Chapter 1
Introduction
INTRODUCTION

Gonorrhoea is a common sexually transmitted disease in India and other developing countries. Gonorrhoea is caused by bacterium *N. gonorrhoeae* & is easily curable. However, undetected, untreated infections can lead to complications like pelvic inflammatory disease, ectopic pregnancy, tubal factor infertility, adverse pregnancy outcomes in females and testicular and prostate infections and infertility in males. Gonorrhoea has gained tremendous importance in the last few decades because of its role as a co-factor in increasing HIV infections (Cohen et al 1997, Laga et al 1993). Thus it is important to undertake appropriate and timely treatment of gonorrhea. The emergence of resistance to antimicrobial agents in gonococci has complicated its treatment and control and hence inspite of availability of number of effective antibiotics, control of gonorrhea has remained a formidable public health challenge. A major contributing factor to the continued spread of gonococcal infection is the remarkable ability of *N. gonorrhoeae* to acquire resistance to antibiotics. Over the last two decades, *N. gonorrhoeae* strains have developed high level resistance against several antimicrobial agents like penicillin, tetracycline and quinolones in different countries (Stathi et al 2006, Wang B et al 2006, Tapsall et al 2005). In 1989, in response to the increasing frequency of isolation of chromosomally & plasmid-mediated tetracycline & penicillin *N. gonorrhoeae* strains throughout the world, the Centres for Disease Control and Prevention (CDC) recommended the use of broad spectrum cephalosporins or fluoroquinolones for the primary treatment of uncomplicated gonorrhea (CDC, MMWR 1989). Oral fluoroquinolone (quinolones) became the first line recommended therapy for gonococcal infection in 1993 (CDC, MMWR 1993). Reports on antibiotic resistance in
N. gonorrhoeae from India have described a varying resistance pattern and also increasing trend for all antibiotics (Khaki et al 2007, Bala M et al 2008, and Kulkarni et al 2011). During the past decade quinolone resistant Neisseria gonorrhoeae have been reported from Asia, Africa, United Stae and Europe (Su X et al 2001, Tompkins et al 2001, Arreaza et al 2003, De Jongh et al 2007, Lewis et al 2008, Wang et al 2007). A high resistance to ciprofloxacin (95-100%) has been reported in different parts of India (Bala M et al 2007, 2008, Khaki et al 2007).

The genetic mechanism for the evolution of antimicrobial resistance in gonococci is either chromosomal mutations or the acquisition of R plasmids. Penicillinase producing strains of N. gonorrhoeae (PPNG) are reported to carry β- lactamase enzyme coding plasmid, six of which have been reported so far. Penicillinase production in these plasmids is mediated by a β-lactamase gene encoded by a truncated TnA transposon. Strains with mutations in chromosomal genes emerged in the late 1950s. (Willcox RP 1970). Two types of chromosomal resistance have been described. The first type is drug specific; it is due to single-step mutation to high level resistance. The second type involves mutations at several chromosomal loci. The combination of mutations at these various loci determines the level as well as the pattern of resistance (Lind et al 1997).

