Review Article

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An insight into the drug resistance profile & mechanism of drug resistance in *Neisseria gonorrhoeae*

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Among the aetiological agents of treatable sexually transmitted diseases (STDs), *Neissseria gonorrhoeae* is considered to be most important because of emerging antibiotic resistant strains that compromise the effectiveness of treatment of the disease - gonorrhoea. In most of the developing countries, treatment of gonorrhoea relies mainly on syndromic management rather than the aetiological based therapy. Gonococcal infections are usually treated with single-dose therapy with an agent found to cure > 95 per cent of cases. Unfortunately during the last few decades, *N. gonorrhoeae* has developed resistance not only to less expensive antimicrobials such as sulphonamides, penicillin and tetracyclines but also to fluoroquinolones. The resistance trend of *N. gonorrhoeae* towards these antimicrobials can be categorised into pre-quinolone, quinolone and post-quinolone era. Among the antimicrobials available so far, only the third-generation cephalosporins could be safely recommended as first-line therapy for gonorrhoea globally. However, resistance to oral third-generation cephalosporins has also started emerging in some countries. Therefore, it has become imperative to initiate sustained national and international efforts to reduce infection and misuse of antibiotics so as to prevent further emergence and spread of antimicrobial resistance. It is necessary not only to monitor drug resistance and optimise treatment regimens, but also to gain insight into how gonococcus develops drug resistance. Knowledge of mechanism of resistance would help us to devise methods to prevent the occurrence of drug resistance against existing and new drugs. Such studies could also help in finding out new drug targets in *N. gonorrhoeae* and also a possibility of identification of new drugs for treating gonorrhoea.

Key words Epidemiology - mechanism of drug resistance - *Neisseria gonorrhoeae*

Introduction

Despite the recent advances in diagnosis, surveillance and treatment, sexually transmitted diseases (STDs) remain one of the leading diseases throughout the world. Increased promiscuity and onset of sexual activity at an early age are two important contributing factors to the spread of sexually transmitted diseases. *Neisseria gonorrhoeae* (also known as the gonococcus) colonizes primarily in the human genitourinary tract, giving rise to the sexually transmitted infection gonorrhoea. It causes both symptomatic and asymptomatic genital and extragenital tract infections. Disease caused by this organism is a significant public health problem despite continual advances in treatment[1,2]. Worldwide, there

are an estimated 62 million new cases a year, with an average of 22 million cases at any given time[3-5]. *N. gonorrhoeae* inhabits mainly mucosal surfaces of the urethra in males and the cervix in females. As the signs and symptoms of infection are often absent or obscure, complications such as pelvic inflammatory disease (PID), infertility, ectopic pregnancy arise[6]. Infection in pregnant women may lead to crucial perforation and blindness in the newborn. Gonococcal infections have also been documented to facilitate acquisition and transmission of HIV and HPV infections[7,8]. Asymptomatic infections by *N. gonorrhoeae* largely contribute to the persistence and transmission of disease in a community[9]. Therefore, to eliminate *N. gonorrhoeae* infections and in turn to control HIV and HPV infections it is important not only to screen high-risk population but also to treat them immediately with most effective drugs. Control of gonococcal infections has relied on effective single-dose antibiotic therapy given at the initial clinical visit, prior to any knowledge of the organism's susceptibility pattern. In the recent past, there has been an alarming increase in the number of isolates of *N. gonorrhoeae* resistant to commonly used drugs[10-14]. Surveillance is, therefore, necessary to understand ongoing resistance trends and to ensure the success of any therapy. The irrational and injudicious use of antibacterial agents, especially in the developing countries is encouraging this trend and the situation is expected to worsen unless appropriate steps are initiated. Thus, resistance of the gonococcus to antibiotics has been the cause of much concern in recent years and has been the subject of extensive investigation. The present review summarizes and trends of drug resistance in *N. gonorrhoeae,* mechanism of drug resistance and discusses the treatment regime. In addition, the need to look for new and alternative antibacterial agents is also emphasized.

