clinic visitors. In view of the extensive resistance against doxycyclin and ciprofloxacin, these antibiotics are not appropriate treatment options for gonorrhoea; instead, extended spectrum cephalosporins are advised.

Disclosure of interest statement The study is funded by Indonesian government through Beasiswa Unggulan (The Excellence Scholarship Program), Ministry of Education and Culture Republic of Indonesia and Public Health Service (GGD) of Amsterdam, The Netherlands. The authors declare that there is no conflict of interest.

P05.06 PROLONGED INFECTION OF PHARYNGEAL GONORRHOEA AFTER TREATMENT WITH CEFTRIAXONE

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Introduction Previous studies reported that in a considerable proportion of pharyngeal gonorrhoea cases treated with extended-spectrum cephalosporins, the infection remains detectable after several weeks. We examined the occurrence of prolonged pharyngeal gonorrhoea infections after treatment with ceftriaxone at a sexually transmitted infection (STI) outpatient clinic in Amsterdam.

Methods A retrospective cohort study was conducted based on routine electronic medical records at the STI clinic of the Public Health Service (GGD) of Amsterdam. Eligible for inclusion were: adults diagnosed with pharyngeal gonorrhoea between January 2012 and July 2013, who were treated with ceftriaxone (500 mg IM), and who returned for a test of cure (TOC) between 7 and 21 days after treatment.

Definitive diagnosis of gonorrhoea was based on Gen-Probe Aptima-Combo 2 Assay™ using Tigris DTS™ system. Some patients also received additional antibiotics with ceftriaxone.

Information on patients' characteristics and clinical history were available, but data on sexual re-exposure after treatment were not.

Results In the study period, 880 pharyngeal gonorrhoea cases were diagnosed; 290 cases (32.9%) returned for a TOC visit and were eligible (255 males and 35 females, median age 34 and 25 years, respectively). In 17 cases (5.9%) *N. gonorrhoeae* infection was detected again. Prolonged infection was not associated with gender ($p = 0.49$) or age ($p = 0.87$), but appeared to be associated with sex work (OR = 3.24 [95% CI 0.83–12.45], $p = 0.07$). Prolonged infection was significantly more common among those who were treated with ceftriaxone only vs a combined-regimen (OR = 4.07, [95% CI 0.90–18.39]; $p = 0.048$).

Conclusion Prolonged pharyngeal gonorrhoea infection after appropriate treatment was not uncommon, and was more often observed in those who were treated with ceftriaxone only. This could be the result of re-infection after treatment or of treatment failure possibly due to poor tissue penetration. Treatment failure due to antimicrobial resistance seems unlikely.

Disclosure of interest The study is fully funded by Public Health Service (GGD) of Amsterdam, The Netherlands. The authors declare that there is no conflict of interest.

P05.07 *NEISSERIA GONORRHOEAE* MULTIANTIGEN SEQUENCE TYPING (NG-MAST) OF ISOLATES COLLECTED FROM STD PATIENTS IN DELHI, INDIA

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Background *Neisseria gonorrhoeae* multiantigen sequence typing (NG-MAST) is a highly discriminatory technique for assessing the genetic diversity of *N. gonorrhoeae* and has also been put forward as a tool for predicting specific antimicrobial resistance (AMR) phenotypes. Therefore, the present study was undertaken to investigate the molecular epidemiology of *N. gonorrhoeae* isolates using NG-MAST in Delhi and to examine if it can be used as a means for predicting AMR. This is the first such research performed in this country.

Methods 100 consecutive gonococcal isolates between April 2010 to October 2013 were investigated. Antimicrobial susceptibility testing was done using disc diffusion method and E test and the results interpreted using the breakpoint criteria of CDS technique. NG-MAST was performed as described previously. WHO *N. gonorrhoeae* reference strains F, G, K-P were used as controls. Association between NG-MAST sequence type (ST) and antimicrobial susceptibility was probed using chi-square and fisher's exact tests.

Results Rates of resistance to classical antibiotics were high. Decreased susceptibility to ceftriaxone (MIC 0.032–0.25 μg/ml) was demonstrated in 5% while azithromycin resistance (MIC ≥1 μg/ml) was seen in 4% isolates. *N. gonorrhoeae* isolates were assigned into 60 different STs and 43 (71.6%) have not been reported previously to the international database. The most common ST was 6058 (21%), followed by ST 9774 (4%), ST9875 (4%), ST9783 (4%) and ST2990 (3%). The majority of the STs (76.6%) were represented by a single isolate. There was significant association between ST6058 and resistance to penicillin ($p = 0.00$) and tetracycline ($p = 0.002$). In all the other antibiotics, no association was found.

Conclusion Our work reflects a highly diversified gonococcal population in Delhi. Further, NG-MAST has a limited applicability as a tool for predicting AMR in our region. A detailed investigation on a large number of representative isolates may provide insight into sexual networks in the city.

Disclosure of interest statement None.

