

P3.270 ANTIMICROBIAL SUSCEPTIBILITY AND MOLECULAR CHARACTERIZATION OF NEISSERIA GONORRHOEA STRAINS FROM SLOVENIA 2008–2012

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Objectives Antimicrobial resistance in *Neisseria gonorrhoeae* is a major public health problem worldwide. Slovenia is among the countries with highest prevalence of decreased susceptibility and resistance to extended-spectrum cephalosporins (ESCs) in Europe. Herein, the phenotypic and molecular characterization of Slovenian *N. gonorrhoeae* strains from 2008–2012 is presented.

Methods *N. gonorrhoeae* isolates cultured 2008–2012 in Slovenia (n = 138) were examined for antimicrobial susceptibility with Etest methodology for 8 antimicrobials (cefixime, ceftriaxone, penicillin, ciprofloxacin, azithromycin, tetracycline, gentamicin and spectinomycin). Furthermore, all isolates were investigated with *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST) for molecular epidemiology, and sequencing of major ESC resistance determinants; penA, mtrR and penB.

Results The overall prevalence of resistance and decreased susceptibility to cefixime and ceftriaxone (MIC₅₀ ≥ 0.125 mg/L) was 16% and 7%, respectively. The resistance and decreased susceptibility showed an epidemic peak during 2009–2011 when it reached 28% for cefixime and 12% for ceftriaxone, however, the prevalence subsequently decreased to 6% and 4%, respectively, in the year 2012. NG-MAST sequence types 1407 (13% of all isolates), 21 (8%) and 225 (6%) were the most common STs during 2008–2012. ST1407, previously stated as an internationally spread successful clone with resistance or decreased susceptibility to ESCs, was most prevalent in 2009 (43%). However, the ST1407 prevalence declined in 2010 (15%) and 2011 (11%) and the clone was rare in 2012 (4%). Instead, in 2012 the ESC susceptible ST21 was the predominant ST (21%). During 2008–2012, a penA mosaic allele (mainly penA XXXIV, associated with ST1407) was detected in 25% of isolates.

Conclusion The prevalence of gonococcal resistance to ESC in Slovenia has fluctuated, which is mainly due to the longitudinal prevalence of a few gonococcal clones. Fortunately, some ESC susceptible clones, such as ST21, now appear to replace the main ESC resistant clone ST1407, a replacement that is indicated in several European countries.

P3.271 IDENTICAL MULTILOCUS SEQUENCE TYPING (MLST) ANALYSIS IN SEQUENTIAL SAMPLES FROM PATIENTS WITH PHARYNGEAL CHLAMYDIA INFECTIONS

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Introduction Pharyngeal *Chlamydia trachomatis* (PcT) must persist to contribute to ongoing transmission. In a retrospective study, we examined MLST-types of PcT in patients who had a positive

pharyngeal swab on two visits, and had not been treated for this infection at first visit.

Methods From 1/1/2008 to 14/7/2010, pharyngeal swabs from patients at risk for pharyngeal gonorrhoea were tested with the AC2 (Hologic-GenProbe) test. Since at that time PcT detection was not considered to represent an infection, PcT results were not reported and patients were not treated, unless they had a chlamydial infection at another anatomic site. We looked for patients who had a positive PcT test on two different occasions with an interval of at least 3 weeks. For inclusion in the study, patients were required to have no *Chlamydia* infections at other anatomic locations at first visit and therefore received no treatment. PcT typing was done by MLST on stored specimens.

Results Sixteen patients could be included and paired pharyngeal samples from four of those patients contained enough DNA for MLST analysis. The intervals between the two visits were 112, 168, 207 and 268 days, respectively. In all four patients MLST types of both pharyngeal samples were completely identical. Patients were two women and two men who had sex with men (MSM). At second visit one woman and one MSM reported commercial sex work and had 30 and 150 sexual partners in the last 6 months, respectively. The second woman reported sex with two known persons and the second MSM reported sex with 15 known persons. None reported sex with a steady partner.