Characteristics of gonoccocus

N. gonorrhoeae is a Gram-negative diplococcus and is known to infect humans only. It is closely related to and probably derived from *Neisseria meningitidis*, but has become highly adapted to survival in the genital tract. It is transmitted by human-to-human contact and survives poorly outside the human body. *N. gonorrhoeae* is a very successful pathogen as it can evade or adapt to host defences, persist without severely damaging the host, and be transmitted to and infect other hosts, thereby maintaining itself. Particularly in women, gonococci may produce only mildly symptomatic or asymptomatic disease. This adaptation allows the organism to persist and disseminate over long periods[15].

The most important characteristics of *N. gonorrhoeae*, in the context of antimicrobial resistance pattern, are its phenotypic and genotypic variability which enables it to evade the host response. Phenotypic variability occurs through differential expression of existing parts of the genome. Genotypic variation is achieved by incorporation of new genetic material, which can be acquired either by conjugation or transformation. It is because of this feature that *N. gonorrhoeae* has acquired penicillinase producing plasmids. Another important feature of *N. gonorrhoeae* is its antigenic variability. This helps the bacterium to survive in its limited host, *i.e.,* humans. Antigenic variability of *N. gonorrhoeae* is partially due to its ability to acquire genetic material from related organisms[16,17].

Epidemiology

Single dose therapy for *N. gonorrhoeae* infection has become the norm in most of the countries throughout the world. The basic reason behind this is that single dose therapy is most effective and assures adequate treatment. World Health Organization (WHO) recommendations for selecting any treatment for gonorrhoea states that the antimicrobial prescribed should be such that the cure rate is about 95 per cent[18,19]. Moreover, during the past few years *N. gonorrhoeae* has started developing resistance against most of the antimicrobials that are prescribed for its therapy. Therefore, surveillance of the antimicrobial resistance becomes very important in monitoring the emergence and spread of resistance and in planning appropriate treatment regimens. Gonorrhoea is a disease mainly found in resource-poor settings where laboratory facilities are limited or unavailable. Due to this reason, culture and antimicrobial susceptibility testing of *N. gonorrhoeae* is hardly done in the developing countries. Developed countries have collected the data in proper manner due to availability of adequate resources. Such planned studies are always of help in monitoring resistance pattern in the bacteria. However, resistance data obtained from developing countries are mainly from point prevalence studies, which cannot be used to follow the trend. Such epidemiological studies need to be done on a regular basis because the prevailing strains of the bacteria and their antimicrobial susceptibility profiles keep on changing. The data obtained on *N.*

gonorrhoeae susceptibility, so far, are incomplete and there is an urgent need for proper surveillance throughout the world.

Pre-quinolone era

Sulphanilamide was introduced as an antimicrobial against *N. gonorrhoeae* as early as in 1937[20,21]. However, the bacteria became resistant quickly against sulphanilamides within a span of two years. At the same time, when sulphanilamides were given as treatment for gonorrhoea, Alexander Fleming also documented the ability of penicillin to inhibit the growth of *N. gonorrhoeae* in his 1929 paper describing penicillin discovery[22]. Thereafter, penicillin became the choice of antimicrobial for the treatment of gonorrhoea in 1943 and remained so for decades[23]. Suceptibility profile of *N. gonorrhoeae* against penicillin (and other antimicrobials) was monitored throughout the world, and *in vitro* resistance to penicillin was expressed in a uniform manner in terms of minimum inhibitory concentrations (MIC). During the initial years of treatment of gonorrhoea with penicillin, all the isolates had an MIC of <0.0125 mg/l (0.02 IU/l) and were considered to be sensitive to the treatment[24,25]. However, *N. gonorrhoeae* began developing low level resistance to penicillin. The MIC values of *N. gonorrhoeae* isolates gradually increased to >0.12 mg/l[24,26] and gradually most of the strains became resistant to penicillin (MIC >0.5 mg/l)[27]. Due to this increase in the MIC values, it was necessary to increase the effective dose of penicillin from 50,000 units in 1945 to 4.8 million units by 1970s[24,28]. This increase in penicillin resistance was proved to be the additive effect of multiple chromosomal mutation resulting in altered penicillin binding proteins, increase in the antibiotic efflux system and probably decrease in the antibiotic uptake from the membrane[29]. Chromosomal mediated penicillin resistance was found to be of low level as determined by the MIC values. Simultaneous to the development of chromosomal mediated resistance, high-level plasmid mediated resistant isolates of *N. gonorrhoeae* were also observed in various countries[24,30,31]. These isolates were termed as penicillinase producing *N. gonorrhoeae* (PPNG) as these harboured a plasmid having a gene of β-lactamase, the product of which conferred resistance towards penicillin. Reports of such high level penicillin resistant isolates were documented in Africa, Asia, North America, Europe and Australia[32]. Due to the emergence of penicillin resistant isolates (both chromosomal and plasmid mediated) of *N. gonorrhoeae*, penicillin was no longer considered an effective treatment for gonorrhoea by 1989 and, therefore, penicillin was also prohibited in most parts of the world[24,32,33].

