

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.

## 021.5 UNDERSTANDING THE MOLECULAR MECHANISM OF MTRR IN THE REGULATION OF ANTIMICROBIAL RESISTANCE IN NEISSERIA GONORRHOEA USING *IN VITRO* AND *IN SILICO* STUDIES

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**Background** *Neisseria gonorrhoeae*, a major STD causing pathogen, 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 in altered conformation of the protein leading to its decreased binding to DNA. This leads to enhanced expression of *MtrCDE* efflux pump resulting in increased efflux of drug.

## 021.6 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 (*tp047* gene) was confirmed with PCR. Next, using published techniques, the 23S rRNA gene was PCR-amplified for a point-mutation assay and *tp0548*, *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.

## 0.22 - Alternative screening tools and screening sites

### 022.1 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 Reagin 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%