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Antimicrobial resistance in *Neisseria gonorrhoeae*

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A BACKGROUND DOCUMENT FOR THE WHO GLOBAL STRATEGY FOR CONTAINMENT OF ANTIMICROBIAL RESISTANCE

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Preface

This technical review provides information on antibiotic resistance (AMR) in the gonococcus from a laboratory perspective. AMR is discussed in the broader perspective of STD control, and the relationship between gonorrhoea and HIV transmission is emphasized, as it is relevant to understanding the importance of gonorrhoea. The review is divided into five sections, dealing with disease incidence, trends and diagnosis; AMR and its mechanisms in *Neisseria gonorrhoeae*; the rationale, purpose and methods of AMR surveillance; treatment; and prevention. A summary precedes and a discussion of research and implementation needs follows each section. The latter are expanded upon in a separate concluding section. Aspects of the biology of the gonococcus are discussed where relevant. The bibliography is meant to be illustrative and informative rather than exhaustive. The subject is broad and, inevitably, not everyone will agree with the emphasis. Comments, suggestions and criticisms are therefore welcome. The review was completed in September 1999.

Glossary of terms and abbreviations

AGSP	Australian gonococcal surveillance programme
AMR	antimicrobial resistance
CMRNG	chromosomally mediated resistance in *Neisseria gonorrhoeae*
CSW	commercial sex worker
DFA	direct immunofluorescence assay
DGI	disseminated gonococcal infection
DNA	deoxyribonucleic acid
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
GASP	gonococcal antimicrobial surveillance programmes (WHO)
GISP	gonococcal isolate surveillance programme (USA)
HAM	homosexually active male
HIV	human immunodeficiency virus
IC_{50}	the antibiotic concentration required to inhibit 50% of the inoculum
IM	intramuscular
LCR	ligase chain reaction
LOS	lipooligosaccharide
LPS	lipopolysaccharide
MIC	minimal inhibitory concentration
MIC_{50}	antibiotic concentration required to inhibit half the isolates tested
MIC_{90}	antibiotic concentration required to inhibit 90% of isolates tested
NAA	nucleic-acid-based amplification assays
NAD	nucleic acid detection
PAHO	Pan American Health Organization
PCR	polymerase chain reaction
PID	pelvic inflammatory disease
PPNG	penicillinase producing *Neisseria gonorrhoeae*
QA	(external) quality assurance
QC	(internal) quality control
QRDR	quinolone resistance determining region
QRNG	quinolone-resistant *Neisseria gonorrhoeae*
RNA	ribonucleic acid
RTI	reproductive tract infection
SEAR	South-East Asia Region (of WHO)
STD	sexually transmitted disease
STI	sexually transmitted infection
TRNG	tetracycline-resistant *Neisseria gonorrhoeae* (high level, plasmid-mediated)
WHO	World Health Organization
WPR	Western Pacific Region (of WHO)

SECTION A

Gonococcal disease – incidence, trends and diagnosis

Summary of Section A

Gonorrhoea is a sexually transmitted disease caused by the bacterium *Neisseria gonorrhoeae* for which humans are the only natural host. It is transmitted by human-to-human contact and is highly adapted to the genital tract, surviving poorly outside the human body. Nevertheless, it is versatile in resisting attack, for example in its ability to develop resistance to antimicrobials and in the antigenic variability by which it evades host defences, thus persisting and often causing asymptomatic (and undetected) infection.

Among the etiological agents of treatable STDs, *N. gonorrhoeae* (along with *Haemophilus ducreyi*) stands out because of the extent to which antibiotic resistance compromises the effectiveness of individual case management. Resistance also affects control programmes. This is discussed in Section B.

The symptoms of gonorrhoea are similar to those caused by other agents, most notably *Chlamydia trachomatis*. *N. gonorrhoeae* causes infections principally of the urethra in men and the endocervix in women, although it may also infect extragenital mucosal sites, including the oropharynx and anorectum. Ocular infections also occur, and in neonates can cause blindness. Genital infection in men usually presents with a urethral discharge, but silent infections are common in women and in the case of extragenital infections. Disseminated gonococcal disease is uncommon.

Genital tract gonorrhoea gives rise to well recognized complications. These include upper reproductive tract infections in women (such as pelvic inflammatory disease—PID) with possible sequelae including infertility, ectopic pregnancy; fetal wastage, neonatal ophthalmia and blindness, and disseminated gonococcal infections. In addition, gonorrhoea is associated with significant enhancement of transmission of the human immunodeficiency virus (HIV) (by up to 500%).

Rates of gonorrhoea vary greatly among countries in the developed and developing world. The highest rates are in South and South-East Asia, sub-Saharan Africa and Latin America. Available data are often incomplete or inaccurate and are usually prevalence rather than incidence data. Difficulties in compiling data include:

Clinical presentation not specific enough for diagnosis based solely on symptoms;

Inadequate facilities, materials or personnel for laboratory-based diagnosis;

Lack of reporting mechanisms even when diagnostic facilities are in place;

Reluctance to report STDs to public health authorities.

In many countries, incidence or prevalence data are not complete enough to provide an accurate picture of disease distribution. The distribution of gonorrhoea is affected by many interrelated factors, including sociogeographical factors, which produce multiple microepidemics. There are a diversity of gonococcal subtypes in different patient subgroups and geographical areas, often with differing antibiotic resistance profiles. Factors influencing the prevalence of particular gonococcal subtypes include overall rates of disease and transmission, and the availability and misuse of antibiotics. This has obvious implications for management of the disease. The highest rates of gonorrhoea are found in lower socioeconomic groups and, in developed countries, among homosexually active men (HAM), travellers and clients of commercial sex workers (CSW). The concept of a 'core group' of high-frequency transmitters of STD who exhibit risk-defining behaviour was originally developed for gonorrhoea.

Control of gonorrhoea (and other STDs) requires a complex, integrated and comprehensive strategy of education, counselling, diagnosis, treatment and case-finding. Key elements are prevention (through promotion of safer sexual practices) and the availability of health care services.

The ability to control the disease is limited by the significant proportion of infections that remain undiagnosed. Factors contributing to this include the large number of asymptomatic infections, especially in women, failure to recognize or act on

symptoms and deficiencies in diagnostic facilities. A syndromic approach to diagnosis and disease management is often effective for STDs, although this is of limited value in vaginal discharge syndromes in women and, by definition, cannot reach asymptomatic patients.

Laboratory diagnosis still relies mainly on conventional test methods. Problem areas include invasive specimen collection procedures, loss of viability of the fragile gonococcus especially when specimen transport is required, and lack of suitable reagents and trained personnel. Non-culture-based assays have some advantages, but they are expensive, require specialized equipment, and do not permit susceptibility testing.

Gonorrhoea remains a significant disease globally. While it is more frequent in poorer countries (and in marginalized groups in all countries), disease rates remain unacceptably high in developed countries, and appear to be increasing at present, at least in some population subgroups, such as HAM. Consequences of a high disease rate include a high incidence of complications and long-term morbidity, as well as increased HIV transmission.

Antibiotic resistance increasingly compromises effective treatment of gonorrhoea. Inexpensive treatment regimens have been rendered ineffective while efficacious ones are often unaffordable. The temptation to use ineffective but cheaper remedies (which are ultimately more expensive because of failure to control disease spread and the cost of inevitable complications) must be resisted. It is necessary to demonstrate the benefits of appropriate treatment. For gonorrhoea, this will require continuing, high-quality susceptibility surveillance.

A1. Rates and patterns of gonococcal disease

A1.1 Overview

Reliable data on the incidence of gonorrhoea are difficult to obtain. Recent global estimates by WHO suggest that of the estimated 62 million new cases in 1995, most occurred in South and South-East Asia, sub-Saharan Africa and South and Central America. The incidence was significantly higher than the 1990 estimates of 25 million new cases. However, there were significant methodological differences between the two studies. What was evident was the paucity of information actually available and the importance of collecting incidence and prevalence data (*1*).

In 1995, the estimated prevalence of gonorrhoea (for adult males aged 15 to 49) was 2% in sub-Saharan Africa, 1% in South and South-East Asia and 0.6% in Latin America and the Caribbean. Rates in developed countries were approximately one-tenth of those in developing nations (Western Europe, 0.07%; North America, 0.1%; Australasia, 0.1%). Prevalence rates for females were even higher, reaching 2.8% in sub-Saharan Africa, 1.4% in South and South-East Asia and 1.1% in Latin America and the Caribbean. Again, the more developed nations had lower prevalences: 0.2% in Western Europe; 0.4% in North America and Australasia (*1*). These investigators, while acknowledging deficiencies in the quantity and quality of the data, estimated the incidences for 1995. The highest rates per 1000 population (aged 15–49) were in sub-Saharan Africa (males 57.71, females 65.47), South and South-East Asia (males 30.03, females 31.80), and Latin America and the Caribbean (males 27.56, females 29.23). The lowest rates were in East Asia and the Pacific (males 4.35, females 3.79), Western Europe (males 5.59, females 6.01), and North America and Australasia (10.85 for males and 12.04 for females).

The incidence of gonorrhoea, as of other STDs, is influenced by many factors, including patterns of sexual behaviour, population demographics, and economic and social conditions (*2*). A consideration of some of these factors, which is a separate topic and the subject of reviews in its own right, is nonetheless relevant to understanding the role of appropriate antibiotic therapy in the treatment and control of gonorrhoea. Thus, brief references to some pertinent points are made below.

A1.2 Rates of gonorrhoea in developed countries

In industrialized countries the increased incidence of gonorrhoea seen during and after World War II was followed by a decline in the 1950s, then an increase until the 1970s, followed by another decline. The increases reflected the 'sexual revolution' of the 1960s, during which period there was a decrease in the ratio of infections in males relative to females, and the 'baby boom' of the 1950s, which led to an increased incidence of the disease in young adults 20 years later. The decline in incidence from the 1970s on has been attributed to a number of factors including the decline in the size of the 18–24 age group as the baby boom generation aged, better education, improved diagnostic facilities, better antibiotic treatment, and better contact tracing (*2*).

The rates of gonorrhoea continued to decline in the 1980s and early 1990s in Sweden and some other European countries and gonorrhoea virtually disappeared as an endemic disease (*3*). In Sweden the incidence rate was 487 cases per 100,000 population in 1970 and less than 5 per 100,000 in 1992. This was attributed to a continuing programme of information and education, extensive use of diagnostic facilities to detect asymptomatic as well as symptomatic infections, and effective antibiotic treatment (*4*).

In some other industrialized countries the decline in gonorrhoea was less marked until the appearance of AIDS. The publicity surrounding sexual transmission of HIV and the behavioural changes which accompanied this increased awareness led to substantial decreases in the rates of gonorrhoea in Western Europe, Australia and the United States in the 1980s and early 1990s (*5*, *6*, *7*, *8*, *9*, *10*). Some illustrative examples include the abrupt decrease in rates of disease in parts of Greece in 1986 and sustained low rates thereafter (*10*); the decline in incidence in Finland from 265 to 6 cases per 100,000 population over the two decades ending in 1995 (*11*); the estimated 1990 incidence in France of 74.8/100,000 in men and 14.2/100,000 in women aged 15–59 (*12*); the decline in the number of reported cases in Norway from more than 10,000 in 1981 to less than 300 in 1993 (cited by Gerbase et al (*1*)); the decrease in incidence from 104 to 23 per 100,000 population in the Netherlands from 1981 to 1988 and then to about 9 per 100,000 in 1995 (*9*).