A few documented reports of antimicrobial susceptibility data from India suggested a slow step-wise increase in penicillin resistance[34-36]. Low level resistance of *N. gonorrhoeae* to penicillin was observed first in India as early as 1981 in Madras, where the first case of β-lactamase isolate of *N. gonorrhoeae* was documented[37]. Thereafter, various other reports indicated the increase in the resistance profile of *N. gonorrhoeae* towards penicillin. Most of the isolates resistant to penicilline were found to be harbouring β-lactamase producing plasmid[38,39]. Use of penicillin for the treatment of gonorrhoea was discontinued in India in 1990[36]. As a result, penicillin resistance (both chromosomal and plasmid mediated) decreased subsequently[36]. Thereafter resistance towards penicillin once again showed a steep rise to 42.4 and 66.7 per cent in 2000 and 2001 respectively, along with increase in isolation of penicillinase producing *N. gonorrhoea* (PPNGs)[40]. Study conducted by Ray *et al*[40] showed high level of penicillin resistance from Hyderabad (79%) and Chennai (62.5%), while low level of resistance (20-33%) for penicillin was observed from isolates obtained from Kolkata, Nagpur and Pune.

Coincident with the development of resistance to penicillin, gonococci also developed resistance to several other antibiotics, including tetracycline, chloramphenicol, erythromycin and streptomycin[41-44]. Tetracycline was also considered as another important antimicrobial during the pre-quinolone era. Since tetracycline was not very expensive and thus was a widely used antimicrobial, and most importantly tetracycline was given as an adjunct therapy for *Chlamydia trachomatis,* it was not possible to evaluate the contribution of tetracycline in the management of gonorrhoea. Same was the case with azithromycin, even though it was considered to be a more expensive alternative. Gradually through constant use of tetracycline to treat such co-infections, *N. gonorrhoeae* acquired low-level resistance towards this antimicrobial. High-level chromosomally mediated tetracycline resistance emerged in the 1970s along with chromosomally mediated penicillin resistance[24]. Plasmid mediated tetracycline resistant *N. gonorrhoeae* (TRNG) emerged in 1985 in Atlanta and The Netherlands and was probably a result of the acquisition on a plasmid, a *tet*-M determinant from streptococcal species[29-32,45,46].

Various reports from USA also indicated the presence of both chromosomal mediated tetracycline resistance and TRNG[24,47]. In 1997, 25.6 per cent of isolates from USA were tetracycline-resistant, of which 17 per cent were chromosomally mediated and 8.6 per cent were TRNG[48]. Regional data from USA showed an increase in TRNG from less than 5 per cent in 1990 to early 15 per cent in 1995. In the WHO Western Pacific study, TRNG were widely but unevenly distributed. In 1998, particularly high proportions of TRNG were seen in Singapore (84%), the Solomon Islands (74%) and Vietnam (35.9%), continuing a pattern observed in earlier years[18]. In all other regions TRNG distribution was below 10 per cent. Reports of high level tetracycline resistance were also documented in Africa, Europe and Netherlands[18].

