PATTERNS OF SEXUAL AND SOCIAL MIXING AMONG HETEROSEXUAL COUPLES LIVING TOGETHER IN ENGLAND: ANALYSES OF A PROBABILITY SAMPLE SURVEY

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Background Patterns of social and sexual mixing are a major determinant of STI transmission. In particular, discordant mixing is an important driver of STI dissemination when high risk populations mix with low risk populations. However patterns of mixing are poorly understood.

Method We analysed data from a probability sample survey of households in the Health Survey for England 2010. 1,891 heterosexual couples living together were included, all individuals were aged 16–69 years. Self-completion questionnaires were used to collect data on previous STI diagnosis/es, same-sex experience, condom use, age at first sex, and number of sexual partners.

Results Males were on average 2 years older than their female partners, though this age difference ranged from a mean of 0 years in those aged 16–24 to a mean of 3 years in those aged over 55. 85.1% of couples had matching characteristics of reporting previous STI diagnosis/es. After adjusting for age, socio-economic class and marital status, an association was found between males reporting previous STI diagnosis/es and their female partners also reporting the same, OR: 3.02 (95% CI: 1.78–5.15). Males who reported 10+ partners were more likely to be in a couple with a female who also reported this AOR: 2.71 (95% CI: 1.79–4.11). A positive correlation was found between men and women with respect to their age at first sex. There was also a correlation in socio-economic class but with greatest mixing between intermediate and higher/lower categories. A correlation was also found with respect to education level and drinking alcohol.

Conclusion We found evidence of significant levels of assortative mixing amongst heterosexual couples living together in England with respect to reporting previous STI diagnosis/es, numbers of partners, frequent drinking, socio-economic class and education. These analyses of probability sample survey data support the observed skewed distribution of STI transmission in the population.

SEXUAL PARTNERSHIP PATTERNS AMONG YOUNG PEOPLE IN RURAL TANZANIA

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Background Sexual partnership patterns influence risk of STI transmission. We describe the pattern of partnerships reported by youth in a survey in rural Tanzania and calculate the UNAIDS recommended measure of concurrency.

Methods In 2007/8, sexual partnership histories were collected, through a face-to-face questionnaire, from 13,814 15–30 y-olds (90% aged 19–25 y) in 20 communities, in Mwanza, Tanzania. Partnership patterns of sexually active participants were described based on reported dates of first and last intercourse with their last 3 partners in the past year. One-off partnerships had the same date of occurrence with greatest mixing between intermediate and higher/lower categories. A correlation was also found with respect to education level and drinking alcohol.

Results Males and males had mean age of 21 and 22 years respectively. In the year prior to the survey, 87% of females and 79% of males reported at least one sexual partner, and 15% of females and 44% of males reported >3 partners. Among those reporting 1–3 partners, 47% of one-off partnerships started within 4 months of the survey suggesting reporting bias and/or censoring of data. Only 3% of females reported >2 new partners in the previous year compared to 26% of males, and 3% of females reported >1 partner in the last 4 weeks compared to 18% of males. The point prevalence of concurrency was 2.3% for females and 10.7% for males. Partnership patterns varied by sex and marital status (Table). The ‘Previously married’ group were the most likely to report multiple partners.

Conclusions High levels of multiple and concurrent partnerships were reported by males and the ‘previously married’. Further analysis of the characteristics of the specific partners and partnerships will be completed to understand the risk associated with each pattern of partnerships. Analyses will also be adjusted for the bias introduced by restricting questions to the last 3 partners.

0.21 - Antimicrobial resistance mechanisms in STI pathogens

MYCOPLASMA GENITALIUM INFECTION LOAD AND TREATMENT FAILURE DUE TO SELECTED MACROLIDE RESISTANCE

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Background Treatment failure due the development of macrolide resistance seen at Melbourne Sexual Health Centre (MSHC) has risen from 15 to 46% over the last six years (p < 0.01). Macrolide resistance is conferred through point mutations in the M. genitalium 23S rRNA gene. Treatment failure with 1g azithromycin is either due to an infection with a pre-existing resistant strain or resistance seen at MSHC has not yet fully understood.

Methods A subset of M. genitalium positive cases seen at MSHC between 2007–9 (n = 67) and 2012 (n = 70) underwent detection of resistance mutations via high resolution melt analysis, with real-time PCR quantifying M. genitalium infectious load.

Result Of those M. genitalium cases that successfully responded to 1g azithromycin, the pre-treatment median loads for 2007–9 (n = 40) and 2012 (n = 48) were 8.5 x 102 and 1.7 x 103 copies per reaction respectively. For M. genitalium strains that possessed resistance in the pre-treatment sample, the loads were remarkably similar to those successfully treated, with 2.2 x 103 copies per reaction detected for 2007-9 (n = 9) and 5.7 x 103 copies for 2012 (n = 22). However, for M. genitalium cases that appeared to develop resistance following
to mutate to still higher MIC values.

**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


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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 *penA*, *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 ceftazidime 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 μg/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 ceftriaxopil, 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 *PEN A* ALLELES FROM CEFTRIAZINU-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. Gonorrhoea 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) 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 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) was replaced by *penA*41 or *penA*89 to create mutants FA19*penA*41 and FA19*penA*89, 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*penA*41 and FA19*penA*89 demonstrated a fitness defect, as measured by competitive index. Mutants were attenuated relative to the parent strain during competitive murine infection. However, only CRO89 bacteria were recovered at later time points from 3 of 7 mice co-inoculated with FA19*penA*41 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 Gc.

**021.4** 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 0.064–5 μg/ml for AZ and 0.012–2 μg/ml for TX was tested for in vitro synergy, using both Etest and agar dilution checkerboard methods. Combinations of cefixime with AZ, cefotaxime, 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. MICs were read 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