, TPE), a high quality sequence of the Bosnia A genome was determined using 454-pyrosequencing, Illumina, SOLiD and traditional Sanger sequencing. Combined average coverage of these sequencing methods was greater than 340x.

Results Compared to other TPA and TPE treponemes, the genome of Bosnia A (1,137,653 bp) was smaller in size (~2 kb) but structurally almost identical to other TPA and TPE strains. The Bosnia A genome clustered with TPE strains (nucleotide identity excluding indels ranged between 99.91 – 99.94%) while TPA strains were more distantly related (99.79 – 99.82%). More than 400 Bosnia A-specific nt changes (i.e. sequences different from TPA and TEN genomes) were found as the result of our analysis.

Conclusions The Bosnia A genome showed similar genetic characteristics as other TPA and TPE strains. Genetic differences found between TPA strains and Bosnia A genome could be used for identification of potential virulence factors of syphis treponemes. Moreover, genetic changes specific for Bosnia A genome could help develop molecular diagnostic tests for endemic syphilis.

P1.012 URINARY CYTOKINE PROFILES IN NON-SPECIFIC, MYCOPLASMA GENITALIUM AND CHLAMYDIA TRACHOMATIS URETHRITIS

doi:10.1136/sextrans-2013-051184.0233

[1]**M J Pond**, [1]A V Nori, [2]Y Hou, [3]S Patel, [1]K G Laing, [1]R L Allen, [3]P Hay, [1]P D Butcher, [1,3]S T Sadiq. *[1]Centre for infection and immunity, Division of Clinical Sciences, St George's University of London, London, UK; [2]Division of Biomedical Sciences, St George's University of London, London, UK; [3]Department of Genitourinary & HIV Medicine, St George's Healthcare NHS Trust, London, UK*

Background Previously, we demonstrated that urinary white cell count increases in proportion to pathogen load in cases of urethritis caused by *Mycoplasma genitalium* but not *Chlamydia trachomatis*. We further investigated urethritis pathogenesis caused by these organisms by comparing concentrations of 23 cytokines present within first void urine (FVU) specimens of male urethritis cases.

Methods FVUs from 52 symptomatic male patients (all underwent Gram stain urethral smear) were collected and patients stratified into those with non-specific urethritis (n = 12), *M. genitalium* urethritis (n = 13), *C. trachomatis* urethritis (n = 14) and non-urethritis controls (n = 13). Cytokines measurements from FVUs specimens were obtained using a Human 30-Plex Luminex assay. Concentrations were obtained for 23 of the 30 cytokines analysed and compared between the four groups using multivariate ANOVA.

Results Overall, there was a significant difference in urinary cytokine profile between groups (p = 0.042). For individual cytokines, clinical group was associated with differences in concentrations of IL-1β (p = 0.007), GCSF (p = 0.042), CCL11 (p = 0.012), MIP-1α (p = 0.029), TNF-α (p = 0.026), IL-7 (p = 0.029), EGF (p = 0.030), VEGF (p = 0.049) and IFNa (p = 0.008). When compared with uninfected non-urethritis controls, cytokine concentrations in: *M. genitalium* samples, were increased for IL-1β (p = 0.017), GCSF (p = 0.010) but decreased for EGF (p = 0.017); *C. trachomatis* samples, were decreased for EGF (p = 0.049); and in non-specific urethritis samples, were increased for CCL5 (p = 0.049), IL-1β (p = 0.05), IL-1RA (p = 0.033) and decreased for EGF (p = 0.032). No significant differences were demonstrated in cytokine concentrations between *C. trachomatis* and *M. genitalium* groups.

Conclusion The increased levels of pro-inflammatory cytokines present in the urethritis groups when compared to non-urethritis controls reflect the acute inflammatory state. The data suggests that *M. genitalium* genital infection may be associated with a discrete mucosal immunological profile potentially explaining the link between cellular inflammatory response and bacterial load, previously observed

P1.013 EMERGENCE OF *NEISSERIA GONORRHOEAE* ISOLATES WITH *IN VITRO* DECREASED SUSCEPTIBILITY TO CEFTRIAXONE IN ARGENTINA

doi:10.1136/sextrans-2013-051184.0234

[1]M Vacchino, [1]R Gianecini, [1]C Oviedo, [2]L Piccoli, [3]L Fernandez Canigia, [4]N Pereyra, [5]M Machain, [6]A Famiglietti, [1]**P G Galarza**. *[1]National Institute of Infectious Diseases, Buenos Aires, Argentina; [2]Perrando Hospital, Chaco, Argentina; [3]Aleman Hospital, Buenos Aires, Argentina; [4]Central Hospital, Formosa, Argentina; [5]Piñeyro Hospital, Junin, Argentina; [6]Clinicas Hospital, Buenos Aires, Argentina*

Background *N. gonorrhoeae* (Ng) has developed resistance to most antimicrobial used for treatment. The report of the first resistant isolates of Ceftriaxone (CRO) in Japan, France and Spain, highlighted the lack of alternatives for syndromic treatment of Ng. In Argentina, although no resistance has been reported, the first isolates with decreased susceptibility to CRO (CRO^{LS}) appear in 2011.

