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treatment, (ie. mutations only detected in follow-up test of cure sample), there was a significantly higher load detected with 3.1 x 104 copies per reaction for 2007–9 (n = 8) and 1.8 x 104 copies for 2012 (n = 8), when compared to either treatment success cases or those with baseline resistance (one sided $p < 0.01$).

Conclusions The higher infectious load in pre-treatment M. genitalium cases that developed detectable resistance after 1g of azithromycin compared to those with baseline resistance and those cured raises the possibility that heterotypic resistance and/or induced resistance may be contributing to macrolide failure in M. genitalium. These findings have implications for current recommended treatment for M. genitalium.

O21.2 EFFECT OF MUTATIONS IN *PILQ* ON THE SUSCEPTIBILITY OF *NEISSERIA GONORRHOEAE* TO CEPHALOSPORINS

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Background The susceptibility of *N. gonorrhoeae* to beta-lactam antibiotics is determined by mutations or the presence of mosaic sequence in *penA*, which codes for PBP2. The level of susceptibility is influenced by the presence of mutations in *ponA*, *mtrR*, *por*, and *pilQ*. Here we investigate the potential for isolates of *N. gonorrhoeae* that give elevated MIC values to both penicillin and cephalosporins to mutate to still higher MIC values.

Methods Mutations in gonococcal isolates were determined by DNA sequencing. MIC values were determined by agar dilution. Mutants exhibiting higher MIC values were selected on GC base agar that contained either a gradient or uniform concentration of cefpodoxime or ceftriaxone.

Results Examination of mutants of *N. gonorrhoeae* with exhibited elevated MIC values to cephalosporins revealed SPL4 3–4. Unlike previous, similar mutants, SPL4 3–4 did not possess additional mutations in *penA*. Genetic transformation experiments and genomic sequencing indicated the presence of a two base insertion mutation in *pilQ* that created a termination codon at amino acid 159 which resulted in a truncated protein and an increase in the ceftriaxone MIC from 0.03 to 0.5 ug/ml. Additional transformation and sequencing experiments using amplified *pilQ* DNA from SPL4 3–4 confirmed that the insertion mutation in *pilQ* was responsible for the increased resistance to cephalosporins as well as to penicillin. Further experimentation by amplification mutagenesis of *pilQ* with Taq polymerase yielded three additional *pilQ* mutants which exhibited increased MICs to cephalosporins, and all caused premature termination of the translation of the *pilQ* protein.

Conclusion Most of the studies examining increased MICs to cephalosporins in the gonococcus have focused on additional mutations in a mosaic *penA* gene. However, in this study we have been able to generate mutations in *pilQ* that resulted in increased MICs. Future studies will look for similar mutations in gonococcal clinical isolates.

O21.3 FITNESS STUDIES ON *NEISSERIA GONORRHOEAE* HARBORING MOSAIC *PENA* ALLELES FROM CEFTRIAXONE-RESISTANT ISOLATES PREDICT THE SPREAD OF RESISTANCE TO EXTENDED-SPECTRUM CEPHALOSPORINS

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Background Approximately 106 million cases of gonorrhoea occur worldwide each year. Gonorrhea significantly affects reproductive health and increases transmission of HIV. Antibiotic treatment is a critical control measure; however, this strategy is threatened by the rapid evolution of resistance in *Neisseria gonorrhoeae* (Gc). Gc susceptibility to ceftriaxone, the last remaining option for antibiotic monotherapy, has decreased globally over the last decade. Recently Gc has been elevated to "superbug" status due to the emergence of ceftriaxone-resistant (CRO^R) strains. Dual antibiotic therapy is now recommended in the USA and Europe. Ceftriaxone resistance in Gc is conferred primarily by mosaic *penA* alleles that encode an altered penicillin-binding protein 2 with up to 70 amino acid substitutions. Whether acquisition of these mosaic alleles is accompanied by a fitness cost is unknown.