TRNG strains were also identified in the WHO South East Asia Region, and Thailand alone accounted for about 16 per cent of isolates in 1994-1997[18]. An additional 55 per cent of strains had chromosomal-mediated resistance. Indonesia had particularly high rates of TRNG, and virtually all *N. gonorrhoeae* isolates show one or the other forms of resistance[49]. In India, decreased susceptibility towards tetracycline was reported as early as in 1971 in Mumbai with 28 per cent of the isolates with decreased susceptibility towards tetracycline[50]. Bhalla *et al*[34] found 28 per cent of 50 consecutive isolates in New Delhi to be TRNG. In 1997, 10 per cent of 94 isolates from Bangladesh were TRNG[51]. In 2000/2001, a study conducted by Ray *et al*[40] reported high percentage resistance in three centers of India (Hyderabad, Nagpur and Pune).

Another important antimicrobial, the aminocyclitol (spectinomycin) was introduced for the therapy against antibiotic resistant gonococci or in patients who were allergic to other drugs. Most gonococci have remained sensitive to spectinomycin, except for the report describing three strains in Europe, which are apparently single-step high-level resistant mutants[52]. First case of spectinomycin resistant *N. gonorrhoeae* isolate was reported in India in 2002[53].

Quinolone era

Quinolones in the treatment of N. gonorrhoeae: In 1989, in response to the increasing frequency of isolation of penicillin, tetracycline, streptomycin and spectinomycin-resistant strains of *N. gonorrhoeae* in the United States and also throughout the world, the Centers for Disease Control and Prevention (CDC) recommended the use of broad-spectrum cephalosporins or fluoroquinolones for the primary treatment of uncomplicated gonorrhoea[54]. Similar policies were also adopted by various national organizations in other parts of the world. The quinolones most widely used for the treatment of gonorrhoea are second generation antimicrobials such as ciprofloxacin, norfloxacin and ofloxacin[55]. The fourth-generation quinolones, such as trovafloxacin, have been tested for the treatment of gonorrhoea, but information on resistance to this antimicrobial is not available. It was noticed that among the quinolones, fluoroquinolones had excellent oral absorption and good tissue distribution, achieved excellent interstitial fluid levels and adequate penetration into macrophages, were free from any serious toxic side effects and induced low frequency of spontaneous single-step mutations. Due to their excellent safety and tolerability, these have become popular alternatives to penicillin and cephalosporin derivatives in the treatment of various infections including gonococcal infection. Soon fluoroquinolones were regarded to be as close as possible to the 'ideal' antimicrobial agent, since these possessed a broad spectrum of antimicrobial activity. By 1993, ciprofloxacin was recommended as the first line therapy to treat gonorrhoea throughout the world[56].

Quinolone resistance profile in N. gonorrhoeae: Initially most of the isolates of gonococci were found to be extremely susceptible to quinolones and more importantly, fluoroquinolones. Widespread use of fluoroquinolones in last 20 years and often misuse, coupled with emerging resistance, gradually compromised their utility[54]. Since the importance of proper documentation of antimicrobial susceptibility studies was understood, data became available from all parts of the world[57]. Knapp's criteria for *in vitro* resistance to quinolones have been followed in almost all the studies carried so far[58]. In most of these studies, it was evident that the resistance towards fluoroquinolones, which is almost chromosomal mediated, develops in an incremental manner. Most of these studies indicate that the initial isolates which were less susceptible towards ciprofloxacin were found to have MIC values of 0.06 mg/l, which gradually increased to 1 mg/l (such strains being referred to as intermediate resistant) and later to as high as 16 mg/l (classified as resistant isolates). Such strains were referred to as quinolone resistant *N. gonorrhoeae* (QRNG). Strains with MIC value >4 µg/l were considered as high level resistance (HLR)

strains. Patients infected with these isolates showing decreased susceptibility or intermediate resistance towards ciprofloxacin, usually responded very well to 500 mg of ciprofloxacin. Later on, a few reports of treatment failure also appeared[59].