There is evidence that the incidence of gonorrhoea has risen again during the late 1990s in some subpopulations. In the USA, although overall rates continue to decline, the incidence in HAM appears to be increasing in some US cities (*13*, *14*, *15*, *16*). In Europe and Australia similar trends have been reported (*17*, *18*). In Australia in 1998 rates in some centres increased by 50%, having risen by 20% in previous years. The sustained increase over several years suggests that this is not merely a cyclical phenomenon. Endemic transmission of gonorrhoea was once again significant in Sweden in 1997 and 1998 (*19*) and the incidence of gonorrhoea in Denmark increased sharply in 1998, especially in HAM in Copenhagen (*20*).

In former Eastern bloc countries there has been a substantial increase in the incidence of gonorrhoea and other STDs, although in some instances this has been followed by an apparent rapid decline, which may actually reflect changes in data collection methods. In the Slovak Republic, it was reported that sexual behaviour had changed considerably, as manifested by male homosexual activity, 'sexual liberty in general, enhanced promiscuity and prostitution'. Between 1990 and 1996 the number of cases reported decreased by almost 90%. It is not known whether this decrease was real because of 'fear of HIV infection (frequent use of condoms)' or due to 'failure in notification' by treating practitioners (*21*). Estonia generally followed Western European trends up to and through the 1980s, but with higher incidence rates: 250/100,000 population in 1981, declining to 100 in 1988. The incidence increased substantially, reaching 233 in 1993, and then declining in 1994 and 1995. This may have been due to the introduction of more effective antibiotic treatment (such as quinolones and cephalosporins) as more than half the isolates examined in one 1994 study were PPNG. However, incomplete case reporting may also be a factor in the apparent decline (*22*). Surveillance problems were also reported from the Russian Federation. The rates in St Petersburg increased to 468/100,000 in 1993 and a sudden apparent drop in 1994 was attributed to failure of notification (*23*). There are doubts about the reliability of current surveillance in Eastern Europe and Central Asia due to a marked decrease in mass screening and contact tracing (*24*). Additionally, attendance at state clinics has declined and many patients are treating themselves or visiting private practitioners. It is believed that only about 5% of cases of gonorrhoea are currently being reported and it is anticipated that reporting will decrease even further so that 'STD epidemics will de facto disappear on paper'.

A1.3 Rates of gonorrhoea in less developed countries

It is generally accepted that rates of gonococcal disease are high in less developed countries, but estimates are usually based on prevalence rather than incidence data (*2*). Disparate rates are reported from different countries and subgroups, but are always much higher than in developed countries (*1*, *2*). Control of STDs in developing countries is comparable to the situation existing in industrialized nations at the beginning of this century (*25*).

Estimated prevalences of gonorrhoea in South Africa are 10 to 12% in attendees at family planning or antenatal clinics (*26*) and 14.3% in female sex workers (*27*). Core groups (see below) are re-

sponsible for much of the transmission of gonorrhoea (*28*). In Malawi 'Cervical infection with *Neisseria gonorrhoeae* was found in 5% of urban antenatal women, comparable to infection rates in low-risk populations in other countries in the region' (*29*).

In developing countries, the publicity given to HIV has had less of an effect than in more developed countries. However, control programmes in Thailand, which successfully targeted HIV transmission rates, also led to declines in gonorrhoea and other STDs. From 1987 to 1993 the number of notified cases of gonorrhoea declined by more than 80% (*30*). Gerbase et al (*1*) cite examples of steady declines in STD rates in Costa Rica, Chile and Zimbabwe, but note that other countries have reported increasing numbers of cases since 1990. They caution that the apparent declines might reflect variations in the quality of surveillance or in access to health care. In a longitudinal 6-year study ending in 1994, rates of gonorrhoea declined in three Latin American countries (Mexico, Argentina and Brazil) but increased in Venezuela (*31*).

For 10 countries in the WHO WPR, working estimates of gonorrhoea rates were deduced from prevalence surveys published between 1990 and 1997. Only properly designed studies with laboratory support were analysed. In 8 of the 10 countries prevalence rates were below 1%; higher rates were reported only in Papua New Guinea (4%) and Cambodia (3%) (*32*). However, rates in high-risk groups differed markedly from those in the general population or in low-risk groups. For example, in the Philippines prevalence rates in female CSWs were 10.6 to 22.2% and in a female prison population the prevalence was 9.2%. In contrast, in antenatal women (considered low risk for STIs) the prevalence was 1% or less (*33*). Similarly, in Viet Nam 1997 estimates were 10% in CSWs but 0.5% in the general population aged 15 to 49 (*34*).

In China, acquisition of data was hampered by poor surveillance at the time gonorrhoea re-emerged in the 1980s. An apparent increase in crude incidence rates was due in part to better surveillance and improved diagnostic methods (*35*). Rates in the southern region were higher than in other parts of the country, and increased substantially up to 1989 (*35*). The prevalence in China as a whole was still estimated to be less than 1% in 1998 (*32*). In Mongolia, following enormous social upheavals after 1990, the number of cases of gonorrhoea increased substantially from 51 per 100,000 in 1983 to 142 in 1995. At this time there was a decline in active surveillance, suggesting that the latter figures may be underestimates (36). Recent prevalence data from Indonesia, based on laboratory-confirmed cases using molecular techniques (*37*), indicated a 15% gonorrhoea rate in CSWs in Jakarta, Surabaya and Menado.

A2. Limitations of available data

Gonorrhoea prevalence and incidence data are subject to biases and limitations. Available data may be derived from clinical or laboratory-based diagnosis and are of variable quality.

When reporting is based on clinical presentation incorrect diagnosis is common, as several infectious agents cause similar symptoms. When reporting is based on laboratory diagnosis, the sensitivity and specificity of the tests used affect the accuracy of diagnosis.

Underdiagnosis is also a significant problem because, particularly in women, *N. gonorrhoeae* infection may be asymptomatic or produce symptoms of insufficient magnitude ('oligosymptomatic' patients) to prompt health-seeking behaviour. Thus, robust screening programmes and laboratory-based diagnosis are needed to avoid underreporting.

Another problem is the underreporting of disease, even when it is accurately diagnosed, so that notified disease may represent only a fraction of cases (*25*). In some estimates, as few as 10% of diagnosed cases are reported in developed countries (*38*). Recent reports from Eastern Europe suggest that less than 5% of cases are reported (*24*). The reasons include privacy/stigmatization concerns, flaws in reporting systems, a perception by patients and practitioners that little is to be gained from the notification process, and general lack of interest. Thus 'report based systems tend to underestimate substantially the total number of new cases' (*1*).

Furthermore, clinic-based notification systems cannot provide complete data if only public health clinics are included or when the specialist services are not used by all social strata. For example, in London and Leeds STD rates were found to be extremely high in certain ethnic groups that did not use public facilities (*39*, *40*). When surveys are conducted in specific population groups, care needs to be taken in extrapolating the results more generally (*1*). This is especially true for gonorrhoea because the disease is concentrated in 'core groups' whose incidence and prevalence rates are not representative of the general population or of the reproductive age group (see A3 below).

Different surveillance mechanisms have been tried. One approach has been a shift from clinician-based to laboratory-based notification. While this led to improved notification rates for some diseases, there was still significant underreporting of gonorrhoea, largely for the same reasons that clinician-based notification failed.

Newer diagnostic techniques which use easily obtained self-collected samples are more accessible to patients and more sensitive (see A4.2 below) and offer the possibility of more precisely determining disease rates. Together with risk assessment data, the information generated can be used to develop a profile of those most likely to be at risk of disease and, therefore, to establish criteria for effective screening programmes (*41*). Any innovation that improves either diagnosis or notification will of course lead to an apparent increase in disease rates.

While primary gonorrhoea rate data are incomplete, data on sequelae and complications (such as ophthalmia neonatorum and PID) are even fewer. Mid-1980s data from Cameroon indicate that up to 30% of newborns developed ophthalmia neonatorum when their mothers had gonorrhoea at the time of delivery. A 17% rate of gonococcal infection in young women attending antenatal clinics in the same country (in 1984) is believed to correlate with the 40% prevalence of infertility in older women (*42*). A longitudinal Swedish study (*43*) compared the incidence of PID over two periods and noted a significant decrease in gonococcal PID over a period during which the incidence of gonorrhoea decreased. A longitudinal study in Argentina (*31*) also found that decreasing rates of PID correlated with trends for gonorrhoea.

Accurate estimates of the burden of gonococcal disease are important for a number of reasons, not least of these to increase awareness of the problem. Advocacy is needed to increase the resources allocated to STD control, especially in developing countries (*2*). The current paucity of data can lead to underestimation of disease incidence, with consequent reduction in the allocation of resources (*24*). Outcome parameters for intervention programmes are particularly difficult to determine in the absence of incidence data; increasingly, 'control programmes are data driven' (*25*). Our inability to provide accurate estimates of incidence and prevalence makes it difficult to perform cost-benefit analyses of interventions. The secondary benefits of preventing sequelae are even more difficult to quantify given the lack of data.

Even with the inadequacies of many reporting systems, trend data from longitudinal studies are useful as long as the limitations of the sample (size and representativeness) are taken into account. However, apparent trends are affected by any factors that change over the course of the survey, such as surveillance and diagnostic methods, the thoroughness of data collection, access to health care, or health-seeking behaviour. None of these is likely to remain constant for prolonged periods.

A3. Transmission and distribution of gonorrhoea

The dynamics of transmission of gonorrhoea are affected by both organism-dependent and organism-independent factors.

Organism-dependent factors, infectivity and virulence, determine its intrinsic ability to colonize, persist on and infect mucosal surfaces (see A4 and E4.1 below). Particular strain subtypes become associated with sociogeographical patient groupings, e.g. HAM (*44*). These associations of gonococcal subtypes with patient subgroups may influence antibiotic susceptibility patterns (*45*).

The recognition of organism-independent influences on transmission dynamics led to the concept of 'core groups' of patients who are 'high frequency transmitters' of gonorrhoea (*46*, *47*) and have a disproportionate influence on disease rates. The core groups may be identified by occupation (CSWs, long-distance truck drivers) or by sexual orientation (e.g. HAM). It is now understood that the behaviour of individuals within a group defines the risk. There has been continuing refinement of the approaches to behaviour modification, with distinctions being made between groups and individuals (*48*). The 'core group' concept, originally developed for gonorrhoea, has also been applied to other STDs. The aim is to recognize those who should be targeted for intervention (*49*). Targeted intervention, including appropriate antibiotic therapy, has a greater impact on an epidemic when the prevalence is low and the disease is still concentrated in core groups (*50*).

Gonorrhoea may thus be regarded as occurring in a series of microepidemics, each in a group of individuals with shared characteristics such as age, sex, race, sexual preference and geographical location (*51*). The extent to which disease occurs, spreads and is maintained in these groups, i.e. the size of the microepidemic, depends on complex events described as 'risk behaviours in risk space' (*52*). Within a microepidemic, individuals are

infected with particular subtypes of gonococci which have adapted to or been selected by this milieu (*45, 53*).

The effects of appropriate antibiotic treatment may be explored, to some extent at least, using the well-established formula

$$R_o = \beta cD$$

where R_o is the reproductive rate of the disease, β = 'transmissibility' of the organisms, c = the rate of partner exchange and D = the duration of infectiousness. When $R_o = 1$ the disease is endemic and stable. Values greater than or less than 1 indicate, respectively, increasing or decreasing disease rates. Typical values for D are 0.5 years and for β 0.5, meaning that the rate of partner exchange needed to maintain stable disease is 4 per year (*54*). (This formula is provided only for illustrative purposes. For details the reader should consult the original articles.)

For most individuals in a community the rate of acquisition of gonorrhoea is too low to maintain the survival of the organism in the population. In a core group, however, the rate of partner exchange is high enough for the reproductive rate R_o to remain 1 or above. Within core groups, some individuals restrict themselves to intragroup contact while others have additional contacts outside the group. Potterat has used the term 'sociogeographic space' to illustrate how particular subsets of the community socialize, interact and transmit gonococci.

