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.

021.2 EFFECT OF MUTATIONS IN *PILQ* ON THE SUSCEPTIBILITY OF *NEISSERIA GONORRHOEAE* TO CEPHALOSPORINS

doi:10.1136/sextrans-2013-051184.0204

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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.

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

doi:10.1136/sextrans-2013-051184.0205

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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.

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

doi:10.1136/sextrans-2013-051184.0206

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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