

Introduction Antimicrobial resistant *Neisseria gonorrhoea* (NG) is important to monitor as a potential global public health threat. The Thailand Enhanced Gonococcal Antimicrobial Surveillance Programme (EGASP) was started in November 2015 as a collaboration between the Thailand Ministry of Public Health, the US Centres for Disease Control and Prevention and the World Health Organisation. As a part of this surveillance activity, Thailand conducted an internal quality assessment (QA) of clinical and laboratory data in order to improve surveillance data quality.

Methods EGASP Thailand occurs in 2 sentinel sites: Bangrak Hospital and Silom Community Clinic at Tropical Medicine. Men with symptoms had demographic and clinical data collected as well as a urethral specimen collected for NG culture. A random selection of 10% of EGASP IDs were sampled from November 2015 to June 2016. We assessed clinical and laboratory findings using a standardised review tool that compared the EGASP database to source documents. We describe key findings from the review activities.

Results Overall, 699 specimens were collected for EGASP and 70 (10%) EGASP IDs were randomly sampled by SQL command for review. Results from the quality review included: differences in laboratory findings (6%), differences in interpretation of the clinical abstraction tool between sentinel sites (10%), missing data in the EGASP database after chart abstraction and laboratory testing (14%), differences in the recording of clinical data (19%), and differences in the recording and tracking of laboratory variables (47%). As a result of this evaluation, staff updated missing data on records sampled, conducted an overall refresher training for staff and established a new laboratory tracking process.

Conclusion EGASP Thailand is the first coordinated global project to conduct comprehensive surveillance for NG resistance from symptomatic men. An internal QA helped direct efforts to improve surveillance. Ongoing NG surveillance and periodic quality assessments help ensure high quality surveillance data.

Oral Presentation Session 13

Biomedical and Systems Biology

013.1 SURVEY OF ANTIMICROBIAL RESISTANCE IN CLINICAL *NEISSERIA GONORRHOEAE* ISOLATED OVER FOUR YEARS IN NAIROBI – KENYA

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Introduction Systematic antimicrobial resistance (AMR) surveillance of *N. gonorrhoea* (GC) from local to global level are being intensified to inform and design a monitoring system for its control. High-level resistance to previously recommended quinolones is widespread and decreased susceptibility to the extended-spectrum (third-generation) cephalosporin has

been report. The Gonococcus antimicrobial surveillance program (GASP) in Kenya and the region carried out a study to determine the frequency and diversity of antimicrobial resistance of GC isolates from a Sex Workers Outreach Program (SWOP) Clinic in Nairobi over a period of 4 years.

Methods The study tested 238 GC isolates from participants presenting with cervical/vaginal discharge. Samples collected were inoculated directly on modified Thayer martin media (MTM), transported to GASP Laboratories at KAVI-Institute of Clinical Research for processing by standard bacteriological procedures. Antibiotic susceptibility testing was performed using diffusion gradient method. The strains were defined as susceptible, intermediate and resistant using E-test as guided by WHO, all the findings were validates at WHO Collaborating Centre for Gonorrhoea and other STIs, Örebro University Hospital in Sweden.

Results GC isolates, 41 in 2012, 119 in 2013, 24 in 2014 and 54 in 2015 showed 100% susceptibility to cefixime, ceftriaxone and spectinomycin in four years with a mean susceptibility of 82%, 37.7%, 19.5%, 1.6% and 0% for azithromycin, erythromycin, ciprofloxacin, penicillin and tetracycline respectively. Over the period ciprofloxacin showed a rise in resistance from 56% in 2012, 58.8% in 2013, 66.7% in 2014 to 68.5% in 2015.

Conclusion Spectinomycin, cefixime, ceftriaxone, azithromycin are useful drugs, while Ciprofloxacin the most prescribed antibiotic is no longer reliable for treatment of GC in the region. Continued surveillance will enables the public health managers to modify the national treatment guidelines. Worsening GC drug resistance will compromise effective treatment and decrease disease control efforts.

013.2 MOLECULAR EPIDEMIOLOGY IN RELATION TO AZITHROMYCIN RESISTANCE IN *NEISSERIA GONORRHOEAE* ISOLATES FROM AMSTERDAM, THE NETHERLANDS, BETWEEN 2008 AND 2015 – A CASE-CONTROL STUDY

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Neisseria gonorrhoeae resistance to ceftriaxone and azithromycin increases, which threatens the recommended dual therapy based on these antimicrobials. We used molecular epidemiology to identify *N. gonorrhoeae* clusters, and associations with azithromycin resistance in Amsterdam, the Netherlands. *N. gonorrhoeae* isolates were selected from patients visiting the Amsterdam Sexually Transmitted Infections Clinic, from January 2008 through September 2015. We included all azithromycin resistant isolates (minimum inhibitory concentration [MIC] ≥ 2.0 mg/L), and frequency matched susceptible controls (MIC ≤ 0.25 mg/L). All isolates were tested using 23S rRNA sequencing, *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), and multilocus variable-number of tandem repeat analysis (NG-MLVA). A hierarchical cluster analysis of NG-MLVA

related to resistance and epidemiological characteristics was performed. We analysed 143 isolates (69 resistant and 74 susceptible); 81% was from men who have sex with men (MSM). Azithromycin resistant isolates had significantly more often C2611T mutations of 23S rRNA ($n=62$, 89.9%, $p<0.001$), an NG-MAST genogroup G2992 ($p<0.001$), G5108 ($p<0.001$), or G359 ($p=0.02$), and were more often part of NG-MLVA clusters ($p<0.001$). Two resistant isolates (2.9%) had A2059G mutations, and five (7.3%) were wild-type 23SrRNA. Four of the five NG-MLVA clusters contained resistant and susceptible isolates, and isolates from HIV-positive and HIV-negative patients. Two of the clusters consisted mainly of resistant isolates and were strains from MSM, heterosexual men and women. Co-occurrence of resistant and susceptible strains in NG-MLVA clusters and frequent occurrence of resistant strains outside of clusters suggests that azithromycin resistance develops independently from the 'background genome'.