The transmissibility of the organism, β, and the duration of infectiousness, D, are both affected by availability of appropriate treatment. It has been shown that gonococci are no longer cultivable from the male urethra 12 hours after appropriate therapy (*55*, *56*), whereas the patient remains infectious for longer periods in the absence of effective therapy. Brunham & Plummer (*54*) estimated D to be approximately 6 months for untreated gonorrhoea.

Transmissibility is dependent at least to some extent on strain subtype as well as on the infecting inoculum (*57*). Thus, male-to-female transmission (by genital-to-genital contact) is more efficient than the reverse, due in part to the much higher numbers of organisms present in male urethral discharge than in vaginal secretions. Holmes et al (*58*) estimated the risk of female-to-male transmission to be about 22%; this risk increased with the number of exposures (*59*). The infecting inoculum, and thus β, is rapidly reduced following effective treatment, but may also be somewhat reduced, decreasing transmissibility at least temporarily, even with suboptimal treatment. (Other important behavioural factors, such as condom usage and rate of partner exchange, are not considered further in this review.)

Improved diagnosis, particularly case-finding of asymptomatic individuals, can also decrease disease rates in the absence of behavioural change. In a recent study of indigenous people in South Australia (*60*), a programme of improved testing and treatment reduced disease prevalence by half over a three-year period. Despite minimal changes in condom use, and no change in the rate of partner exchange, the value of D decreased. Perhaps significantly, gonococci in this region remain susceptible to the penicillins, facilitating cheap and effective therapy.

Gonorrhoea has been described as a disease of the marginalized (*61*) and this concept is useful when considering regional disease distribution and populations at risk.

The concept of marginalization at the international level reflects the fact that the burden of gonorrhoea falls disproportionately on less developed countries. 'First world' nations have had low rates of gonorrhoea for many years, the greatest decline in most cases occurring after the advent of the AIDS epidemic, apparently as a welcome by-product of efforts to contain the spread of HIV. (However, in some Western nations, such as Sweden, the rate of gonococcal disease had already declined significantly before the appearance of HIV; see A1 above). This is in stark contrast with the situation in less developed countries. Where data are available (*1*) it is evident that rates of gonorrhoea vary inversely with the degree of economic development. This is illustrated by data emerging from former Eastern bloc countries, and in the lower rates of gonorrhoea achieved in some Asian countries as economic conditions improved.

Marginalization occurs within countries, in that individuals lower on the socioeconomic scale have higher rates of gonococcal disease (*61*). Examples include indigenous populations in Australia, and the urban poor in the United Kingdom and the United States. In less developed countries also, those in lower social strata appear to have higher disease rates. Additionally, disproportionately high rates occur in HAM in developed countries.

Another important factor in the spread of gonorrhoea and of antibiotic-resistant gonococci is the increase in travel, both within and between countries. The influence of travel on the spread of STDs was recently reviewed (*62*, *63*). There has been an

exponential rise in travel for pleasure in the past few decades, as well as in movements of migrant workers and refugees (*63*). The amount and speed of travel favours transmission of strains from one country to another during the presymptomatic incubation phase of infection (*62*). Individual travellers spread new strains of gonococci upon returning to their country of origin. For example, as the rate of gonorrhoea declined in Sweden, endemic PPNG disappeared, but resistant strains continued to be isolated from patients with overseas contacts. Similarly, in Finland, which had a higher rate of disease than other Nordic countries, while there was a significant decrease in the overall rate of gonorrhoea from 1990 to 1995, the number of imported cases remained constant and thus represented an increasing proportion of the total (*64*).

Certain core groups of travellers are particularly associated with spread of STDs, including gonorrhoea, and of resistant strains: female 'international' CSWs, long-distance truck drivers, sailors and migrant workers (*62*, *63*). Illegal immigrant CSWs were responsible for importation and maintenance of PPNG and QRNG in Sydney, Australia (*65*, *66*). Socioeconomic differences between former Eastern bloc countries and Western Europe have led to increased travel by sex workers and their clients (*62*). Long-distance truck drivers and sailors constitute a significant male core group. There is also a separate group of 'sex tourists' (usually older men) who travel 'specifically for the purpose of sex' (*62*).

A4. Relevant characteristics of the gonococcus

The biology of the gonococcus is fascinating. Since to the non-expert the extent of knowledge may seem overwhelming, this section attempts to provide brief insights into some characteristics of the gonococcus that are critical to understanding the epidemiology, management and treatment of gonorrhoea. Some of these points will be treated in more detail in other sections of this document.

Neisseria gonorrhoeae is a Gram-negative diplococcus. It is found in 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. The gonococcus is a very successful pathogen. 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. The host's attempt to eliminate the organism sets up 'pathogen-host coevolution… a Darwinian process that involves both the generation of genetic diversity in the pathogen and the operation of immune selection at the molecular level by the host' (*67*).

One of the noteworthy characteristics of the gonococcus is its phenotypic and genotypic variability, which enable 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. Some examples of the adaptability of the gonococcus follow.

As a result of its evolutionary adaptation to the genital tract, the gonococcus has special requirements when grown *in vitro*, e.g. CO_2, sulfur in the form of cysteine, and iron. Gonococci often have additional, multiple requirements, hence the difficulty in formulating media to support optimal growth. The fastidious culture requirements of the gonococcus have implications for traditional laboratory diagnosis and antibiotic susceptibility testing. However, determination of growth factor requirements provides a means of differentiating gonococci into auxotypes (*68*).

The gonococcal cell wall has many similarities to those of other Gram-negative cell bacteria. Studies of its structure have helped explain aspects of the host/parasite interaction. Components such as pili, outer membrane proteins and lipopolysaccharides are involved in the attachment and binding of the bacteria to epithelial surfaces, their passage through the epithelium and their interaction with phagocytes. Variations in the outer membrane porin protein (P1, Por) affect permeability and penetration of antibiotics into the organism. The variability of the epitopes of this protein, sometimes in combination with other phenotypic characteristics, has served as another means of differentiating gonococci for epidemiological purposes and of analysing the spread of antibiotic-resistant subtypes (*69*, *70*).

Another important feature of the gonococcus is its antigenic variability. This is a survival mechanism for an organism with a very restricted host range, whose chameleon-like ability to alter those parts of the organism that are in contact with the

host has frustrated attempts to produce a vaccine. The antigenic variability of *N. gonorrhoeae* is due in part to its ability to acquire genetic material from related organisms. This capacity for genetic recombination makes it a prime example of a 'panmictic' or non-clonal organism.

In the context of antibiotic resistance, the most obvious example of incorporation of new genetic material is the acquisition by the gonococcus of penicillinase-determining plasmids. This led to dispersion of the penicillinase genes by conjugation, and PPNG quickly spread throughout the world, reducing the utility of this group of antibiotics. Gonococci can also acquire DNA by transformation, which is as important to antibiotic resistance as conjugal transfer of plasmids.

Development of antibiotic resistance is but one of many adaptations the gonococcus is continually making to ensure its survival. Resistance alone is not sufficient to ensure its survival and may be less important than other components of the 'coevolution' process.

A5. Diagnosis

A5.1 Clinical diagnosis

Neisseria gonorrhoeae causes both symptomatic and asymptomatic genital and extragenital tract infections. There is a broad spectrum of clinical presentations and the symptoms overlap those of other STDs, most notably *Chlamydia trachomatis* infection. It is thus difficult to diagnose gonorrhoea on clinical grounds alone. Some examples of the more common clinical presentations and the problems associated with clinical diagnosis follow.

Uncomplicated gonococcal infections

Gonococcal disease of the genital tract most often presents as urethritis in men and cervicitis in women. Men with urethritis complain of a urethral discharge in about 80% of cases and of burning on micturition about half the time (*71*). Although about 10% of patients may be asymptomatic at the time of diagnosis, many of these are actually in the pre-symptomatic stage of gonorrhoea (*71*). The incubation period of gonorrhoea in men was estimated by these authors to range from one to 57 days (mean 8.3; median 5.8), which is longer than previously reported. Women with cervicitis sometimes complain of abnormal or increased vaginal discharge. However, asymptomatic genital infections are more common in women than in men. When specifically asked, many women acknowledge the presence of a discharge which was not uncomfortable enough to prompt them to seek treatment.

Extragenital gonorrhoea

Extragenital sites of infection include the anorectum, oropharnyx and eyes. Proctitis, with anorectal pain, tenesmus and rectal discharge is one presentation of anorectal gonorrhoea, although many of these infections are asymptomatic. Pharyngeal infections are rarely symptomatic. Ophthalmic infections in neonates present as conjunctivitis with discharge. Eye infections may also occur in adults. In both age groups there is risk of disease progression with corneal involvement and perforation. A non-progressive form of ophthalmic disease in children has been seen in recurrent epidemics in Australia (*72*).

Complicated gonococcal disease

The more common complications of both genital and extragenital gonorrhoea occur in women and neonates. Ascending reproductive tract infections in women (pelvic inflammatory disease, PID) have multiple presentations (endometritis, salpingitis, tubo-ovarian abscess, pelvic peritonitis) and may be caused by a number of different organisms, alone or in combination. However, *N. gonorrhoeae* is a leading cause of PID. Sterility or damaged Fallopian tubes, leading to ectopic pregnancy, are well-recognized sequelae.

About 1–2% of mucosal infections give rise to disseminated gonococcal infections (DGI), with women disproportionately affected. The percentage of DGI in a community is dependent on the subtype of the prevalent gonococcus (*73*, *74*). Presentation is usually with different combinations of mild systemic symptoms, infective arthritis, rash and tenosynovitis (*75*).

To this list of long-recognized complications may now be added the significant enhancement of HIV transmission (*76*). This is thought to result from an increase in the viral load in the semen (*77*) or cervico-vaginal fluids (*78*, *79*) those co-infected with gonorrhoea and HIV, and to an increase in the number of target cells for HIV in the inflammatory exudate present in symptomatic bacterial STDs (*80*).

Thus, gonorrhoea has a wide variety of clinical presentations, none of them uniquely caused by the gonococcus. A significant proportion of gonococcal infections is not recognized because they occur in asymptomatic or 'oligosymptomatic' patients or

are still in the incubating ('presymptomatic') phase. Causes other than gonorrhoea account for most cases of vaginal discharge. Gonorrhoea may account for up to 20% in high-prevalence settings, but often 10% or less, even in high-risk groups. Simultaneous infection with more than one agent, for example co-infection with *N. gonorrhoeae* and *C. trachomatis* is common in some settings, although this may be less frequent than first thought in other groups, such as HAM (*71*, *81*). Multiple infections and the non-specific nature of symptoms complicate the diagnosis and management of the individual patient as well as the implementation of public health measures to control gonorrhoea. Simple tests, such as microscopy, are reliable for etiological diagnosis in male urethritis but in other cases, diagnosis may require costly and sophisticated laboratory services. Since material and human resources for differential diagnosis are often not available where STDs are most prevalent, treatment algorithms for the syndromic management of STDs have been devised for resource-poor settings. In the absence of adequate laboratory facilities, these algorithms can be used to treat infections caused by all organisms likely to be involved in male urethritis or vaginal discharge. This approach, while clinically effective, necessitates prescribing multiple antibiotics. Evaluations of these syndromic approaches to management have also highlighted gaps which occur in the detection and treatment of asymptomatic infections, especially in women. However, syndromic management systematizes the approach to treatment of STDs in resource-poor settings.

Combining the assessment of STD risk factors with syndromic management algorithms increases the utility of this approach.

Different algorithms may be applied, depending on the level of diagnostic facilities available, and can be adapted to local disease prevalence. Intermittent prevalence studies using sensitive techniques are recommended to determine the relative contribution of various STDs to the total disease burden and to different syndromes in a given locality. Susceptibility surveillance is an essential component.

