To Study the Development of Diagnostic Assay for Co-detection of *Neisseria gonorrhoeae* and *C. trachomatis* & Elucidating the Molecular Mechanism of Antimicrobial Resistance in *Neisseria gonorrhoeae*

ABSTRACT

Submitted to the University of Delhi
For the Award of the Degree of
DOCTOR OF PHILOSOPHY
(Biomedical Sciences)
2013

*Under the Supervision of*
Prof. Daman Saluja

DIVYA SACHDEV

Dr. B.R. Ambedkar Center for Biomedical Research
University of Delhi
Delhi-110007
India
Neisseria and Chlamydia, two major STD causing pathogens, tends to pose high burden of morbidity that is borne disproportionately by women and infants with approximately 2/3\textsuperscript{rd} of cases from developing countries. Symptoms of infection include cervicitis, epidymiitis, salphingitis, PID, ectopic pregnancy and infertility. In the absence of appropriate vaccine and rapid, easy economical test, antibiotic therapy is only recommended for treatment given on the basis of clinical symptoms. Syndromic management not only misses out asymptomatic patients but uninfected individuals with similar symptoms are also recommended treatment. This overtreatment leads to the emergence of antibiotic resistant strains.

In present study we developed an easy, economic, rapid cost effective PCR based test for co-detection of both the pathogens in single test. Further we have modified our test and developed visual assay for detection of single pathogen using molecular beacon probe. This reduces the detection time as PCR products could directly be visualized under dark reader. This also reduces the chance of cross contamination and eliminates the use of carcinogenic compounds like EtBr for running of agarose gel. Stability of master mix was also standardized at 4°C for 4 months. We also found that our gDNA isolation method and PCR works equally good when dry swabs were used for the collection of cervical swabs. This will make our method, choice of test at peripheral laboratories with limited infrastructure.

Since increasing antimicrobial resistance makes Neisseria as super bug, we have tried to elucidate the mechanism of development of antibiotic resistance. This will help development of newer antibiotics. Clinical isolates (n=28) were studied for mutational analysis of various genes (porB, penA, mtrR and mtrE) responsible for imparting antimicrobial resistance. We have observed that mutations in various chromosomal genes act synergistically to impart high resistance to Neisseria. Majority of penicillin resistance was found to be plasmid mediated with African type plasmid found in all the isolates. Single or double mutations at G120 and A121 position are frequent and N122K is a novel mutation observed in few resistant isolates. We also found that mutations in PorB alone are not responsible for imparting high resistance to Neisseria.
and act in conjunction with mutations in MtrR. L33V is a novel mutation observed in MtrR along with G45D mutation which makes the isolate hypersusceptible as compare to isolate with G45D mutation alone. Mutations in PenA and MtrE also play an important role in imparting enhanced antimicrobial resistance.

Mutations both in DNA binding domain (G45D) and dimerization domain of MtrR (H105Y) as well as in promoter region of MtrR (A/T deletion) leads to enhanced expression of MtrCDE efflux pump. We have tried to elucidate the mechanism of action of H105Y mutation in C Terminal domain of MtrR. Attempts have been made to in-silico model the structure of MtrR to understand how mutations affect its interaction with DNA. Protein structure of wild type and mutant MtrR was compared to understand the structural effect of mutations. We also studied the binding of wild type and mutant MtrR with penicillin as well as DNA using both in silico approach and fluorimetric assay. Our in silico results suggest altered conformation of the mutant protein and enhanced binding of penicillin with mutant MtrR. 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. Fluorimetric results showed that binding of penicillin with mutant MtrR leads to alteration in conformation. These two mechanisms may act together to cause altered binding of the mutant with DNA. Fluorescence assay and electrophoretic mobility shift assay were carried out to study the effect of mutations in MtrR on its biological activity. Our EMSA results and fluorimetric results suggest decreased binding of mutant MtrR with its promoter. This is further supported by in silico DNA protein binding experiments.