Conclusion Our findings of identical MLST types are consistent with persistent PcT infection for a period of 3–9 months, although repetitive exposure to untreated partners with identical *C. trachomatis* strains can not be excluded.

P3.272 PREVALENCE OF CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHOEA AND UREAPLASMA UREALYTICUM IN PREGNANT WOMEN OF SABZEVAR - IRAN

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Background One approach to eliminating sexually transmitted Infections (STIs) in a community is to screen high-risk persons, followed by the treatment and education of people who test positive. We examined a Triplex PCR assay to detect urine samples in pregnant women. major goal of this study was to determine the prevalence of *Chlamydia trachomatis* (Ct.), *Neisseria gonorrhoeae* (Ng.) and *Ureaplasma Urealyticum* (Uu.) and to apply a Triplex PCR techniques to detect 3 pathogens in one specimens.

Methods A cross-sectional study was conducted among 399 pregnant adolescents women. All participants received prenatal care and delivered at urban hospital in Sabzevar- Iran. A socio- demographic questionnaire was completed. A Triplex PCR with universal primers was developed for screening of subjected women in this study. Descriptive and univariate analyses were performed to describe disease prevalence.

Results Of pregnant adolescents, 15.28% were diagnosed with either Ct., Ng. or Uu. infection. The prevalence of Ct. was 12.28%, and that of Ng 1.25% and finally 19.54% of pregnant had Uu. infection.

In univariate analysis, Ct. was associated with having had any level of education (P < 0.05), abortion (P < 0.05), and Uu. was associated with PTD (P < 0.05). Multivariate analysis did not show any significant association.

Conclusions Compared with available data, a decline of STIs prevalence was observed in our setting. This might be the result of

community-based education programmes focusing on changes to sexual behaviour. However, STIs rates are still high, and the problem needs more concrete and sustained efforts for its control. Screening for Ct., Ng. and Uu. is recommended during pregnancy. Based on our finding in this study, the overall high incidence of Ct. and Uu. support screening recommendations for pregnant women in Sabzevar- Iran.

P3.273 MOLECULAR TYPING AND DETECTION OF MACROLIDE RESISTANCE MUTATIONS IN *T. PALLIDUM* STRAINS FROM CALI, COLOMBIA

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Background Cali is a syphilis-endemic area, with a very high prevalence of gestational syphilis (14.7/1000 live births) and congenital syphilis (7.7/1000 live births). Molecular typing techniques are useful for studying bacterial strain diversity, molecular epidemiologic patterns and antimicrobial resistance patterns. The study objective was to determine the *T. pallidum* (*Tp*) strain diversity and analyse for the mutations associated with macrolide-resistance in this syphilis-endemic region.

Methods 19 secondary syphilis (SS) patients, 15 (RPR+, FTA-ABS+, HIV-) and 4 (RPR+, FTA-ABS+, HIV+) were enrolled in the study. *Tp* DNA was extracted from syphilis skin lesions and quantified by real-time-qPCR targeting *polA* gene. Molecular typing was performed using established typing and subtyping methods (*arp*, *tp*, *tp0279*, *tp0548*) and strains were analysed for A2058G and A2059G mutations within the 23S rRNA gene. Strain diversity in Cali strains (*arp* and *tp*) was compared with other published *Tp* molecular studies using the Shannon index.

Results 14/19 SS patients were *polA* PCR positive (range 5.4 – 38.920; mean 3.227 copies/ug total DNA). Among 8/14 samples that were fully typed, 7 strain subtypes (21a11/d, 12d9/f, 10p9/f, 14j10f, 5p9/f, 14k9/f, 14d9/f) were identified with only 2 strains exhibiting identical patterns (21a11/d). The A2059G mutation was found in 1 specimen from an HIV-negative subject. Cali *Tp* strain types had a Shannon index of 2.2, higher than all 13 studies in other localities reported in two recent reviews.