A5.2 Laboratory diagnosis

One regrettable consequence of the introduction of syndromic management has been the loss of support for laboratory-diagnostic facilities in some settings, usually as a cost-cutting measure.

General remarks

Laboratory diagnosis has been the subject of a recent review (82). This section incorporates relevant portions of a WHO Western Pacific Region manual of tests for the detection of reproductive tract infection (*83*).

The ideal diagnostic test is rapid enough for the results to be available while the patient waits, as well as being easy to perform, inexpensive, highly sensitive and specific, and requiring no specialized equipment. It should use samples obtained by non-invasive procedures. The results should be reliable, reproducible and robust. Additionally, in the case of gonorrhoea, it should include determination of antibiotic susceptibility. Clearly, a single test with all of these characteristics does not yet exist. Thus, a variety of culture-based and other methods are used, singly or in combination, depending on the needs, resources and objectives.

In the case of gonorrhoea, the objectives of laboratory testing are often not simply the diagnosis and treatment of the individual patient, but the control of disease transmission. Therefore, testing may be used to screen asymptomatic patients for infection (case-finding) and to generate prevalence and/or incidence data for epidemiological purposes or to validate syndromic management algorithms. The benefit to gonorrhoea control of using diagnostic laboratory protocols in case-finding has recently been demonstrated (*60*).

The choice of test(s) is greatly influenced by cost, as well as by logistical factors such as access to facilities and availability of suitable transport. Appropriate collection and transport of samples is particularly critical for gonorrhoea. In the female genital tract, gonococci reside in the endocervical glands and not the vaginal squamous epithelium, so that sampling for culture requires an endocervical swab under direct vision. This requires privacy, proper lighting and a vaginal speculum that can be reliably resterilized, facilities that are not always available. Additionally, some women are reluctant to undergo a pelvic examination, for personal or cultural reasons.

When direct inoculation and incubation of cultures is not possible, efficient transport is needed to maintain the viability of gonococci. Delayed culture is more suited to diagnosis in men than in women because there is a much higher number of gonococci in urethral discharges than in endocervical material and because urethral specimens are relatively clean, whereas the female genital tract has an extensive normal flora.

Microscopy techniques

Examination of a Gram-stained smear requires a well-maintained, good-quality microscope and the services of a competent microscopist. For urethritis in men, this is a sensitive and specific test that is inexpensive and rapid enough to be performed while the patient waits. The sensitivity of the Gram stain is greatly reduced in asymptomatic infections. In endocervicitis, the sensitivity of this test is less than 50%, when compared with properly performed culture. Gram-stained smears are less useful in diagnosing rectal gonorrhoea, and the best results are obtained when samples are taken under direct vision with a proctoscope. Pharyngeal specimens are not suitable for smears. Gram stain is not optimal for diagnosis of gonorrhoea at other sites, such as the conjunctiva and skin. In general, detection rates are lower when Gram-stained smears are examined by inexperienced microscopists (*84*). In developing countries, where skilled microscopists and well-maintained microscopes may not be available, performing the test may only complicate diagnostic algorithms (*85*).

Culture-based methods

Culture based systems have a high degree of specificity, but are expensive and require personnel trained in handling the fastidious gonococcus. Even seemingly basic skills, such as inoculation of culture plates, may be inexpertly performed (*84*). Culture media vary widely in quality; optimally prepared and quality controlled GC agars are critical to the success of this diagnostic method.

The two basic requirements for reliable gonococcal culture media are that they be enriched enough to support the growth of the fastidious *N. gonorrhoeae* and selective enough to suppress the often extensive normal flora. Both the nutrient and selective properties of gonococcal culture media have been steadily improved, so that the sensitivity of culture as a diagnostic tool has significantly increased. In the clinic, newer molecular techniques offer no significant advantage over optimized culture techniques in terms of positivity rates and specificity (*86*).

In addition to isolation media of demonstrable quality, elevated CO_2, humidity, and an incubation temperature of 35–37 °C are required to support gonococcal growth. Carbon dioxide can be supplied by a variety of means (including the low-tech candle jar), but the initial cost and maintenance of a reliable incubator, and even the availability of a reliable electricity supply, may pose difficulties in resource-poor settings.

Identification of *N. gonorrhoeae* can be problematic. A number of techniques are available, and their applicability and use depend on resources, expertise and circumstance. For a review see Knapp (*87*).

Presumptive identification, usually based on growth on selective GC agar, Gram stain morphology of the growth, and simple and inexpensive tests for 'oxidase' and 'superoxol' positivity (*88*), is regarded as sufficient for diagnosis in many settings, particularly for samples from the genital tract.

In developed countries, definitive identification is more usual, particularly for isolates from extragenital sites. Definitive identification relies on the above criteria plus one or more tests that explore carbohydrate utilization patterns, immunological characteristics or enzyme profiles. Carbohydrate utilization tests are the most widely used. These tests may be growth-dependent (in which acidification of media containing glucose, but not media supplemented with other sugars, is diagnostic for *N. gonorrhoeae*), or growth-independent (based on constitutive enzyme activity). The latter are easier to perform, less expensive and more reliable. Nucleic-acid-based techniques for identification of gonococci are also available, but are not practical because of their cost.

The choice of tests for definitive diagnosis depends on the objectives of the testing laboratory. For STD clinics, distinguishing between gonococci and non-gonococcal Neisseriae may be sufficient. Immunological methods such as co-agglutination or, previously, fluorescent antibody tests, have often been used for this purpose. For laboratories that have broader goals, or when diagnosing non-genital infections, complete speciation is usual.

As discussed below, antibiotic susceptibility testing should be part of the diagnostic process if at all possible.

Enzyme immunoassay

Tests based on antigen detection and enzyme immunoassay have been developed and extensively investigated. However, a meta-analysis of studies conducted in low- and high-prevalence settings and in symptomatic and asymptomatic men and women concluded that 'there are few to no situations for which this assay is recommended' (*86*).

Nucleic-acid-based techniques

General remarks

Because culture-based diagnosis of gonorrhoea has specimen collection and transport requirements that may pose difficulties, there has been an impetus to develop tests based on nucleic acid detection tests, some of which reportedly have high sensitivity and specificity. For the diagnosis of urethritis and endocervicitis, a non-invasive first-catch urine sample usually suffices.

Extensive use of nucleic acid detection tests is limited by their cost, even in developed countries. This technology has been shown to be cost-effective in screening for *Chlamydia trachomatis* in the United States, where appropriate therapy is available. The long-term sequelae of this infection, such as infertility and PID are significant and costly (*89*). Whether these methods will also be cost-effective for gonorrhoea in developed countries, where disease has declined considerably, is still a matter of debate. In developing countries the cost of performing a single test of this kind may exceed the total annual per capita health budget, and there are also considerable infrastructure and maintenance costs. Another major disadvantage of these tests is that they do not, at present, permit determination of antimicrobial susceptibility, information that is essential for disease control. (See also C1).

Nucleic acid hybridization tests ('probe' tests)

In tests of this type a specific probe binds to complementary nucleic acid present in the sample. The signal is amplified by means of a chemiluminescent label on the probe. A commercial test for *N. gonorrhoeae* is available that includes specimen collection kits and a lytic transport medium that stabilizes the preparation for one month. The current version is a combined test that also detects *C. trachomatis*. A review of a number of studies concluded that the sensitivity of the test is about 85% and the specificity 99% (*86*), although the sensitivity was as low as 54% in one report (*90*). Hybridization tests can also be used to confirm culture-based diagnosis of *N. gonorrhoeae*, but they are expensive for this use.

Nucleic acid-based amplification assays (NAA)

A number of techniques are available for the amplification of gonococcal DNA using cycling probe technology. Theoretically, even a single DNA copy, from live or dead organisms, can be amplified to detectable levels. The sensitivity of this test, for all anatomical sites, has been estimated to be about 95% (*86*). False positive results may arise due to specimen cross-contamination (e.g. during transport of leaking urine containers) or environmental contamination of samples (*41*). Although it is claimed that new laboratory procedures minimize the latter problem, Shapiro (*91*) has suggested using probability theory to estimate the likelihood of contamination. False negative results may arise from the presence of inhibitors of NAA in body fluids. Additionally, patient specimens that are self-collected may be inadequately sampled, sometimes deliberately. Some of these problems have not been addressed in commercial test protocols (*92*).

More recent data suggest that the specificity of some commercial NAA tests is lower than reported by Koumans (*86*). In high-prevalence populations not previously tested with these products, 16% of positive results failed to confirm on retesting and 82% of equivocal results were not confirmed. This may be due to differences among Neisseriaceae in different geographical areas (*93*).

Often with the aid of external funding, NAA has been used in developing countries at facilities where syndromic management is in place but laboratory facilities are lacking. The goal of these programmes is to determine the frequency of occurrence of gonorrhoea in order to design and refine algorithms for managing STDs (*94*). In developed countries, NAA assays are particularly useful in outreach programmes that target groups who do not utilize existing services, such as alienated urban youths (*95*) and populations in remote areas where culture-based testing is impractical. In these settings, self-collected samples other than urine (e.g. tampons or swabs) have been demonstrated to be useful in women (*92*).

Since the most effective applications for NAA tests still need to be established, it has been suggested that initially they be used broadly, with continuous monitoring of their performance (*41*).

Summary of laboratory diagnostic methods

A table of available test protocols, modified from the WHO Western Pacific document on detection of reproductive tract infections (*83*) follows. Some commercial tests based on nucleic acid detection combine testing for *C. trachomatis* and *N. gonorrhoeae* at a much lower cost than when these assays are performed separately.

There is as yet no test that allows rapid examination of samples from all the major sites of

gonococcal infection while the patient waits. A rapid, inexpensive and reliable near-patient test would improve case-finding and, thus, efficient treatment and disease management. While syndromic management has been successful in some patient presentations, such as urethral discharge in men, algorithms for vaginal discharge have resulted in overtreatment because even in high-prevalence settings only about 10% of cases are caused by *N. gonorrhoeae* or *C. trachomatis* (*41*).

While the NAA tests provide a means of increasing the sensitivity of testing, they require expensive equipment, usually located at a centralized laboratory, and are most economical when performed in batches. For these reasons it is unlikely that the current generation of NAA kits will provide point of care testing. Some commercial assays use cycling probe technology under isothermal conditions. Miniaturized assays using reagents that are stable at room temperature are now being developed (*41*) and this initiative is actively encouraged (*96*). International programmes are under way to provide inexpensive diagnostics to developing countries (*97*).

Section A. Research and implementation needs

1. Incidence, prevalence and cost-benefit data

Gonorrhoea is grossly underreported, particularly in areas where the incidence is high. Accurate and reliable incidence and prevalence data are essential

CHARACTERISTICS OF *N. GONORRHOEAE* DETECTION ASSAYS [(a)]

	Microscopy	Culture	DNA Detection	Amplification & Detection	
			PACE 2C	PCR	LCR
Sensitivity[1]	95%	85%–100%	85%	89%–97%	95%–100%
Specificity[1]	98%–100%	100%	98%	94%–100%	98%–100%
Advantages	rapid, inexpensive	gold standard * **susceptibility testing available**	rapid, viable organisms not required	viable organisms not required, extremely sensitive, allow non-invasive sampling, can detect *C. trachomatis* in same sample	
Disadvantages	insensitive for females, rectal samples; ineffective for pharyngeal samples	stringent handling, usually requires 48hrs for growth and additional time for identification multi-staged QA necessary	expensive * **susceptibility testing not possible** approved for urethral samples from men and endocervix samples	expensive, requires expertise * **susceptibility testing not possible**	no test for sample inhibitors
Level of use	on-site lab	on-site lab, intermediate	intermediate, referral lab	intermediate, referral lab	intermediate, referral lab
Training	moderate	moderate	moderate	moderate to extensive	moderate
Equipment	light microscope	incubator, light microscope, candle jar	water bath, luminometer	microfuge, thermal cycler, incubator, microwell reader	heat block, thermal cycler, microfuge, lmx processor
Ease of performance	easy	moderate	moderate	moderate to difficult, automated	moderate, automated
Cost	US$ 0.50	US$ 1.00 (+$1–3 to confirm positive isolates) maintenance costs usually low	US$ 6.00	US$ 11.00 (US$ 14.00 for simultaneous *C. trachomatis* detection) high capital and maintenance costs	US$ 14.00 high capital and maintenance costs

1. Sensitivity and specificity are for detection of *N. gonorrhoeae* in urethral, endocervical and urine samples by culture, except for microscopy, which is for detection in urethral samples from symptomatic men.