The genetic mechanisms of N. gonorrhoeae resistance to antibacterials are complicated and not definitely elucidated. However, acquisition of some genes and a number of mutations in structural genes and regulatory regions are considered to be involved unambiguously in resistance development. A number of studies have been performed to determine the mechanism of antibiotic resistance in N. gonorrhoeae (Wang et al 2006, Ruiz et al 2005, Dewi et al 2004, Chisholm et al 2010, Warner et al 2008, Allen et al 2011, Ohneck et al 2011). Various studies
have investigated the mechanism of resistance to penicillin, tetracycline, spectinomycin, and azithromycin, ceftriaxone resistance (Galimand et al 2000, Wang et al 2006, Ilina et al 2008, Allen et al 2011, Chisholm et al 2010 and Caramante et al 2012). The rapid demise of sulphonamides in treating gonorrhea, penicillins became the mainstay of global therapy for almost 40 years, initially at very low dosage and subsequently given at higher doses with probenecid. Tetracyclines or erythromycin were used during this period for the management of penicillin allergic patients and sometimes for cases of chromosomally mediated penicillin-resistant *N gonorrhoeae* infection. The global spread of high-level plasmid-mediated penicillin and tetracycline resistance among *N gonorrhoeae* isolates in the 1980s effectively sealed the fate of these antibiotics in terms of gonorrhoea treatment (Lewis et al 2010). Spectinomycin provided a temporary solution to the problem of penicillinase-producing *N gonorrhoeae* but resistance rapidly developed with first-line use (Boslego et al 1987). Spectinomycin is now seldom used due to the high cost and practical unavailability but remains useful in special instances, such as treating pregnant women with severe penicillin allergy or cephalosporin-resistant gonorrhoea. In most countries, quinolones rather than intramuscular ceftriaxone replaced penicillins and spectinomycin as first-line oral therapy for gonorrhoea in the 1980s and were used with success for over a decade before resistance developed, initially in the Asia Pacific region and subsequently in the USA, Europe and Africa (Dan 2004, Lewis et al 2010). The quinolones most widely used for the treatment of gonorrhoea were second generation antimicrobials such as ciprofloxacin, norfloxacin and ofloxacin (Andriole VT 1998). Gatifloxacin, the third generation quinolone was used for treatment in *N gonorrhoeae* patients showing non-susceptible to ciprofloxacin (Fung-Tomé et al 2001). The fourth generation quinolones, such as trovafloxacin, have been tested for treatment of gonorrhoea but information on resistance to this antimicrobial is
Quinolone resistance is almost exclusively mediated by chromosomal mutations, which affect either the target sites or the access of the antibiotic to the cell (Tapsall WHO 2001). These altered proteins can no longer be bound by the fluoroquinolone and therefore the drug is unable to inhibit DNA replication and the bacterium becomes less susceptible or resistant. The targets of the quinolone resistance are gyrA gene, coding for DNA gyrase, and in the analogous region of the parC gene coding for DNA topoisomerase IV. High-level clinically relevant resistance is mediated by alteration of the target sites, initially, via mutation in the gyrA gene. Multiple amino acid substitutions have been described which, when combined, result in high-level resistance. Multiple mutations also occur in the parC gene which codes for the production of topoisomerase IV, a secondary target for quinolones in gonococci, but again found in association with high-level resistance. (Su X et al 2001, Zhang et al 2009, Unemo et al 2009, Illina et al 2008, Dewi et al 2006, Tanaka et al 2004). Changes in parC seem to arise in the presence of mutations affecting gyrA. Quinolone resistance mutations in N. gonorrhoeae at codon 67,75,84,91,95,120 in gyrA and at 85,86,87,88,91,92, 100,116 in parC have been reported (Dewi et al 2004, Illina et al 2008, Zhang et al 2009, Unemo et al 2009).

Multiple mutations are required to generate clinically important resistance (Hooper DC 2001) and up to 4 simultaneous amino acid alterations in gyrA and parC have been identified in high-level fluoroquinolone resistance strains of N. gonorrhoeae (Choudhary et al 2002, Illina et al 2008, Tanaka et al 2004, Shutz et al 2001, SuX et al 2001).

The efflux mediated resistance to quinolones, which is well recognized in a number of Gram negative bacteria (Poole 2000), has also been reported in N. gonorrhoeae. Mutations in MtrRCDE (Mtr, multiple transferable resistance) efflux system, specifically in the promoter and the coding sequence of the transcriptional repressor (mtrR) cause the enhanced expression of
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efflux pump (Zarantonelli et al 2001, Veal et al 2002, Dewi et al 2004, Illina et al 2008). The efflux-mediated resistance to fluoroquinolones has also been reported in *N. gonorrhoeae* (Dewi et al 2004, Vereshchagin et al 2005). Earlier studies have showed that the mutation in the *mtrR* efflux system was due to the alteration in Ala-39→Thr, Gly-45→Asp, and Tyr-105→His region (Dewi et al 2004, Hagman et al 1995, Unemo et al 2009, Illina et al 2008,). The fluoroquinolones have broad-spectrum bactericidal activity, excellent oral bioavailability, good tissue penetration and favorable safety and tolerability profiles. As the effect of each mutation in an isolate is not equivalent for all fluoroquinolones, due to variations of the chemical structures among this class of agents this study was performed on quinolone resistant strains of *N. gonorrhoeae*.

Various studies on antimicrobial susceptibility, auxotyping, serotyping and plasmid analysis have been carried out in India (Divekar et al 1999, Ray et al 2000, Khaki et al 2007, Bala M et al 2008, Bala M 2010, Kulkarni et al 2011) but only one study has been carried out on quinolone resistance mutations in *gyrA* and *parC* in India (Chaudhry et al 2002). The information regarding the quinolone resistance mutations in *MtrRCDE* efflux system however is very limited and no report is available on Indian strains. Therefore this study was conducted to identify and characterize mutations in the *gyrA, parC & mtrR* genes in *N. gonorrhoeae* isolates resistant to different antibiotics in the quinolone group and to compare these mutations with the level of MICs.