Materials and Methods: We studied 5649 Ng isolates since 1993 derived from Argentine Gonococcal Antimicrobial-Susceptibility Network. MIC was determined by agar dilution according to CLSI recommendations. We studied extended spectrum cephalosporins (ESCs) resistance determinant (*mtr*, *pen*A, *por*B) by sequencing and carry out the molecular typing by Ng multi-antigen sequence typing (NG-MAST).

Results We detected 10 CRO^{LS} isolates, 4 showed a CRO MICs of 0.064 μg/ml and 6 of them of 0.125 μg/ml. All isolates were also resistant to two or more antimicrobial agents (Penicilin and Tetracycline and/or Ciprofloxacin) and showed decreased susceptibility to Cefixime with MICs between 0.125 and 0.5 μg/ml.

Six NG-MAST sequence types (STs) were detected, with ST925 (n = 3) and ST1407 (n = 3) being most common. Also found ST225, ST3620 and two new STs: ST8508 and ST8509.

The *pen*A gene analysis revealed three different no mosaic alleles patterns: V (n = 1), IX (n = 1) and XII (n = 1) and two mosaic alleles patterns: XXXIV (n = 4) and X (n = 3). Nine isolates showed mutations in G120 and A121 positions in *por*B1b allele and one isolate revealed A121G substitution in allele *por*B1a, both previously described. All isolates carried a nucleotide (A) deletion in the inverted region of *mtr*R gene.

Conclusion The evidence of the first isolates with decreased susceptibility to ESC in Argentina and the presence of ST1407 involved in the extensively drug resistant Ng raise the need for emphasise surveillance studies to ESC and know the distribution of ST1407 in our Region and its associated resistance.

P1.014 HERPES SIMPLEX VIRUS TYPE 2 UL23 THYMIDINE KINASE: MOLECULAR DETECTION OF POLYMORPHISM AND MUTATIONS ASSOCIATED WITH ACYCLOVIR RESISTANCE IN SOUTH AFRICA

doi:10.1136/sextrans-2013-051184.0235

[1]**E E Muller**, [1]M P Magooa, [1,2,3]D A Lewis. *[1]NICD, NHLS, Johannesburg, South Africa; [2]Department of Internal Medicine, Faculty of Health Sciences, University of*

Sex Transm Infect: first published as 10.1136/sextrans-2013-051184.0235 on 13 July 2013. Downloaded from http://sti.bmj.com/ on June 7, 2023 by guest. Protected by copyright.

the Witwatersrand, Johannesburg, South Africa; [3]Division of Medical Microbiology, University of Cape Town, Cape Town, South Africa

Background Acyclovir (ACV) is the first-line treatment for symptomatic primary and recurrent genital herpes in South Africa. Resistance to ACV is mainly due to mutations in the HSV-2 viral *UL23* gene that codes for thymidine kinase (TK), resulting in an inability for the drug to inhibit viral replication. The objectives of this study were to obtain genotypic data on the frequency of HSV-2 TK resistance mutations and natural polymorphism as well as to assess the prevalence of ACV-resistant HSV-2 among participants in the genital ulcer aetiological studies conducted between 2007 and 2011.
Methods We amplified and fully sequenced the *UL23* gene of 254 HSV-2 positive specimens obtained from participants in genital ulcer aetiological studies conducted between 2007 and 2011 in Gauteng Province, South Africa. Additionally, intratypic differences between the HSV-2 were determined based on the number of reiterated sequences located in the non-coding regions of the *US1* and *US12* genes of HSV-2.
Results We identified 63 single and/or double nucleotide mutations in the *UL23* genes analysed, that resulted in 30 silent mutations and 32 amino acid changes. 41% of these amino acid changes were due to natural polymorphism. In addition, we identified 19 unknown amino acid changes in 30 samples that have not been described before. All unknown mutations detected were outside the TK resistance "hotspots", which are renowned sites for ACV resistance mutations to occur. No frameshift mutations or mutations causing stop codons were identified in the *UL23* genes of the samples analysed.
Conclusions No evidence was found of known ACV resistance mutations in HSV-2 following the addition of ACV as first-line therapy for genital ulceration in South Africa. Genotyping of HSV-2, based on the length of reiterated sequences in the *US1* and *US12* genes of HSV-2, revealed a high degree of HSV-2 heterogeneity in this population.