(a) Modified from "Laboratory tests for the detection of reproductive tract infections"(*83*).

to define the true scope of the problem, to monitor the success of intervention programmes, and to provide a basis for informed advocacy. While 'improving global estimates of the prevalence and incidence of STDs will require concerted efforts by international agencies, national health services, private companies, and the research community' (*1*), epidemiological sentinel surveys are probably more realistic goals in the short term. Newer diagnostic tests may facilitate such surveys, which can be conducted in carefully selected groups of patients representative of the general population, such as pregnant women or military recruits. However, gonorrhoea is usually concentrated in subpopulations that have much higher incidence and prevalence rates.

Even in developed countries the introduction of new diagnostic tests must be justified by a cost-benefit analysis (*41*) and such analyses have helped justify intervention programmes in developing countries (*98*). Economic data provide a rationale for adequate treatment regimens.

2. Diagnosis

There is a great need for low-cost, effective, simple, near-patient tests both for diagnosis and for case-finding. The NAA tests currently available should be used selectively in developed countries to improve diagnosis rates and, in combination with risk assessment, to help define patient risk markers. A future goal is optimal targeting of NAA tests based on cost-benefit analysis (*41*).

In developing countries NAA tests can be used to improve local syndromic management by more reliably defining etiologies (for example, the contribution of gonorrhoea to vaginal discharge syndromes in the patient population). These tests also have potential in screening programmes aimed at determining prevalence and treating asymptomatic and oligosymptomatic individuals.

It is hoped that newer NAA tests being developed with cycling probe technology will not require expensive test equipment and centralized facilities; issues of cost, availability, distribution, and how best to use such tests will need to be addressed.

SECTION B

Antibiotic resistance in *Neisseria gonorrhoeae*

Summary of Section B

Among the etiological agents of treatable STDs, *N. gonorrhoeae* and *Haemophilus ducreyi* stand out because of the extent to which antibiotic resistance compromises the effectiveness of individual case-management and disease-control programmes. The gonococcus continues to develop resistance both to older, less expensive antimicrobials and to more recently introduced agents. Local and international trends need to be documented.

Obstacles to obtaining quality data include: limited use of culture-based diagnosis, which is essential for susceptibility testing; a high degree of variability in sampling and test methods; reliance on point prevalence studies, which may quickly become outdated.

Data may be obtained from national and regional surveillance programmes (including those sponsored by international organizations), local studies in developing countries (which are often externally funded), and case reports of unusual resistant strains or of treatment failures.

A standard treatment regimen is expected to cure 95% or more of gonorrhoea infections. Because of the close correlation between *in vitro* resistance and clinical failure, in general, an antibiotic should not be used when more than 5% of strains are resistant to it. The rationale of this recommendation will be considered, as well as: treatment strategies and expected outcomes for groups at high risk; the relationship between in vitro susceptibility and clinical outcome; use of pharmacokinetic data. For approved antibiotics, the breakpoint method is practical for determining susceptibility patterns and guiding treatment regimens.

Major resistance problems exist in regions where gonorrhoea is most prevalent. In much of the world gonococci are resistant to penicillins and tetracyclines, and resistance to multiple agents is common. In some developed countries the penicillins are still used effectively, but imported infections must be identified and treated accordingly. There is no reliably documented resistance to the third-generation cephalosporin antibiotics recommended for treatment of gonorrhoea (cefixime–oral and ceftriaxone–injectable), but the cost of these agents limits their use in many countries. There is cross-resistance between penicillins and the less expensive earlier-generation cephalosporins.

Significant quinolone resistance has emerged in the WHO Regions of the Western Pacific and South-East Asia and has spread to countries on the Pacific rim. The use of these agents needs urgent review. Spectinomycin appears to have retained its effectiveness. Data for other agents are sparse. Even though they are not recommended, co-trimoxazole, chloramphenicol/thiamphenicol and aminoglycosides are used, usually in resource-poor settings; available data suggest that resistance to these agents sometimes reaches unacceptable levels.

Resistance may be mediated by alteration of the antibiotic's target or by exclusion or destruction of the antibiotic. The emergence and spread of resistance in *N. gonorrhoeae* has occurred mainly by the acquisition of new DNA via conjugation and transformation and determinants may be located on the chromosome or on extrachromosomal elements (plasmids). In contrast to plasmid-mediated resistance, chromosomal resistance often occurs incrementally. Both forms of resistance can coexist in one organism and resistance to multiple antibiotics is common. Chromosomal alterations which affect permeability can simultaneously reduce susceptibility to penicillins, tetracyclines and macrolides and plasmids often carry the determinants both for penicillinases and high-level tetracycline resistance.

For the penicillins and quinolones there are multiple resistance mechanisms, involving porins (uptake), efflux pumps and the cellular targets of these antibiotics. High-level resistance to spectinomycin and aminoglycosides probably occurs by point mutations affecting their ribosomal target sites. Once antibiotic resistance has emerged, the resistant strains have a selective advantage if treatment regimens are not changed and they become endemic once a critical number of indi-

viduals have been infected. This often occurs as a consequence of infection by the resistant strain of high-frequency transmitters in core groups, e.g. CSWs. The impact of travellers on the spread of resistant strains to new communities has been well documented.

B1. Current drug resistance problems and trends

B1.1 Collection, validity and relevance of susceptibility data

The highest rates of gonorrhoea are found in resource-poor settings where laboratory facilities are limited or unavailable. In these circumstances, susceptibility testing or even culture of *N. gonorrhoeae* is problematic. Susceptibility testing requires standardized sampling and assay methods to ensure accuracy and to enable comparison with data generated elsewhere (see C1 below). Serious technical errors, such as the use of susceptibility discs of inappropriate potency, have led to incorrect estimates of resistance in a number of studies.

Monitoring of gonococcal susceptibility is not universal in developed countries because the incidence of endemic gonorrhoea is so low. Nevertheless, the most current data are from some of these countries because they have the resources for adequate testing and some of them have longitudinal surveillance programmes (*9*, *99*, *100*, *101*) which permit continuing assessment of resistance patterns and monitoring of emerging trends (*66*). In the developing world, resistance data are often obtained from point prevalence studies, which, while valuable, cannot be used to follow trends. The prevailing gonococcal strains and their antibiotic susceptibilities can change very rapidly; this means that short-term studies need to be repeated regularly to be useful in defining treatment regimens.

The enthusiasm for gonococcal susceptibility surveillance decreased in some locations as a consequence of the emphasis on syndromic management, and in developed countries, the introduction of non-culture-based diagnostic tests has led to decreased availability of isolates for susceptibility testing.

Even when isolates are cultured, the strains available may be limited to geographical areas in close proximity to the laboratory, or to a particular subgroup of patients. Data are most often obtained from major population centres or from screening of subgroups such as CSWs. Strains from specialized clinics to which patients are referred after treatment failure may be over-represented, introducing a bias into the sample. As long as any biases in the sample are recognized, and the sampling procedure remains relatively constant over a long period, it is still possible to use the data to analyse resistance trends (*102*).

In conclusion, global data on gonococcal susceptibility are incomplete and the available data must be viewed in the context of the sample of strains used.

B1.2 Sources of data

For many years WHO has recommended establishing a global surveillance programme for gonococcal susceptibility (*103*, *104*). The aim of this gonococcal antimicrobial susceptibility programme (GASP) is to create 'a series of networks of laboratories based in WHO regions that will monitor gonococcal antimicrobial susceptibility and disseminate information on trends in susceptibility and emergence of resistance. These data can then be used to direct the choice of appropriate treatment regimens (*105*) (see also section C3).

The most effective surveillance programmes merge data generated at various levels. Local data are needed to establish antibiotic regimens for syndromic management; regional or global data provide early information on the emergence and spread of resistance in adjoining countries and elsewhere in the world that may soon affect the local situation (*106*). Developed countries have an interest in effective intervention 'on the grounds that it's better to stop it there than stop it here' (*107*).

The progress of GASP has been slow due to a delay in establishing laboratories, networks and infrastructure for activities such as quality assurance. However, programmes have been established in Latin America and the Caribbean, and in the WHO Regions of the Western Pacific and South-East Asia (*105*, *106*, *108*). Within these regions, a number of national networks are at various stages of development.

In addition to data generated by GASP, there have been numerous independent studies of gonococcal susceptibility, conducted over short periods in more limited geographical areas, some of which have been merged into more broadly based surveillance programmes (*109*). Some of these studies involved only small numbers of isolates from selected groups of patients, whereas others have provided substantial information about the local situation.

Case reports of unusually resistant strains are

useful because they may signal new or emerging resistance. When the resistant infection was acquired abroad, such reports may provide an early warning of the emergence of resistance in areas where surveillance is lacking.

B1.3 Use of susceptibility data for epidemiologically based treatment regimens

When and on what basis should a standard treatment regimen be altered? Since, for gonorrhoea, the clinical success of a treatment regimen correlates closely with the susceptibility of the organism, standard regimens can be based on *in vitro* susceptibility determinations and should be modified when susceptibility patterns change.

i. Overall rationale

A standard treatment regimen is expected to cure 95% or more of gonorrhoea infections, a criterion that has been accepted for many years (*110*, *111*, *112*), although the precise origins of this principle are unclear to this reviewer. Thus, in general, an antibiotic should not be used when more than 5% of local isolates are resistant to it. While the rationale for this choice was not explained, McCutchan et al (*113*) recommended that, in the United Kingdom, a change from standard penicillin treatment of gonorrhoea would be needed when PPNG demonstrated 'a rise to more than 5% in indigenous disease'. The authors carefully distinguished between endemic and imported cases. In the USA, McCormack (*114*) suggested that 'if the prevalence of PPNG in any area…constitute a substantial minority (perhaps 5 per cent)…penicillins will cease to be the drugs of choice'. However, a lower 'trigger' level of 3% was later suggested as cause for a change in the standard regimen in the USA (*115*). Various panels of specialists have also recommended that 'regimens yielding lower cure rates (i.e. than 95%) should only be used with great caution since…they may select for resistant strains' (*111*, *112*).

Although the 5% failure rate criterion may have been derived empirically, and some experts suggest that 'there is no recognized level at which a therapeutic regimen should be changed' (*116*), 5% resistance is the cut-off now most generally applied (*9*).

ii. Treatment strategies for groups at high risk

A more stringent criterion is recommended for 'high-risk groups'. In groups 'such as sex workers and their clients…treatment regimens…should be nearly 100% effective' in order 'to reduce the risk of development and transmission of resistant strains …to the general population' (*112*). McCutchan et al (*113*) recommended targeting patients failing to respond to treatment, contacts of those known to harbour resistant strains and travellers from areas where resistance is endemic, even when the level of resistance is below 5%. Ison et al (*116*) suggested that a differential approach based on patient characteristics (ethnic origin, sexual orientation and travel history) be used in combination with resistance data. Such approaches require a degree of sophistication that may not be available in less affluent settings. However the 'zero tolerance of resistance' approach for high-frequency transmitters of disease is consistent with strategies for gonorrhoea control.

iii. Relationship between *in vitro* susceptibility and clinical outcome: MIC breakpoints

While the correlation between *in vitro* estimations of susceptibility/resistance and likely clinical outcome is extremely strong for gonorrhoea, it is not absolute. Occasionally, gonorrhoea is self-limited, with spontaneous elimination of the organism. The duration of carriage is site specific (*117*), being longer for anal and cervical colonization and shorter for pharyngeal infection. Therefore, care must be taken not to credit a 'cure' to treatment with an inadequate regimen. Inadequate therapies may temporarily suppress symptoms and lead to transient culture negativity, but symptoms may reappear after cessation of the antibiotic.