The ciprofloxacin resistant isolates were reported in mid 1980s from many parts of the world[60,61]. By the end of 1992, more than 40 per cent ciprofloxacin resistant isolates were documented in Japan[61]. Thereafter, ciprofloxacin resistant strains spread very quickly from Asia to Australia, Hawaii and North America[60,62-64]. Studies from USA also indicated a rise in ciprofloxacin resistant isolates especially in California[65]. Significant ciprofloxacin resistance emerged simultaneously from the WHO Western Pacific Region[66] and SEAR[18]. In these countries, it was thought that the emergence of ciprofloxacin resistance was accelerated mainly because of its use for the treatment of other diseases as well. There were two reports of increasing ciprofloxacin resistance from Bangladesh[67,68]. In India, the use of ciprofloxacin, as the first-line therapy for gonorrhoea started in 1990[5]. It was also included in the syndromic management in cases of suspected gonorrhoea. Resistance to norfloxacin soon appeared in 1996 from New Delhi, India[34]. By the end of 2000, a burst in ciprofloxacin-resistant isolates was observed in India[13,34-36,38,40,69]. Interestingly, with the emergence of fluoroquinolone resistant strains in India, a rapid decline of PPNGs was observed[14]. Similar observation was also reported from other countries indicating a penicillinase producing plasmid curing effect on an ecological scale[5,70]. In most of these studies, the molecular basis of antibiotic resistance was not investigated. In a study from India, decrease in ciprofloxacin resistant strains was observed which may be due to ciprofloxacin not being used for treatment of gonorrhoea in India[14]. Studies from developed countries such as Australia, Canada and US suggest that quinolone resistant strains were introduced sporadically over many years. Once introduced into sexual networks, these subtypes spread and eventually achieved endemic transmission. In response to the increase in ciprofloxacin resistant isolates from throughout the world, the use of this antimicrobial to treat gonorrhoea was discontinued in early 2000s from most of the countries[65]. In 2004, CDC discontinued the use of ciprofloxacin to treat gonococcal infections[71]. The use of ciprofloxacin was continued in Europe till 2004 and was discontinued only in 2005[45,72]. Simultaneously, the use of quinolone group of antimicrobials for the treatment of gonorrhoea was also discontinued in India[14,34,40,73,74].

Post-quinolone era

Consequent to the increase in the resistance profile of *N. gonorrhoeae* towards quinolones, third-generation cephalosporins, both injectable (ceftriaxone) and oral (cefixime and cefdinir), were the only available treatment recommended by the CDC and other national organizations for the gonococcal infections[33,40,71,72,75]. In patients allergic to cephalosporins, spectinomycin was recommended as the drug of choice. Cephalosporins were discovered[76] in 1945. These are known to work as other β-lactams, by inhibiting the cell wall synthesis through binding and inhibiting the action of enzymes responsible for inserting peptidoglycan cross-linkage structures into the cell wall.

Cephalosporins are known to be important antimicrobials for the last 10 years. Despite their historic reliability, resistance to cephalosporins also started developing in Asia and later on in other regions of the world as well. The resistance towards cephalosporins was documented as early as in 1996 and then later in 2000 in Japan[77,78]. Several subsequent reports from Japan also indicated much higher MIC values for cephalosporins[79-84]. Similar results were also documented from other countries like China, Hong Kong, Taiwan, Europe, US and Africa[66,84-90]. A surveillance report from India, wherein isolates collected from different laboratories of India, Bangladesh, Nepal and Sri Lanka during 1999-2001, documented significant increase in the isolates with decreased susceptibility to ceftriaxone[40]. In India, Bala *et al*[14] reported nine isolates with ceftriaxone MIC of 0.064 mg/l among the 382 isolates studied during 2002-2006. All cases were treated with ceftrixone 400 mg and there were no treatment failures observed. Some *N. gonorrhoeae* isolates demonstrating reduced cephalosporin susceptibility also have reduced susceptibility to multiple drug classes, including quinolones, macrolides, penicillins, and tetracyclines[91]. These ceftriaxone less sensitive strains almost always exhibited resistance to quinolones or quinolones and penicillin as reported from Australia, Japan and India suggesting increasing prevalence of these multi-resistant strains in these countries[14,92,93].

Potential alternatives in the treatment of N. gonorrhoeae infections: Until 1980s, there was a parallel and consistent development of the new antibacterials, which were active against most of the resistant strains

of bacteria. The increasing drug resistance in almost all bacteria in the recent past, has prompted scientists to look for possible alternatives such as immunotherapy, vaccination, identification of novel targets for drugs, probiotics, *etc*[94]. Attacking virulence mechanisms rather than the whole bacterial structure offers a wide range of possibilities. An advantage of such a strategy is that it seems less likely to apply selection pressure. Although no work has been done for *N. gonorrhoeae*, in other bacteria, targets that have been investigated include receptor sites, sortases, quorum sensing signals, Shiga toxin, and staphylococcal enterotoxinB[95-99]. In addition, the therapies derived from complementary and alternative medicine (CAM) used by the general public, need to be explored[100,101].