Methods and Results Here we examined the impact of mosaic *penA* alleles from two well-characterised CRO^R clinical isolates, H041 (MIC = 2–4 µg/ml) and F89 (MIC = 1–2 µg/ml), on Gc fitness *in vitro* and *in vivo*. The wild-type *penA* allele of laboratory strain FA19 (CRO^S) was replaced by *penA41* or *penA89* to create mutants FA19*penA41* and FA19*penA89*, respectively. Acquisition of the mosaic alleles increased ceftriaxone resistance ≥ 500-fold. Both mutants grew significantly slower than FA19 in liquid culture. When cultured competitively with the parent strain, FA19*penA41* and FA19*penA89* demonstrated a fitness defect, as measured by competitive index. Mutants were attenuated relative to the parent strain during competitive murine infection. However, only CRO^R bacteria were recovered at later time points from 3 of 7 mice co-inoculated with FA19*penA41* and FA19, suggesting selection of compensatory mutations *in vivo*.

Conclusions Acquisition of mosaic alleles significantly reduced fitness of Gc, but compensatory mutations can be selected *in vivo* that alleviate fitness defects while maintaining resistance. Our studies may be useful in predicting the national and international spread of CRO^R Gc.

O21.4 *IN VITRO* SYNERGY DETERMINATION FOR DUAL ANTIBIOTIC THERAPY AGAINST RESISTANT *NEISSERIA GONORRHOEAE* USING ETEST® AND AGAR DILUTION

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Background Antimicrobial resistance (AMR) of *Neisseria gonorrhoeae* (Ng) is increasing. With recent resistance to last resort extended-spectrum cephalosporins, combination therapy of azithromycin (AZ) and ceftriaxone (TX) is now widely recommended. We used 2 methods to study *in vitro* synergy of recommended and new dual antibiotic combinations.

Methods A panel of 15 Ng strains with a minimal inhibitory concentration (MIC) of 0.064–8 for AZ and 0.012–2 for TX was tested for *in vitro* synergy, using both Etest and agar dilution checkerboard methods. Combinations of cefixime with AZ, colistin, ertapenem, gentamicin and moxifloxacin were also tested using the Etest method on 10 stains of the panel. Etests were placed crosswise at the MIC of each antibiotic in a 90° angle. All tests were performed in duplicate. MIC's were red after 16–18 hours (Etest) or 24–48 hours (checkerboard) incubation. Synergy was defined as a fractional inhibitory concentration index (FICI) ≤ 0.5.

Results Using the Etest method no synergy was found in any strain for any of the used combinations. Mean FICI for each combination was between 0.77–1.27. Individual FICI's varied between 0.49–2.00. Values ≤ 0.5 could not be confirmed in repeat testing. No antagonism was found. Mean FICI for AZ+TX was 1.27 (0.58–2.00). The results

of the checkerboard for AZ+TX indicated synergy for only 2 of the 15 strains (FICI: 0.16 and 0.5). The mean FICI of all strains was 0.64 (0.16–1.01). Adding AZ to TX could not lower the TX MIC below 0.25 for one TX resistant strain (MIC for TX alone: 2).
Conclusion The recommended combination therapy against Ng (AZ+TX) showed no *in vitro* synergy using either the Etest or the agar dilution method. Other combinations of antibiotics from various groups showed no indication of *in vitro* synergy using the Etest method.

O21.5 UNDERSTANDING THE MOLECULAR MECHANISM OF MTRR IN THE REGULATION OF ANTIMICROBIAL RESISTANCE IN NEISSERIA GONORRHOEAE USING *IN VITRO* AND IN SILICO STUDIES

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Background Neisseria gonorrhoeae, a major STD causing pathogens, tends to pose high burden of morbidity that is borne disproportionately by women and infants with approximately 2/3rd of cases from developing countries. In the absence of appropriate vaccine and rapid, easy, economical test, antibiotic therapy is recommended for treatment on the basis of clinical symptoms. This has led to the emergence of antibiotic resistant strains. Since increasing antimicrobial resistance makes Neisseria as super bug, we have tried to elucidate the mechanism of development of antibiotic resistance.
Methods Mutational analysis of mtrR gene and its DNA binding site was carried out for 28 clinical isolates resistant to multiple drugs. Wild type and mutant mtrR were cloned, expressed and purified. Fluorescence assay and electrophoretic mobility shift assay (EMSA) were carried out to study the effect of mutations in MtrR on its biological activity. Using discovery studio, structure of MtrR was modelled in-silico to understand how mutations affect its interaction with DNA.
Results Mutations in DNA binding domain (G45D) and dimerization domain of MtrR (H105Y) as well as in promoter region of MtrR (A/T deletion) were observed in clinical isolates (n = 28). EMSA and fluorimetric results suggest decreased binding of mutant MtrR with its promoter. In silico modelled structure of wild type and mutant MtrR proteins suggest altered conformation of the mutant protein. Altered conformation leads to difference in the posture of homodimer formed and increased centre to centre distance of helix 1 and helix 1' in two monomers of mtrR. In silico analysis of protein-DNA complex suggest that this increased distance cause altered binding of the mutant with DNA.
Conclusions Mutations in mtrR result is altered conformation of the protein leading to its decrease binding to DNA. This leads to enhanced expression of MtrCDE efflux pump resulting in increased efflux of drug.