013.3 VACCINE DEVELOPMENT TO COMBAT ANTIMICROBIAL RESISTANT GONORRHOEA

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Introduction *Neisseria gonorrhoeae*, the causative agent gonorrhoea, is a major public health problem worldwide with an estimated global incidence of 106 million cases/yr. If left undiagnosed or untreated, infection can lead to severe sequelae that include pelvic inflammatory disease, infertility, neonatal complications, and an increased risk of HIV. It recognised by WHO and CDC as an urgent threat to global health due to the emergence of multi-drug resistant gonococcal strains. There is currently no vaccine, and no new antibiotics or new vaccine candidates in late-stage development.

Methods To facilitate gonococcal vaccine development, we performed mathematical modelling to predict the impact of different vaccine scenarios. We have also identified and characterised a series of potential vaccine candidates.

Results Mathematical modelling of different vaccine scenarios indicates that even a modestly efficacious vaccine could have a substantial impact on gonorrhoea prevalence and sequelae. We have also characterised 2 highly conserved and immunogenic candidate vaccine antigens. *In vitro* assays, using wild type, knock-out and complemented strains, have shown that NGO1958 (gonococcal homologue of the *Neisseria* heparin binding antigen (NHBA) present in the serogroup B meningococcal vaccine) is involved in serum resistance and adherence to cervical epithelial cells. Similar assays show that NGO2139 (methionine uptake receptor) is involved in resistance to killing by human serum, monocytes and macrophages, as well as adherence and invasion of cervical epithelial cells. Antibodies to these proteins are bactericidal and can block gonococcal infection of cervical epithelial cells. Additional studies are underway to determine if antibodies to these proteins can protect against infection in a mouse model.

Conclusion We present two antigens that elicit both bactericidal and functional blocking antibodies, which are valid candidate antigens for possible inclusion in an urgently needed vaccine for the prevention of gonorrhoea.

013.4 MULTIPLE CYTOKINE GENE EXPRESSION DETECTED AFTER HPV VACCINATION

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Introduction Human papillomavirus (HPV) infection does minimal damage and does not induce the production of immune mediators by host epithelial cells. The induction of a pro-inflammatory immune response is necessary to break the tolerance induced by HPV. Therapeutic interventions with vaccines to induce an effective immune response have the potential to treat latent infection as well as clinically apparent lesions. The aim of this study was to evaluate the influence of the human papillomavirus (HPV) vaccination on peripheral blood mononuclear cell (PBMC) proliferation and cytokine gene transcription.

Methods PBMCs isolated after immunisation were incubated with HPV vaccine, phytohemagglutinin (PHA) or buffer. Cell proliferation was assessed by MTT reduction assay. RNA was extracted from PBMCs, and the relative concentration of cytokine messenger RNA (mRNA) transcripts (IFN- β , IFN- γ , IL-12, TNF- α , IL-6, IL-17, or IL-10) relative to transcription of the β -actin gene was determined by real-time polymerase chain reaction.

Results PBMC proliferation in response to HPV vaccine and PHA were greater than that observed in unstimulated cells ($p<0.001$). Cytokine mRNAs were upregulated in stimulated PBMC cultures. The median increase in vaccine-stimulated cultures was: IFN- β =334.4 fold; IL-12=46.33 fold; IFN- γ =12.64 fold; IL-6=9.07 fold; IL-17=7.33 fold; IL-10=6.47 fold; and TNF- α =2.36 fold.

Conclusion The IFN- β expression was significantly higher ($p<0.05$). Proliferative PBMC responses and multiple cytokine gene expression were detected in women who received the HPV vaccine.

013.5 CORE GROUPS OF INDIVIDUALS WITH CHLAMYDIA AND/OR GONORRHOEA REINFECTIONS HAVE INCREASED ODDS OF DIAGNOSIS WITH INFECTIOUS SYPHILIS: A POPULATION-BASED RETROSPECTIVE COHORT STUDY IN BRITISH COLUMBIA, CANADA, 2006–2015

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Introduction The incidence of infectious syphilis (primary, secondary, or early latent) has increased in British Columbia (BC). Identifying core groups at risk for syphilis can inform public health programming. We assessed the odds of syphilis infection among individuals with repeat *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) infections in this population-based analysis.

Methods Surveillance records for all BC residents diagnosed with ≥ 2 CT (CT reinfection) or ≥ 2 GC (GC reinfection) or ≥ 2 infections including CT and GC (CT/GC reinfection) from 1/1/2006 to 12/31/2015 were linked with all infectious syphilis cases from the same time period. Logistic regression models