Spectinomycin can also be considered as a therapeutic option for persons with gonococcal urogenital infection who cannot tolerate cephalosporins[102]. However, it would probably remain as an alternative treatment rather than a recommended one, because high levels of resistance developed when this antimicrobial was widely used in the mid-1980s[103]. Azithromycin, 2 g, taken orally has been shown to be effective against uncomplicated gonococcal infection and could be thought as an option for persons who are allergic to cephalosporins. However, concerns about the development of antimicrobial resistance to macrolides with widespread use restrict current treatment recommendations to limited circumstances. Macrolides such as azithromycin and erythromycin, have also been associated with the multiple transferable resistance efflux system[104,105].

Several naturally occurring bacterial DNA gyrase inhibitors, such as the coumarins, which include novobiocin, clorobiocin and coumermycin A1, have been shown to have antibacterial property[106]. The coumarin derivatives inhibit ATPase activity of DNA gyrase by competing with ATP for binding to the B subunit of the enzyme. Recently activity of some medicinal plants has been evaluated against *N. gonorrhoeae* which seems to have a promising future[107]. Among the compounds that were evaluated, eugenol, a compound from *Ocimum sanctum* was also found to be active against multi-resistant isolates of *N. gonorrhoeae*[108,109].

Mechanism of drug resistance

N. gonorrhoeae, originally highly susceptible to antibiotics can adapt to adverse conditions[110]. A hostile environment in which antibiotics are present may select for the multiple changes which result in resistance and treatment failure. Mechanisms of antibiotic resistance in gonococci may be conveniently grouped as those that involve reduced access of the antibiotic to the target site and those that involve alteration of the target site itself. Access of antibiotics to the target site may be limited by reduced permeability of the cell envelope caused by changes in porin proteins; active export of antibiotics from the cell by means of efflux pumps; and destruction of the antibiotic before it can interact with the target. Alteration or deletion of the target site of the antibiotic results in a reduction of its affinity for the antibiotic. Genetically, these changes may be mediated by either chromosomal or extra-chromosomal elements (plasmids). Multiple resistance determinants may co-exist in a single organism so that the level of resistance can increase incrementally and a single strain can be resistant to a number of different antibiotics.

In gonococci, chromosomally mediated resistance is generally slow to emerge and disseminate. In *N. gonorrhoea*, the process of genetic transformation is known to be responsible for acquiring drug resistance. But such a change is visible only if many such acquisitions of the determinant take place[110]. Plasmid-mediated resistance, at present limited to penicillins and tetracyclines, is transmitted by means of conjugation. This process requires the presence of a conjugative plasmid to mobilize the plasmid carrying the resistance determinants. Since not all strains possess conjugative plasmids, the rate of spread of resistance may be limited to some extent. However, conjugative plasmids are also transferable during conjugation, so that some recipient strains then become donors themselves[110]. Different rates of dissemination of extra-chromosomally mediated resistance have been observed. For example, the 'Asian' PPNG plasmid spread more rapidly than the 'African' PPNG plasmid because initially only strains carrying the former determinant also contained conjugative elements. In *N. gonorrhoeae*, plasmid-mediated resistance spreads more rapidly than chromosomally mediated resistance. Amongst non-quinolone drugs, several studies have been carried out to understand the mechanism of penicillin resistance, which has been summarized below.