For some antibiotics, the correlation between *in vitro* activity and clinical outcome is so strong that in practical terms they can be equated. PPNG will almost invariably fail penicillin treatment. Spectinomycin resistance is always very high level, occurring in a single step, obviating the need for complex interpretive criteria. High-level plasmid-mediated tetracycline resistance correlates with a very high probability of treatment failure. However, the situation may be less straightforward for some CMRNG, where resistance (e.g. to penicillins and quinolones) develops incrementally, producing a broad MIC range in isolates. (See section B2). For strains at either end of this range, which are easily classified as sensitive or resistant, there is an excellent correlation with clinical outcome. For strains with intermediate levels of resistance, an increasing number of treatment failures are observed as the MIC approaches a critical level.

The concept of a MIC 'breakpoint' was devel-

oped to facilitate treatment decisions based on susceptibility testing. Organisms with MIC at the breakpoint are considered too resistant for the infection to be reliably cured with usual doses. While some infections with these organisms will respond to treatment, the breakpoint is usually set conservatively to avoid inadequately treating a potentially resistant infection. Against *N. gonorrhoeae* strains for which the MIC is at the breakpoint, penicillins have about a 10–15% probability of treatment failure. Failure rates rise rapidly, approaching 100%, as the MIC increases (*118*). For the quinolones, there is an even higher rate of treatment failure at the currently accepted breakpoint, which for ciprofloxacin is 1 mg/l. Treatment failures occur in about 60% of patients infected with gonococcal strains for which the MIC is this high, and about 8% treatment failures occur when the MIC is elevated but still below the current breakpoint (*119*).

The breakpoint method provides a reasonable approach to establishing treatment regimens for penicillins and quinolones. For other antibiotics, the situation is less clear; breakpoints are sometimes determined arbitrarily, resulting in potentially weak correlations between susceptibility and clinical response. With azithromycin, treatment failures have been reported even when the infecting strains had MICs below the tentative, empirically derived breakpoints (*120*). For third generation cephalosporins, breakpoints have not been established because treatment failure has yet to occur. There are no published clinical trials of other antibiotics that are occasionally used to treat gonorrhoea, such as co-trimoxazole and gentamicin, and breakpoints have yet to be set. In the absence of such studies, it is important to examine isolates from treatment failures to determine their susceptibility to the antibiotic used.

As discussed in Section C2, the choice of susceptibility test method is critical to the numerical MIC value obtained in mg/l. Different, but equally valid numerical MIC values will be obtained with different methodologies. The categorization of strains as susceptible/resistant is dependent on adherence to the criteria of the particular test method and use of appropriate controls and quality assurance procedures. It is possible to compare results of different properly performed studies with regard to the proportions of resistant strains present. However, direct comparison of the numerical MIC values obtained by different techniques is usually not possible.

iv. Use of pharmacokinetic data

A theoretically more complete approach to establishing susceptibility breakpoints, described by Moran and Levine (*121*), takes into account the pharmacokinetics of the drugs. The authors calculated the 'therapeutic time', an estimate of the number of hours that drug concentrations in blood remained at four or more times the MIC_{90}. (The MIC_{90} is the amount of antibiotic which will inhibit 90% of strains examined and, as the authors themselves point out, its value is sample dependent.) The MIC_{90} is a useful parameter for monitoring trends, but it does not necessarily reflect the range of MICs or the proportion of strains for which MICs are above a critical or breakpoint value. As discussed in section C2, MIC values vary with the test method used, so that the 'therapeutic time' estimates would also vary. Additionally, susceptibility is often assessed by methods other than MIC determination. Despite these limitations, the pharmacokinetic approach has merit because of its inclusion of additional parameters likely to influence clinical outcome.

In addition to efficacy, the choice of a standard treatment regimen must be based on additional factors such as: the cost and availability of the antibiotics; their safety and tolerability; the likelihood of adequate compliance with the regimen by patients; the possibly of compromising the usefulness of the drug in treating other infectious diseases; and strategies for delaying the emergence of resistance. Some of these factors are considered elsewhere in this review, but the reader is referred to the recent document 'Management of sexually transmitted diseases' (*112*).

B1.4 Recent resistance data

Resistance data are often incomplete (see section B1.1). In the discussion that follows, agents that are recommended for the treatment of gonorrhoea (penicillins, cephalosporins, quinolones, spectinomycin) are considered first, followed by the less frequently used agents, and by a consideration of high-level tetracycline resistance. Regional resistance data are organized using the current WHO regional divisions (Africa, Europe, Western Pacific, South and South-East Asia, and the Americas). Data from individual countries are reported when available and pertinent.

As discussed in the previous section, it is generally recommended that an agent no longer be included in treatment regimens when resistance,

determined by *in vitro* testing, is of the order of 5%. However, in more affluent countries treatment regimens may be changed before this level of resistance is reached, while in resource-poor settings an agent may continue to be used as first-line treatment, despite a higher rate of resistance, because there are no other affordable treatments.

Penicillins (penicillin, ampicillin, amoxycillin)

Summary. In much of the world, penicillin resistance is very frequent in gonococci (see B2 below). The usefulness of penicillins is increasingly compromised by chromosomally mediated resistance (CMRNG), while penicillinase-mediated resistance (PPNG) remains a major problem.

In the Americas, excluding the USA and Canada, data from 1995 on 10,500 isolates from 10 countries indicated a PPNG rate of about 10%, up from less than 5% in 1990 (*105*). Data from individual countries in this region confirm this trend. In Argentina, PPNG rose from 1.9% in 1980 to 28% in 1992–1994, declining to 14% in 1996. The proportion of CMRNG also continued to rise and reached 19% in 1996 (*122*). In Trinidad and Tobago 7.6% of 518 strains were reported to be penicillin-resistant in 1997; 70% of them were PPNG (*123*). In the USA, the Gonococcal Isolate Surveillance Programme (GISP) reported 17.9% penicillin resistance in 1997 (5.1% PPNG and 12.8% CMRNG) (*124*).

In the WHO Western Pacific Region, 1998 data are available for about 10,000 isolates from 16 countries. The proportion of isolates resistant to penicillins by any mechanism ranged from 9% in New Caledonia to 90% in the Republic of Korea. Particularly high levels of resistance were recorded in China (62%), Hong Kong Special Administrative Region of China (69%), the Philippines (82%), Viet Nam (77%) and Singapore (59%). PPNG were widely distributed throughout the WPR in 1998; although they represented less than 10% of isolates in a number of centres (Australia, China, Hong Kong SAR, Fiji, Tonga, New Zealand and Japan), their frequency was particularly high in the Philippines (79% of isolates), the Republic of Korea (74%) Singapore (57.2%), Viet Nam (62.7%) and Mongolia (26%). The surveillance programme has detected an increasing proportion of CMRNG since its inception in 1992. In Hong Kong SAR, CMRNG isolates now represent 66.4% of strains, while the proportion of PPNG has declined to 2.6%. This has been ascribed to the widespread use of quinolones, which are able to cure strains of plasmids (*125*). In some countries, data were available that demonstrated different resistance frequencies in urban and rural areas. In urban Australia, penicillin resistance is about 40%, most of it CMRNG. In contrast, in northern Australia, where the rates of gonorrhoea are up to 100-fold higher, only 1–2% of isolates were resistant (*18*).

In SEAR countries PPNG rates are also generally high. Studies of strains isolated in Thailand and Indonesia show high proportions of both PPNG and CMRNG (*102*, *126*) Djajakusmah et al (*127*) confirmed the high rate of PPNG in Bandung, Indonesia, but did not find any CMRNG, despite continuing use of benzathine penicillin in brothels. In India, studies from Mumbai (128) and Delhi (*129*) reported 94% and 8% PPNG, respectively. In Sri Lanka, PPNG rates have declined considerably (*130*), while in Bangladesh 23% of isolates collected in 1997 were PPNG (*131*).

Studies from a number of African countries indicate that a high proportion of isolates are penicillin-resistant; penicillins have been of little use in the region for a considerable time. PPNG first appeared in South Africa in 1977, and in the 1980s reached proportions necessitating withdrawal of the penicillins for treatment (*26*). In the United Republic of Tanzania, PPNG rates ranged from 26 to 74% in different patient subgroups (*132*). An earlier study indicated that CMRNG represented a considerable proportion of isolates (*133*). In Malawi, PPNG rates were reportedly 36% in a 1993 report (*134*) but >83% in a 1996 study (*29*). In the Gambia, 77% of isolates were PPNG in 1997 (135). From 1988 to 1996 PPNG rates in eight African countries (Cameroon, Côte d'Ivoire, Ethiopia, Mozambique, Rwanda, South Africa, Zaire and Zambia) ranged from 47% in Rwanda to 82% in Cameroon (*136*). Bogaerts et al (*137*) followed PPNG in Rwanda from 1985 to 1993, at which time there was a sudden increase. In Ethiopia in 1997 70% of 68 isolates were PPNG (*138*). CMRNG were also common in these countries (136). There was a high rate of PPNG (56%) in Kenyan isolates in 1995–1996 and 9% of the penicillinase-negative strains were CMRNG (*139*). Guyot et al (*140*) reported that 83% of 100 isolates from Liberia were penicillin-resistant.

In some parts of Europe, penicillins are still used effectively to treat gonorrhoea (*141*). However, resistance is frequent in imported cases (*10*, *11*, *142*, *143*, *144*). In Scotland in 1996, 2.6% of isolates

were PPNG and 5% CMRNG (*145*). About one-third of Swedish isolates in 1997 were PPNG, but only a few of these were acquired locally (*19*). Thirty per cent of isolates examined in France in 1997 were penicillin-resistant, with equal contributions made by PPNG and CMRNG (*146*).

Data from the Middle East are sparse. In Dubai (United Arab Emirates) and Saudi Arabia penicillin resistance appeared to be frequent in small samples (*147*, *148*).

Cephalosporins

Summary. The cephalosporins recommended for use in gonorrhoea are third-generation agents. Ceftriaxone is the injectable agent and cefixime the oral cephalosporin most widely used. There is no documented case of treatment failure with these agents. The situation regarding the continued efficacy of earlier-generation cephalosporin agents, which are not standard treatments in most developed countries, is less clear. Resistance to the earlier-generation cephalosporin antibiotics can be expected when chromosomal resistance to the penicillins is present.

Cephalosporin antibiotics are usually classified by 'generations' which roughly correspond to the time of their introduction and activity. Third-generation cephalosporins are unaffected by the TEM-1 β-lactamase of PPNG. Beta-lactamases capable of inactivating third generation cephalosporins are found in other Gram-negative bacteria, but have not yet been detected in *N. gonorrhoeae*. Alteration in gonococcal susceptibility to third-generation cephalosporins is thus chromosomally mediated and step-wise (see section B2). There is evidence of a slight 'shift to the right' in the mean MICs of these agents, i.e. that some decrease in susceptibility has occurred. However, the high activity of third-generation cephalosporins, especially of ceftriaxone, means that there is still considerable 'reserve capacity' in terms of the possibility of increasing the dose of this agent. Actually, some guidelines have decreased the recommended dosage of ceftriaxone from 250 to 125 mg single dose IM injection. One reason for this is that when oral cefixime became available, the bioavailability of its active form was equivalent to that of a 125 mg IM dose of ceftriaxone. The ceftriaxone dose was reduced in the interests of uniformity, although many still prescribe the higher 250 mg dose.