P1.015 ON THE MECHANISMS OF DRUG RESISTANCE IN HIV-1 RT

doi:10.1136/sextrans-2013-051184.0236

B Sharma. *University of Allahabaad, Allahabaad, India*

Human Immunodeficiency Virus Reverse Transcriptase (HIV-1RT), catalysing synthesis of proviral cDNA has been widely exploited as a most suitable target for attack by anti-AIDS chemotherapeutics. The antiHIV-1RT molecules involve dideoxy analogues of dNTP (nucleoside reverse transcriptase inhibitors, NRTIs), which inhibit it competitively by terminating DNA chain extension. The other group of HIV-1 RT inhibitors involves non-nucleoside reverse transcriptase inhibitors (NNRTIs), which bind preferably in the hydrophobic pocket or elsewhere at enzyme surface, inhibit it non-competitively. The AIDS therapy prescribes different combinations of various anti-HIV drugs mainly the antiHIV-1RTs and antiproteases (in highly active antiretroviral therapy; HAART), which significantly reduces HIV count to undetectable level. Unfortunately, due to drug selection pressure drug resistant variants of HIV-1 emerge and preclude chemotherapy of this pandemic. This paper is an endeavour to illustrate possible mechanisms involved in anti-HIV drugs resistance. Apart from attributes of numerous mutations reported in three key enzymes of HIV (RT, protease and integrase) in making antiHIV drugs, one plausible mechanism of nucleoside analogue resistance involves ATP/GTP-based excision to unblock chain-terminated primers; thereby allowing viral replication to continue. Such unblocking has also been reported *in vitro* in presence of excess of pyrophosphate concentration; a reaction known as pyrophosphorolysis. In addition to viral mutants isolated from the AIDS patients, we have shown some artificial mutants of K154, which exhibited antiHIV-1 drugs resistance property and the mechanism involved reduction in binding of the drugs to RT because of conformational change. Our recent report on mechanism of resistance of HIV-1RT against rilpivirin due to E138K mutation proposes a new aspect in this context. However, an extensive study exploring some new targets and drug resistance mechanisms is needed for design and development of novel and potential anti-viral agents to combat this challenge

P1.016 MOLECULAR ANALYSIS OF ANTIMICROBIAL RESISTANT NEISSERIA GONORRHOEA ISOLATES FROM DELHI, INDIA: A FUNCTIONAL GENOMICS APPROACH

doi:10.1136/sextrans-2013-051184.0237

[1]D Sachdev, [2]M Bala, [1]I kumari, [1]R Pandey, [3]**U Chaudhry**, [1]D Saluja. *[1]Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi-110007, India; [2]Regional STD Teaching, Training & Research Centre, Vardhman Mahavir Medical College & Safdarjung Hospital, NewDelhi-110029, India; [3]Bhaskarcharya College of Applied Sciences, University of Delhi, NewDelhi-110075, India*

Background In India, knowledge regarding N. gonorrhoeae antimicrobial resistance profiling is limited, and data concerning genetic characteristics of N. gonorrhoeae is also lacking. Herein, we investigated the genetic resistance determinants for various antimicrobials used against N. gonorrhoeae isolated in Delhi, India. Various studies have shown that this resistance towards antimicrobials could be either plasmid or chromosomal mediated involving mutations in various genes.
Methods Molecular basis of plasmid and chromosomal mediated antimicrobial resistance was analysed by amplifying and sequencing the most target genes, pen A and por B, of N. gonorrhoeae. Attempts have been made to in-silico model the structure of mutant PenA to understand how mutations in these genes affect the drug binding. A PCR assay was also carried out to analyse the penicillinase producing N. gonorrhoeae (PPNG).
Results Out of the 40 clinical isolates of N. gonorrhoeae studied which were resistant to various antimicrobials, twenty eight isolates showed high resistance to penicillin (3–32μg/ml). These resistant isolates were PPNG positive (70%; 28/40) and predominantly harboured the African type of PPNG plasmid. Only two isolates carried the Asian type of plasmid. Mutations were also observed in penA and porB genes which correlate their effects on drug resistance. Through in silico modelling studies, we were able to even show that a single point mutation at G452S in penA gene changed the susceptibility of N. gonorrhoeae towards penicillin and tetracycline.
Conclusions This study clearly shows a cumulative effect of increasing mutations with subsequent increase in resistance towards various antimicrobials. Presence of both African and Asian type of penicillinase producing plasmid gives an indication of extensive travel of patients affected with Gonorrhea. Our in silico modelling studies of mutant proteins provide new insights to access increasing antimicrobial resistance among Neisseria gonorrhoeae.

P1.017 UNIQUE ACTIVITY OF SITAFLOXACIN, ONE OF NEWER FLUOROQUINOLONES, AGAINST CIPROFLOXACIN-RESISTANT N. GONORRHOEAE

doi:10.1136/sextrans-2013-051184.0238

R Hamasuna, T Sho, M Matsumoto, T Matsumoto. *Department of Urology, University of Occupational and Environmental Health, Japan, Kitakyushu, Japan*

Background Fluoroquinolone-resistance in Neisseria gonorrhoeae is spreading in the world and almost 80% of N. gonorrhoeae strains are resistant to fluoroquinolones in Japan. It is known that the resistance to fluoroquinolones is closely related to genetic mutations of quinolone-resistance determining regions (QRDR) on gyrase genes such as gyrA or parC. In 2009, the first national surveillance of antimicrobial susceptibilities of N. gonorrhoeae was performed in Japan. In this surveillance, we found that sitafloxacin, one of newer

Sex Transm Infect: first published as 10.1136/sextrans-2013-051184.0235 on 13 July 2013. Downloaded from http://sti.bmj.com/ on June 7, 2023 by guest. Protected by copyright.