(i) Resistance to penicillins: The penicillins were widely used for the treatment of gonorrhoea for many years (and still are in some regions). Originally, *N. gonorrhoeae* was extremely sensitive to almost all the drugs, known so far, and treatment with 150,000 units of penicillin was effective in most instances. Later on

decreased *in vitro* susceptibility towards penicillin appeared and it was thought to be associated with treatment failure[2]. Increasing the recommended dose of penicillin 'temporarily alleviated the clinical problems resulting from infection with these strains, but almost inexorably levels of resistance increased and large numbers of treatment failures again occurred, even with high-dose regimens[111,112]. This was an example of step-wise accrual of chromosomal changes over a period of many years. The targets of β-lactam agents are the penicillin binding proteins (PBPs), enzymes located in the cell envelope that participate in cell wall metabolism. Alterations in PBP-2 and PBP-1 decreased their affinity for the penicillins, and thus the susceptibility of the organism. PBP-2 is encoded by the *pen*A locus[113]. Changes in other loci such as *mtr* and *pen*B produce additive effects. The *mtr* locus mediates resistance to a wide range of antibiotics, detergents and dyes through an active efflux system[114,115]. Mutations in the *pen*B locus, which affect a porin, result in reduced permeability of the cell envelope to hydrophilic antibiotics and other compounds[110,116]. *N. gonorrhoeae* also has a *por*A 'pseudogene' which is not expressed[117]. In contrast, *N. meningitidis* expresses two porins, PorA and PorB. The combined effect of *pen*A mutations and increased expression of *mtr* is shown to increase the MIC of penicillin by 120-fold[118]. Gonococci exhibiting these changes are termed chromosomally resistant *N. gonorrhoeae* (CMRNG). Reduced susceptibility to cephalosporins, tetracyclines and other agents is also mediated by chromosomal mechanisms in the concerned genes[30,113,119].

In addition to chromosome mediated resistance, resistance to penicillins is also mediated by a plasmid-borne, inducible TEM-1 type β-lactamase. β-lactamase is known to hydrolyze the β-lactam ring of penicillins, thus inactivating them. Chromosome mediated resistance is slow and incremental, on the contrary, resistance mediated by plasmid is a single step process. PPNG were detected at the same time in the United Kingdom and the USA[31]. The first isolates were reported, respectively, from Africa and the Far East. Although the same TEM type of β-lactamase was present in both instances, the gene was carried on plasmids of different sizes, which became known as the 'African' and 'Asian' plasmids. Transmission of the resistance by conjugation required the presence of another mobilizing plasmid, which was already present in the Asian PPNG when it was first isolated, but was not found in the African strains until 1981[120]. Thus, the Asian strain disseminated more widely and more quickly. Lactamase production (PPNG) and chromosomal changes (CMRNG) can co-exist in the same isolate. This is relevant because of the clinical use of penicillins in combination with β-lactamase inhibitors. These substances, such as clavulanic acid and sulbactam, prevent the β-lactamases from inactivating the penicillins. Combinations such as amoxycillin/clavulanic acid are widely used to treat other infections. In theory, and sometimes in practice these represent an effective oral therapy for PPNG infections, but more commonly single-dose regimens of penicillin/inhibitor combinations have failed[121]. This appears to be due to PPNG strains having a high frequency of underlying intrinsic or chromosomally mediated penicillin resistance. Chromosomally mediated resistance can be measured reliably only after the organism is 'cured' of its plasmid and the MICs reassessed[122].

(ii) Resistance to quinolones: As already stated, the quinolone antibiotics most widely used for the treatment of gonorrhoea are 'second generation' agents such as ciprofloxacin and ofloxacin. As in the case of chromosome mediated penicillin resistance, resistance to these antibiotics has developed incrementally over a number of years and multiple chromosomal changes are involved. Access of quinolones to their targets is reduced by changes in cell permeability and possibly by efflux mechanisms. These events produce low-level quinolone resistance. The targets of the quinolones are topoisomerases, including DNA gyrase[123-125]. High-level clinically relevant resistance is mediated by alteration of the target sites, initially via mutation in the *gyr*A gene[12]. Multiple amino acid substitutions have been described which, when combined, result in high-level resistance. Multiple mutations also occur in the *par*C 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. Changes in ParC seem to arise in the presence of mutations affecting GyrA. The more recent (fourth generation) quinolones are more active against strains with altered ParC, but are less effective against GyrA mutants. Thus, these compounds will in theory, be active against some, but not all, ciprofloxacin-resistant gonococci[24]. The newer quinolones have yet to be assessed for efficacy against gonorrhoea. One of these agents, trovafloxacin, has been withdrawn from use in many countries because of toxic side-effects.

