Chapter 7
SUMMARY AND CONCLUSIONS
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In order to provide adequate and rational therapy to the patients with symptoms of gonorrhea, it is imperative to know the in vitro susceptibility of *N. gonorrhoeae*. We, therefore, decided to determine the antimicrobial susceptibility pattern of recently isolated *N. gonorrhoeae* strains from Pune, Delhi, Hyderabad, Nagpur and Mumbai. The study was conducted on 64 *N. gonorrhoeae* strains recently isolated from male and female patients attending STD clinics during the period Jan-2007 to Jun-2009. The minimum inhibitory concentrations for various antibiotics for all the isolates were determined by E test except for enoxacin, agar dilution was performed. Antimicrobial susceptibility testing revealed that 57.8% strains were resistant to tetracycline, 48.4% to penicillin & of these penicillin resistant strains, 15.6% were PPNG. All the isolates were found sensitive to ceftriaxone, cefixime & spectinomycin.

Sero typing & auxotyping of these strains revealed that the strains belonging to AUH group were sensitive to penicillin whereas Bopt strains were resistant to penicillin and tetracycline. Most of our PPNG strains were non requiring and apparently belonged to WI group. We observed 31 auxotype/ serovar (A/S) classes, the commonest being NR/Bopt, NR/AV/Bx, NR/Aost, Pro/ Bopt, Pro/AV/Bx. A combination of serotyping and auxotyping seems to be the best way to differentiate *Neisseria gonorrhoeae* strains in epidemiologic studies, bringing together the most simple techniques and the best discriminatory power among isolates.

High level of prevalence of resistance to ciprofloxacin was observed in last few years in the clinical isolates of *N. gonorrhoeae*. The effect of each mutation in an isolate is not equivalent
for all quinolones, due to variations of the chemical structures among this class of agents. Therefore this study was performed on recently isolated quinolone resistant strains of *N. gonorrhoeae*. This study was carried out to identify and characterize mutations in the *gyrA*, *parC* and *mtrR* genes in isolates resistant to different antibiotics in the quinolone group and for comparing these mutations with the level of resistance. Antibiotic susceptibility tests showed 17.1% resistance to gatifloxacin, 95.3% to lomefloxacin, 96.8% to enoxacin & ciprofloxacin and 98.4% to norfloxacin & ofloxacin. DNA sequence analysis of the PCR amplified regions of *gyrA*, *parC* and *mtrR* genes of these isolates revealed that the isolates resistant to all six antibiotics (n=11) had double mutations in *gyrA*, single mutations in *parC* and multiple mutations in *mtrR* gene. Of the 48 strains with intermediate resistance or susceptible to gatifloxacin, 14% strains showed mutations in all the three regions, 15.6% in *gyrA* and *parC*, 17.1% in *gyrA* and *mtrR*, and 28.1% in *gyrA* gene only. The number and pattern of mutations in *gyrA*, *parC* and *mtrR* in the strains resistant to six and that in the strains resistant to five antibiotics were similar while strains resistant to ≤ 4 antibiotics had single or double mutations in *gyrA* only. The quinolone resistant mutations were detected at codon 91 & 95 in *gyrA*, at codon 91 in *parC* and at codon 39, 45, 47, 105, 77, 78, 79, 86, 98, 105, 110 in *mtrR* gene. The common mutation observed in *gyrA* & *mtrR* gene was Ser-91→Phe (95.3%) & Tyr-105→His (42.2%). Asp-95→Gly was the most prevalent mutation in strains isolated from Delhi and Asp-95→Asn was restricted to Pune strains only. Ser-91-Thr mutation in *gyrA* is first time reported from India. Interestingly, Ser-91-Thr mutation was detected only in MSM population. The difference in the proportion of strains showing mutations in *gyrA* and *parC* was found to be significant. The strains resistant to all antibiotics were significantly (p<0.001) associated with presence of double / triple mutations in the *mtrR* coding region. Proportion of multiple mutations in *gyrA* gene was
100% whereas that in mtrR gene it was much lower (46.8%) and this proportional difference was found to be statistically significant (p<0.01)

The phylogenetic analysis showed that the strains isolated from different geographic areas showed similarity except the strains isolated from Mumbai formed a diverse cluster and these strains were isolated from MSM population.

This is the first study, reporting mutation patterns in gyrA, parC & mtrR genes in quinolone resistant *N. gonorrhoeae* isolates from India. We found significantly more number of *N. gonorrhoeae* isolates showing mutations in gyrA gene as compared to that parC or mtrR (p<0.001). Mutation in gyrA gene was strongly associated with quinolone resistance indicating that mutation at this region contributes largely to the development of resistance. Mutations in parC and mtrR regions were not significantly associated with resistance indicating that these mutations play a complementary role. Thus our results add to the existing knowledge of quinolone resistant mutations in *N. gonorrhoeae* worldwide which will be important in understanding mechanisms of decreased susceptibilities to quinolones as well as in carrying out epidemiological assessments of transmission and spread of quinolone resistant strains, which may be eventually useful for the development of newer drugs for management of gonorrhoea.