O21.6 A TALE OF TWO CITIES: TREPONEMA PALLIDUM MACROLIDE RESISTANCE IN COLOMBO (SRI LANKA) AND LONDON (UNITED KINGDOM)

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Background The bacterium *Treponema pallidum* (*T. pallidum*) causes syphilis. Penicillin is effective treatment, but azithromycin (a macrolide) is a single-dose oral alternative for those with allergy. Unfortunately, macrolide resistance secondary to one of two 23S ribosomal RNA (rRNA) point mutations (A2058G and A2059G) is now wide-spread. Molecular strain-typing suggests that epidemics and macrolide resistance are unlikely the spread of single clones.

We present typing and macrolide resistance data from two geographically distinct populations: Colombo, Sri Lanka and London, UK.
Methods Cross-sectional studies were conducted at the Colombo District STD clinics and St Mary's Hospital, London. Ulcer exudate and/or blood were collected from patients with microbiologically confirmed syphilis. Presence of *T. pallidum* DNA (*tpp047* gene) was confirmed with PCR. Next, using published techniques, the *23SrRNA* gene was PCR-amplified for a point-mutation assay and *tpp0548*, *arp* and *tprE,G&J* amplicons were used for strain-typing.
Results Sri Lanka: 24 *T. pallidum* PCR-positive samples were collected. Patients were men (45.9% MSM) and 91.6% Sinhalese with a mean age of 28 (range 29). None were HIV-1 infected. Two strain types were discovered (14b/f and 13b/f), neither harbouring macrolide resistance.

London: 43 men were recruited, 18 in 2006–8 and 25 in 2011–12. Mean age was 37.5 (range 43); 95.2% were MSM and 62.8% were HIV-1 infected. Half (22/43) were white British. A total of 5 full and 14 partial strain types were identified, of which 6 were unique. Macrolide resistance increased from 66.7%(12/18) in 2006–8 to 80%(20/25) in 2011–12.
Conclusion Colombo *T. pallidum* strains have limited diversity with no macrolide resistance. London strains are more varied and increasingly macrolide-resistant. Ethnic diversity in London exceeds Colombo's and may explain increased strain diversity. In contrast to Sri Lanka, azithromycin is widely used to treat Chlamydia and non-specific urethritis in the UK thus selection pressure may be driving macrolide resistance.

O.22 - Alternative screening tools and screening sites

O22.1 EVALUATION OF SYPHILIS POINT OF CARE TESTS CONDUCTED BY MIDWIVES AT PRIMARY HEALTH FACILITIES IN GHANA

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Background Globally, over two million pregnancies are affected by syphilis annually, resulting in adverse pregnancy outcomes and severe sequelae in the newborn. Cost-effective strategies exist, which prevent vertical transmission. Ghana's Policy recommends antenatal (ANC) syphilis screening and treatment of positive clients, but pregnant women were often not tested especially in areas where laboratory services are unavailable. The study examined the performance of point-of-care (POC) tests for screening ANC attendants for syphilis conducted by midwives at the primary level health facilities in Ghana.
Methods The study was conducted from March to September 2010. In all, 1249 pregnant women attending ANC in 8 sites were recruited and tested using Determine® Syphilis TP (POC) and results compared with Treponema Pallidum Haem-Agglutination Test (TPHA) and Rapid Plasma Reaginin test (RPR).
Results The sensitivity of tests conducted by midwives was 25%, 60% and 75% when compared with TPHA, active syphilis (reactive to TPHA and RPR) and High titre active syphilis (HTS) (greater than 1:8) respectively. A higher sensitivity was noted in detecting active syphilis and high titre infections. The prevalence of syphilis using POC test on whole blood conducted by midwives was 5.5% (70/1282), at the district laboratory on serum samples was 10.1%

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