(iii) Resistance to cephalosporin antibiotics: Altered gonococcal susceptibility to cephalosporin antibiotics is chromosomally mediated and is due to the same changes that account for decreased penicillin

susceptibility[110,119]. There is cross-resistance between penicillins and early generation cephalosporins such as cefuroxime[119,126]. However, this is not the case for the later generation cephalosporins such as ceftriaxone and cefixime. Not all cephalosporins are hydrolyzed by the TEM-1 type β-lactamase, and therefore, some of these compounds are active against PPNG. Other β-lactamases (cephalosporinases), which are constitutively expressed by many other Gram-negative genera, have thus far not been detected in gonococci and there has been no transfer of genetic material encoding production of extended spectrum β-lactamases into pathogenic *Neisseria*. If such an event occurs, it would be devastating for gonorrhoea treatment programmes that rely heavily on the third-generation cephalosporins. In the past five years, gonococci with decreased susceptibility to ceftriaxone has been reported though the mechanism of resistance has not been fully understood[77]. Recent data also suggest that the emergence and spreading of cephalosporins-resitance gonococci is quite similar to the data showing the emergence of quinolone-resistance strains[127].

Conclusion

Despite a high prevalence of uncomplicated gonorrhoea and an increasing incidence of resistant isolates of *N. gonorrhoeae*, throughout the world, standardized monitoring of the antimicrobial susceptibility profile has been restricted to Gonococcal Isolate Surveillance Project (GISP) in United States, Gonococcal Resistance to Antimicrobials Surveillance Program (GRASP) in England and Wales and Gonococcal Antimicrobial Susceptibility Program (GASP) in the America and the Caribbean. There is a need for better control of gonococcal disease including enhanced global surveillance of resistance and improved treatment. The cost associated with culture of gonococci for determination of antimicrobial resistance profile forbids routine determination of MIC before treatment. Hence, there is an undeniable need to simplify and standardize the *in vitro* antibacterial susceptibility procedures. The E-test method though expensive, is an attractive alternative to the earlier, agar dilution technique, for gonococcal antibacterial susceptibility testing. Molecular technology can provide an alternate procedure to the culture method for the surveillance of the antimicrobial susceptibility. It is believed that the single tube assays using techniques like hybridization capture for the detection of wide range of resistance genes would allow their application beyond the laboratory directly in clinical practice[128]. Efforts should be made to design common assay for detection as well as to investigate the drug resistance profile of *N. gonorrhoeae* so that infected patients can be treated immediately. Such methods would facilitate an early treatment with the most effective drugs as well as will control the transmission of infection.

The single dose approach has also contributed to the ability of *N. gonorrhoeae* to develop resistance to commonly used, inexpensive and effective drugs. The capacity and ease for genetic recombination and high transmissibility of resistant genes has made the development of new antibiotics a challenging area of research. The choice of antibacterial agent must take into account the data generated by laboratory based surveillance of susceptibility. This surveillance would be useful not only in deciding the correct treatment but also in helping to detect the emergence of new antibiotic resistant traits and to monitor the effectiveness of prescribed treatment. To make this information reliable, the laboratories need to adopt and use standardized laboratory procedures and take part in external quality assessment programme[129]. Studies addressing the effect of continued suppression of bacteria by an antimicrobial agent (post-antibiotic effect) and the effect exerted at sub-inhibitory concentrations, known as the post-antibiotic sub-MIC effect (PA-SME) need to be carried out for the rational use of antibiotics in *N. gonorrhoeae* infection. Global initiatives are imperative to integrate diagnostics, disease management and control of antimicrobial resistance.

It has also been observed that the problem of antibiotic resistance is greatly influenced by poverty and the factors related to it. Due to the cost involved, treatment of gonorrhoea relies on syndromic management in developing countries including India. The problem of drug-resistance in these countries, though an important issue, is thus not properly addressed, as there are other issues such as basic health care of higher priority. Formulation and implementation of policies related to understanding of the problem and the consequences of lack of control, both by users and policy makers will help in reducing transmission of resistant strains.

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