The utility of earlier-generation cephalosporins in the treatment of gonorrhoea is less clear. Cephalosporins are not equally resistant to the action of the beta-lactamase of PPNG, and earlier-generation cephalosporins are especially susceptible to this enzyme (*149*).

Chromosomally mediated mechanisms affecting the susceptibility of *N. gonorrhoeae* to β-lactam antibiotics include modification of penicillin-binding proteins (PBPs) and decreased penetration of antibiotics into the cell (*150*). Both penicillins and cephalosporins are affected by these resistance mechanisms.

PBPs, enzymes involved in cell wall metabolism, are the targets of the β-lactam antibiotics. Multiple amino acid substitutions within their transpeptidase domains have occurred over time (*150*), leading to 100-fold or greater increases in penicillin MICs (*151*). Cephalosporins, even within the same generation, have different affinities for the various PBPs and this is reflected in differential antimicrobial activity. For example, ceftriaxone is significantly more active than cefotaxime. Clendennen et al (*152*) reported that in 1989 more than 10% of 135 gonococcal isolates from US servicemen in the Philippines were resistant to cefuroxime and cefoxitin, whereas all were susceptible to cefpodoxime and ceftriaxone. It is thus difficult to fully predict the efficacy of cephalosporins, particularly as the highly transformable gonococci continually acquire new genetic information from related species (*153*).

Continuous monitoring of the susceptibility of *N. gonorrhoeae* to cephalosporin antibiotics is required. Because chromosomally mediated penicillin resistance also correlates with resistance to earlier-generation cephalosporins, such as cefuroxime, considerable evaluation is recommended before using these agents to treat gonorrhoea (*149*, *154*). The criteria for resistance to earlier-generation cephalosporins are unclear and not sufficiently standardized to permit meaningful comparisons of different studies. When the same strains were examined in different laboratories substantially different results were obtained (*155*). This study and others cited by Phillips (*149*) indicate that resistance is frequent enough that treatment failure would be common in regions where penicillin resistance is high. Resistance to these agents may be due either to PPNG or to CMRNG. As the two may coexist, reliable monitoring of CMRNG requires that the bacteria be 'cured' of the β-lactamase plasmid. Therefore, stability to β-lactamase is not a sufficient criterion for activity.

(See also discussion on β-lactamase inhibitors below.)

Quinolones

Summary. Quinolone-resistant gonococci (QRNG) have emerged and spread relatively recently in areas with a high burden of gonococcal disease combined with antibiotic overuse or misuse. Emergence and spread of QRNG may have been accelerated by the introduction of the quinolones for the treatment of other diseases. The highest rates of QRNG are found in some WPR and SEAR countries, where emergence and spread of QRNG have been systematically documented since 1992. Bordering regions have recorded the importation of QRNG with resultant sustained domestic transmission. As well as the frequency of resistance, the MICs of resistant strains have increased. The use of these oral agents is now severely compromised in many settings where the rates of gonorrhoea are particularly high.

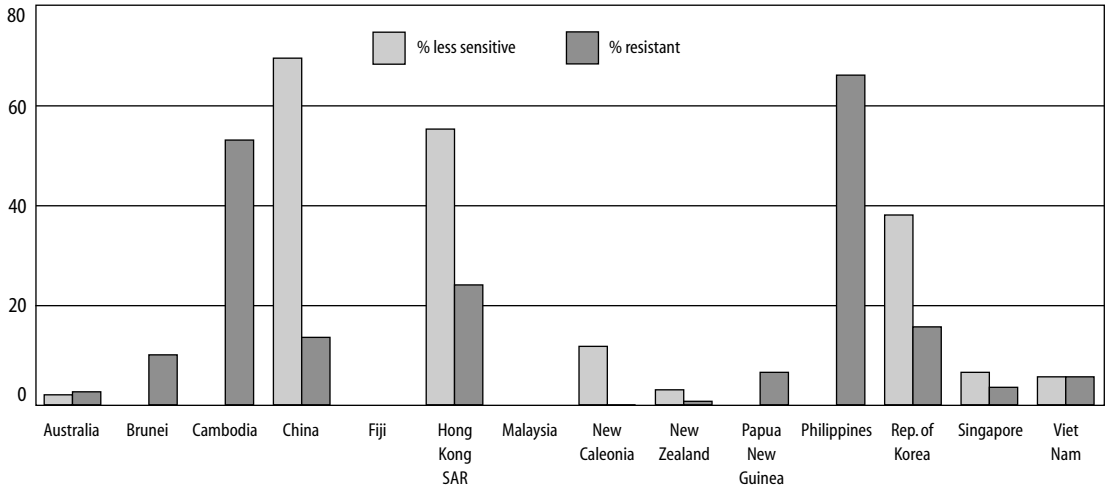
Those quinolones most widely used in the treatment of gonorrhoea (norfloxacin, ciprofloxacin and ofloxacin) are classified as second-generation agents (*156*). The data presented here relate mainly to this group. Fourth-generation quinolones, such as trovafloxacin, have been tested for the treatment of gonorrhoea, but information on resistance to this agent is not yet available. Trovafloxacin has been recently withdrawn from use in many countries because of side-effects.

The use of quinolones to treat STDs and the emergence of resistance in gonococci have been reviewed (*157*, *158*). Criteria for *in vitro* resistance to quinolones (as expressed by numeric MIC values) are method dependent but are more uniform than for some other antibiotics. The criteria of Knapp et al (*159*) are widely used.

Initially, gonococci were extremely susceptible to quinolones. Resistance, which is exclusively chromosomally mediated, has developed incrementally. Ciprofloxacin MICs for the first less susceptible strains were of the order of 0.06 mg/l. MICs of 1 mg/l were later observed and now MICs may be as high as 16–32 mg/l. Patients infected with strains showing decreased susceptibility or intermediate resistance usually respond to currently recommended doses of quinolones (e.g. 500 mg of ciprofloxacin), although treatment failure has occasionally been reported even with these dose regimens (*119*, *160*). Treatment failures are more frequent with the lower doses previously used (e.g. 250 mg single dose ciprofloxacin). Infections with strains for which MICs are very high will not resolve even with the higher doses currently recommended. Because MICs increase in a stepwise manner, due to alterations in multiple targets (see B2 below), it is important to monitor MIC trends. High-level quinolone resistance is more likely to emerge in areas that already have a high prevalence of strains with intermediate susceptibility, suggesting that quinolones should not be used in those regions (*158*). Therefore, wherever data differentiating low- and high-level QRNG are available, they will be provided.

In the Western Pacific Region the WHO WPR

Percentage of *Neisseria gonorrhoeae* isolates that were less sensitive to or resistant to quinolone antibiotics in 14 countries in the WHO Western Pacific Region in 1996. Less sensitive: ciprofloxacin MICs 0.06–0.5 mg/l; Resistant: ciprofloxacin MICs 1 mg/l or more.



GASP has monitored the emergence and spread of QRNG in an increasing number of countries since 1992. In each centre, both the number of QRNG isolates and their MICs have progressively increased. Data for 1996 are summarized in the figure. Note that it differs from a similar figure published previously (*105*), in which the categories were reversed due to an error in the original.

There was some further significant change in 1998 in the proportion of less sensitive strains present in the WPR surveys. The proportion of less sensitive strains remained particularly high in China (36.4%), Hong Kong SAR (44%), Japan (50%) and the Republic of Korea (51.5%). In a large sample from Fiji and a small one from the Solomon Islands, no QRNG were detected. Many WPR centres reported an increase in the proportion of resistant strains seen in 1998 or else maintained the high numbers seen in previous years. The highest proportions of fully quinolone-resistant isolates were seen in the Philippines (63%), Hong Kong SAR (48.8%), China (54.2%) and the Republic of Korea (11.2%). Lower rates, but still cause for concern, were found in Viet Nam (8%), Singapore (7%) and New Caledonia (7.5%). In other centres there was less of an increase in fully resistant isolates. In Australia, resistant strains accounted for 3.2% of all isolates; most of these were from Sydney.

QRNG have been isolated from travellers returning to their home countries from the WPR. These include cases and clusters in North America and the United Kingdom (*161*, *162*, *163*, *164*). In the USA, there has been intercity spread and transmission of isolates with low level quinolone resistance (*165*, *166*).

Both low- and high-level QRNG have been detected in areas of South and South-East Asia, including New Delhi (*129*, *167*), Mumbai (*128*), Sri Lanka, Myanmar and Nepal (WHO SEAR GASP, 1998, unpublished), often at rates that severely limit quinolone use, as in Thailand (*126*) and Sri Lanka (*130*). Over a third of isolates from Bangladesh were QRNG in 1997, 11% having high-level resistance (*131*). Some countries in the SEAR and WPR remain free or relatively free of QRNG, including Indonesia (*102*, *127*), Fiji, and Pacific Island States such as the Solomon Islands. This may reflect low quinolone use in these countries. Quinolones have only recently become the recommended treatment for gonorrhoea in Indonesia. Quinolone-resistant strains isolated in Sydney, Australia from 1994 to 1999 were imported from SEAR countries shown in the figure, as well as from Vanuatu, Malaysia, Thailand, Indonesia, Iceland, Sri Lanka and India. This suggests that QRNG continue to spread.

In many European countries QRNG account for only a low percentage of strains, mostly imported. There have been reports of QRNG in the Russian Federation, England, Scotland, Spain, Greece, Finland, Estonia, Sweden, France, Norway and the Netherlands (*9*, *11*, *19*, *142*, *144*, *145*, *146*, *160*, *163*, *168*, *169*, *170*). Not all of these publications report the proportion of all isolates that QRNG represent. A longitudinal study in London (160) reported only 18 cases of QRNG (four with high-level resistance) among about 5,000 isolates examined between 1989 and 1997. However there was a significant decrease in the susceptibility of isolates to the quinolone agents as measured by MIC shifts. Seventeen per cent of isolates in Sweden in 1997 were QRNG; although most of these were imported from Asia, a small number were acquired locally (*19*).

In Africa quinolones are not extensively used because they are not generally available, and resistance appears to be commensurately low, although data are few. All isolates tested in Malawi by Lind et al (*134*) were fully sensitive and the same was true in 1996 (*29*). Similarly, isolates from the United Republic of Tanzania, South Africa, Côte d'Ivoire, the Gambia, Rwanda and Zaire examined up to 1993 were quinolone sensitive (*26*, *132*, *133*, *135, 171*, *172*). In an earlier study from 1985 to 1990 in Rwanda, Bogaerts et al (*173*) reported the appearance of low-level quinolone resistance and instances of treatment failure, but this was subsequently reversed (*137*). The resistance may have been related to widespread use of early quinolones to treat diarrhoea.

In the Americas case reports document the presence of highly resistant QRNG in Brazil (*174*), but none were found in Argentina in two studies (*122*, *175*). Dillon et al (*176*) noted the presence of low level quinolone resistance in several regions of the Caribbean and the Americas from 1993 to 1995. Among 72 isolates in Honduras (*177*) and 518 in Trinidad and Tobago (*123*) there were no QRNG. In Uruguay between 1994 and 1997, 6% of 119 strains showed decreased susceptibility to ciprofloxacin, with two strains having high-level resistance (*178*).

In the Middle East, 1996 data from Dubai suggested the presence of low level quinolone resistance in a small sample (*147*). Another small sample from Saudi Arabia in 1998 (*148*) did not include any QRNG.