Conclusions There is a high *T. pallidum* strain diversity in Cali, Colombia, probably due to population mobilisation and close proximity to other syphilis-endemic regions (i.e. Buenaventura, Pacific Coast). Contact tracing and cluster identification is difficult to achieve in this setting. Although our sample size was small, the identification of the A2059G mutation suggests that macrolides should be used with caution for syphilis treatment in Cali and close monitoring for macrolide resistant strains should be initiated.

P3.274 CHARACTERIZATION OF VAGINAL LACTOBACILLUS AMONG NON-PREGNANT WOMEN WITH AND WITHOUT BACTERIAL VAGINOSIS IN INDIA AND US

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Background *Lactobacillus* is an integral part of vaginal microbiota that maintains healthy environment and plays an important role in

preventing sexually transmitted infections and HIV. We profile the *Lactobacillus* species present when the women are healthy or have Bacterial Vaginosis among women in US and India.

Method Between February 2010 and November 2011, a sample of 75 women attending Prerana Women's Health Clinic in India or San Francisco City Clinic in USA were sampled and diagnosed for BV based on Amsel's Criteria. In addition, Gram stained smears of vaginal fluid were Nugent scored. Vaginal swabs were then cultured in MRS broth. Gram positive *Lactobacilli* generating about 600–800bp amplicon by 16SrDNA PCR with 16S primers were further characterised by sequencing.

Result Vaginal samples were obtained from 75 women. According to Amsel criteria, 34 women were healthy and 41 women had BV. *Lactobacilli* were isolated from 22 healthy Indian and 10 healthy US women. *Lactobacilli* were also isolated from 4 Indian and 5 US women with BV. Eleven *Lactobacillus* species were isolated from 26 Indian women and 9 species were identified from 15 US women. The common *Lactobacilli* species found in Indian women included *L. Crispatus* (24.3%), *L. Gasseri* (24.3%), and *L. Jensenii* (13.5%), while *L. Crispatus* (32.0%), *L. Jensenii* (20.0%), and *L. Coleohominis* (12.0%) were common in US women. *L. crispatus* was cultured from 44% of healthy and 4.9% of women with BV. *L. jensenii*, *L. gasseri*, and *L. acidophilus* were cultured from 25.6%, 23.5% and 2.9% of healthy women; and 2.4%, 4.9% and 0.0% of BV women, respectively.

Conclusion Our findings showed *lactobacilli* species present in healthy vagina of women in India do not differ from those reported from other countries. This information is useful to development of microbicides for HIV prevention.

P3.275 NEISSERIA GONORRHOEA: SITUATION OF ANTIBIOTIC RESISTANCE IN GERMANY

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Background The dramatic development of antimicrobial resistance in *Neisseria gonorrhoeae* is a serious problem for treatment and control of gonorrhoea. Numerous formerly effective therapeutic agents are no longer appropriate.

High level penicillin resistance and quinolone resistance disseminated globally. The third generation cephalosporins are amongst the last agents to remain effective. Reduced susceptibility to these cephalosporins is increasingly common. Currently the emergence of cephalosporine resistance in gonococci can be observed. There is a grave concern that multidrug resistant *Neisseria gonorrhoeae* strains will develop (MDR-NG).

Methods Non-selected collection of *Neisseria gonorrhoeae* isolates from all regions of Germany.

It is the aim of this surveillance to monitor the antimicrobial resistance to a range of therapeutically relevant antimicrobials as third generation cephalosporins (cefixime, ceftriaxone), azithromycin, gentamicin, spectinomycin, ciprofloxacin and penicillin.

Results We report current results from the 2011/12 antimicrobial resistance (AMR) surveillance in Germany.

More than 10% of the isolates displayed decreased susceptibility to cephalosporins, mostly to cefixime. Nearly 5% of all isolates showed a decreased susceptibility to both cephalosporins. All of these isolates also displayed resistance to ciprofloxacin.

Conclusion Cefixime is no longer recommended as first line therapy agent due to a high number of isolates with decreased susceptibility. Ceftriaxone is an appropriate treatment for gonorrhoea, but a drift to decreased susceptibility can be observed. Azithromycin remains still effective. Rates of ciprofloxacin and penicillin resistance are high across Germany.