P05.08 BETA-LACTAM ANTIBIOTICS INDUCE PROTEIN EXPRESSION CHANGES IN *NEISSERIA GONORRHOEAE* REVEALED BY A PROTEOMIC APPROACH

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Introduction *Neisseria gonorrhoeae* resistance to extended-spectrum cephalosporins as well as treatment failures with ESCs has been increasingly reported in many countries globally. These increasing trends together with the limitation of drugs of choice lead gonorrhoea to become a global health concern. Herein, we aimed to reveal more understanding on the physiological response of gonococci to ESCs using proteomic approach.
Methods *N. gonorrhoeae* reference strain was grown with or without a subinhibitory dose of ESCs. Protein expression was determined by 2-dimensional gel electrophoresis in conjunction with MALDI-TOF/TOF MS analysis.
Results In total, 14 and 13 proteins were significantly altered expression following exposure to ceftriaxone and cefixime, respectively. Most of expressed proteins shared a similar expression pattern in response to ceftriaxone and cefixime reflecting similarities in antibiotic mechanisms. ESC antibiotics triggered proteins in a variety of functions, such as membrane protein, transport system, energy metabolism, and stress response, which would help gonococci to survive under drug stress. Interestingly, the subinhibitory dose of ESCs also triggered the expression of gonococcal virulence factors (e.g. azurin and peptidyl-prolyl isomerase), which might be an adaptation mechanism of gonococci in ESC stress and also affect the outcome of gonococcal infection.
Conclusion The present work might provide new insights into physiological adaptive networks of gonococci to antimicrobial agents and more understanding toward the mechanism of action, which subsequently may benefit for the further drug discovery of new antimicrobials to combat with resistant gonococci.

P05.09 PHENOTYPIC, GENETIC AND GENOMIC CHARACTERISATION OF THE 2015 WHO *NEISSERIA GONORRHOEAE* REFERENCE STRAINS FOR QUALITY ASSURANCE OF LABORATORY INVESTIGATIONS GLOBALLY

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Introduction Gonorrhoea and antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* are major public health concerns globally. Resistance to all antimicrobials available for treatment of gonorrhoea has now been reported in *N. gonorrhoeae*. Enhanced quality assured gonococcal AMR surveillance is crucial worldwide and the WHO Global Gonococcal Antimicrobial Surveillance Programme (GASP) was revitalised in 2009. To obtain reliable and comparable AMR data internationally, appropriate and well-characterised *N. gonorrhoeae* reference strains are essential for quality assurance. The phenotypic and genetic characteristics of the 2008 WHO *N. gonorrhoeae* reference strains (n = 8) were previously published. Here, we describe the phenotypic, genetic, and genomic characteristics of the 2015 WHO *N. gonorrhoeae* reference strains.
Methods In the 2015 WHO *N. gonorrhoeae* reference strain panel (n = 14), six additional strains have been selected to include representation of high-level cephalosporin and azithromycin resistance and *porA* mutant strain. These strains were phenotypically characterised by antibiogram, serovar, and prolyliminopeptidase (PIP) screening; and genetically in regards of resistance plasmid types, polymorphisms in divergent genetic resistance-mediating loci (n = 14), *porB* sequencing, *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), and multilocus sequence typing (MLST). Fully characterised finished reference genomes for all the 2015 WHO *N. gonorrhoeae* reference strains were produced using PacBio and Illumina sequencing technologies.
Results The 2015 WHO reference strains represent all available main phenotypes of resistance and susceptibility to antimicrobials previously and currently used for treatment of gonorrhoea, as well as several considered for future use. All corresponding resistance genotypes and molecular epidemiological types were also elucidated. Finally, references genomes of each strain were obtained and characterised in detail.
Conclusion The 2015 WHO *N. gonorrhoeae* reference strains are intended for internal and external quality assurance in all types of laboratory investigations, i.e. particularly in GASP, but also for phenotypic (e.g. culture) and molecular diagnostics, species determination, genetic AMR detection, molecular epidemiology, and genome sequencing as well as other novel molecular technologies.
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P05.10 ANTIMICROBIAL RESISTANCE OF NEISSERIA GONORRHOEA IN GERMANY, RESULTS FROM THE GONOCOCCAL RESISTANCE NETWORK (GORENET)

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Introduction *Neisseria gonorrhoeae* (NG)-infections are not reportable in Germany and only limited data on NG-epidemiology and antimicrobial resistance (AMR) are available. With GORENET we monitor the NG-AMR and patterns of resistance testing in Germany in order to guide treatment algorithms and targeted prevention strategies.
Methods We recruited laboratories based on geographic distribution and number of NG-isolates. From April 2014 prospective data on all performed NG-AMR-tests together with patient-related information were collected. Laboratories send a part of the isolates to the national reference laboratory (NRL) for culturing and AMR-testing towards ceftriaxone, cefixime, azithromycin, ciprofloxacin, and penicillin by using E-Test, as well as beta-lactamase. Results are interpreted according to European Committee on Antimicrobial Susceptibility Testing 4.0.