Spectinomycin

Summary. This injectable agent retains its activity against *N. gonorrhoeae* in most parts of the world.

Resistance to spectinomycin, previously rare, was notable during the mid-1980s, coinciding with its use to treat US servicemen in the Republic of Korea (*179*). There were other reports of spectinomycin-resistant strains originating in the Far East (*180*). Resistant strains were detected only sporadically prior to this time, but the drug had only been used as a second line agent before the appearance of PPNG (*181*). The emergence of spectinomycin resistance in PPNG isolates and the availability of alternative treatments led to a decrease in its use. Some treatment failures with spectinomycin have been reported despite *in vitro* susceptibility (*182*). A possible explanation is poor distribution of the antibiotic from the intramuscular injection site.

In the Western Pacific Region, Clendennen et al (*183*) reported that in 1989 8% of strains from military personnel in the Philippines were spectinomycin-resistant. Data have been available from the WPR GASP study since 1992. Spectinomycin resistance was detected at that time in China (*184*) but it has since decreased to low levels. Sporadic resistance was reported in Papua New Guinea, New Caledonia, Viet Nam and Australia between 1992 and 1998. In recent years all isolates from the Republic of Korea have been susceptible.

In the South-East Asia Region, Clendennen et al (*152*) reported that in 1990 8.9% of 305 strains from Thailand were spectinomycin-resistant, whereas no resistance was detected in 1994–1995 (*126*). All 94 isolates tested in Bangladesh in 1997 were susceptible (*131*), as were 33 strains in Mumbai (*128*) and 50 isolates tested in Bandung, Indonesia (*127*).

In several African studies, all isolates were reportedly susceptible to spectinomycin (*133*, *137*, *140*, *172*).

In the Americas, the 1996 USA GISP reported that all isolates were susceptible to spectinomycin. Up to 1995, only sporadic resistance was noted in the PAHO GASP data (*105*, *175*). No resistance was found in Trinidad and Tobago (*123*).

In Europe, all 337 Finnish isolates were spectinomycin-sensitive in 1993 (*11*) as were 104 in London (*142*). French isolates from 1997 were all sensitive to this agent (*146*).

In the Middle East, all isolates in two small surveys in Dubai (*147*) and Saudi Arabia (*148*) were spectinomycin-sensitive.

Tetracyclines

Summary. Both chromosomal and plasmid-mediated resistance are widespread. The tetracyclines are no longer recommended for treatment of gonorrhoea because of increasing resistance, as well as the need for multiple dose therapy, which decreases compliance.

However, tetracyclines are cheap, generally available and widely used in the informal health sector of many countries. Additionally they are often used as adjunct therapy in the syndromic management of STD in settings where co-infection with agents such as *C. trachomatis* is likely. In this situation, it is not possible to evaluate what if any contribution tetracyclines may make in the management of gonorrhoea. Azithromycin (see below) is frequently used to treat *C. trachomatis* infections in developed countries, but tetracyclines are less expensive and are therefore more widely used in treatment strategies aimed at both organisms. In *N. gonorrhoeae*, plasmid-mediated tetracycline resistance (TRNG) is high level. The emergence and spread of TRNG have been closely monitored since they were first reported in the United States in 1985 (185, 186).

In the 1997 GISP study in the USA, 25.6% of isolates were tetracycline-resistant (17% chromosomally mediated and 8.6% TRNG) (*124*). In the rest of the Americas, regional data showed an increase in TRNG from less than 5% in 1990 to nearly 15% in 1995 (*105*). A Nicaraguan study from 1989 reported 22% TRNG (187). The proportion of TRNG in Caribbean and Latin American countries in 1993–1995 ranged from 6% to 63% and chromosomally mediated resistance from 5% to 71% (*176*). In Trinidad and Tobago, 10% of strains were tetracycline-resistant, half of these being TRNG (*123*). In Argentina, an increase in TRNG from 2% in 1993 to 8.8% in 1996 was documented by Fiorito et al (*122*). Chromosomally mediated resistance was nearly 25%.

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 Viet Nam (35.9%), continuing a pattern observed in earlier years. In all other centres TRNG was below 10%. Malaysia did not supply data in 1998; TRNG rates in that country have historically been high. The pattern of TRNG distribution has changed only slightly in the WPR in the past seven years. It has been speculated that the use of quinolone antibiotics, which can cure bacteria of plasmids, may

be the reason for a decrease in TRNG in Hong Kong SAR, from 4.5% to 2.1% from 1993 to 1995 (*125*). PPNG also decreased over this period. However, there are no experimental data to support this hypothesis.

TRNG are a significant problem in the WHO SEAR, and in Thailand accounted for about 16% of isolates in 1994–1997 (*126*). An additional 55% of strains had chromosomally determined resistance. Indonesia has particularly high rates of TRNG, and virtually all *N. gonorrhoeae* isolates show one or the other form of resistance (*102*, *127*). In India, Bhalla et al (*129*) found 28% of 50 consecutive isolates in New Delhi to be TRNG. In 1997, 10% of 94 isolates from Bangladesh were TRNG (*131*).

In Europe, TRNG were detected in the United Kingdom and the Netherlands soon after the first American reports (*188*, *189*). In both countries TRNG have increased and represent a high proportion of strains (*9*, *142*). TRNG were not detected in Sweden until 1992, and then only in a small number of imported isolates; but chromosomally mediated resistance to the tetracyclines was already at a high level and increasing (*143*). TRNG were detected in Greece in 1990 (*144*) and in Finland in 1993 (*11*), but only in low numbers; there was also substantial chromosomally mediated resistance. TRNG have also been reported in Denmark, Spain, France and Scotland (*9*, *145*).

Data from Africa are of particular interest because of the continuing use of tetracyclines in the informal health sector. The emergence and spread of TRNG in Africa has been documented in several studies (*133*, *136*, *172*). Up to 1993 TRNG increased rapidly, to 61% in Côte d'Ivoire, 12 % in Rwanda and 30% in Zaire. Among strains from Northern Tanzania, 35% were TRNG in 1992. In Rwanda TRNG spread rapidly after 1989 (*137*). TRNG were absent from strains examined in Durban, South Africa up to 1993 (*170*), although studies cited by Pham-Kanter et al (*26*) suggest that tetracycline resistance is frequent in other parts of that country. In Malawi, the majority of isolates were tetracycline-resistant (*134*). Other data confirm that TRNG are widespread in Africa: 82% in Cameroon, 65% in Ethiopia, 21% in Mozambique, 41% in South Africa, 62% in Zambia, 84% in the Gambia and ≥84% in Malawi (*29*, *135*, *172*).

Other antibiotic agents

Summary. Other agents, some of them not recommended for this purpose, are used to treat gonorrhoea in many parts of the world. The high cost of efficacious antibiotics often leads to the use of suboptimal regimens. Lack of data on resistance contributes to their continued use.

Azithromycin

This new macrolide has been explored in the treatment of gonorrhoea because of its efficacy in the treatment of *C. trachomatis* infections. Even if demonstrated to be efficacious, its cost would be a limiting factor in using it to treat gonorrhoea. Some reports from countries where gonococci remain relatively sensitive to antibiotics have shown azithromycin to be reasonably effective in a 1g oral dose. Other reports indicate that there is an unacceptable rate of treatment failure with this dose (despite initial *in vitro* susceptibility of the isolates) and that higher doses are associated with a high frequency of side-effects (*120*, *190*, *191*, *192*,*193*). Increases in azithromycin MICs have been reported during treatment (*192*, *194*). Parameters for defining azithromycin resistance in *N. gonorrhoeae* and correlations with treatment outcome are not yet available (*120*). This means that susceptibility surveillance is not yet possible. Absorption and distribution issues may explain some of the treatment failures.

Co-trimoxazole (sulfamethoxazole-trimethoprim combination)

Determining susceptibility to this combination is technically more complex than for other antibiotics (see section C2). This agent is rarely if ever used to treat gonorrhoea in developed countries; therefore data sources are limited. The most recent data include those of West et al (*132*, *133*) which indicate increasing resistance in regions of Tanzania where co-trimoxazole is used. Similarly, resistance was found in 18% of strains in the Gambia (*135*) and 64% in Ethiopia (*138*). Guyot et al (*140*) reported that 100 strains from Monrovia, Liberia all showed intermediate resistance.

Aminoglycosides

These agents (mainly kanamycin and gentamicin) are most often used in less developed countries. Criteria for resistance are not standardized and few data are available. One study in Ethiopia estimated

kanamycin resistance to be 17% among 68 isolates (*138*). All fifty isolates tested by Djajakusumah et al (*127*) in Indonesia were susceptible to kanamycin. Gentamicin MICs for 100 Liberian isolates were in a narrow range, 4 to 8 mg/l (140). A Malawi study (*29*) suggested that for strains tested using the assay methods of the US GISP, gentamicin MICs ≤4 mg/l indicated 'highly susceptible', 8–16 mg/l 'moderately susceptible', and ≥32 mg/l 'low susceptibility'. Fifty-seven of 63 isolates were in the moderate or low susceptibility categories. MICs for strains from treatment failures ranged from 4 to 16 mg/l, but treatment success was noted with two strains for which the MIC was 32 mg/l.

Chloramphenicol/Thiamphenicol

According to Moran (personal communication of internal document) criteria for correlating the MIC of these agents with clinical outcome are not well developed. One study (*195*) found that treatment failure with thiamphenicol became significant at MICs of 0.5 mg/l and above. Data are not available on the current prevalence of strains of this type. Using a MIC of ≥2 mg/l as the cut-off for resistance, 12% of 50 isolates from female CSWs in Bandung, Indonesia were resistant (*127*). Thiamphenicol is widely used in Indonesia, and these levels of resistance make its use for gonorrhoea of questionable value. Chloramphenicol is widely used in a number of countries for many types of infections. Some unpublished studies on gonococci isolated from travellers indicate substantial levels of resistance to chloramphenicol. Ten of 46 isolates tested in Delhi in 1996 had chloramphenicol MICs of ≥4 mg/l (*129*).

B2. Mechanisms of antibiotic resistance in the gonococcus

General considerations

The gonococcus, originally highly susceptible to antibiotics (*196*) can adapt to adverse conditions. 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 coexist 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. While genetic transformation, the mechanism of acquisition of these determinants, is common in *N. gonorrhoeae*, clinically relevant resistance requires multiple gene transfers (*197*). 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 (*197*). Different rates of dissemination of extrachromosomally mediated resistance have thus 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.

A description of resistance mechanisms for some individual antibiotics follows. Particular attention is given to the penicillins for illustrative purposes.

Penicillins (Penicillin, ampicillin, amoxycillin, penicillin/β-lactamase inhibitor combinations)

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 and treatment with 150,000 units of penicillin was efficacious in most instances (*198*) decreased *in vitro* susceptibility appeared, it was associated with treatment failure (*196*). Increasing the recommended dose of penicillin 'temporarily alleviated the clinical problems resulting from infection with these strains' (*198*), but almost inexorably levels of resistance increased and large

numbers of treatment failures again occurred, even with high-dose regimens (*55*). This was an example of stepwise 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 decrease their affinity for the penicillins, and thus the susceptibility of the organism (*199*). PBP-2 is encoded by the *penA* locus (*200*).

Changes in other loci such as *mtr* and *penB* produce additive effects. The *mtr* locus mediates resistance to a wide range of antibiotics, detergents and dyes (*201*) through an active efflux system (*202*). Mutations in the *penB* locus, which affect a porin, result in reduced permeability of the cell envelope to hydrophilic antibiotics and other compounds (*197*, *203*). Gonococci possess two alleles for porin, P1A and P1B (WI and WII/III in the Swedish nomenclature(*204*)), but in a given strain only a single porin is expressed, resulting in mutually exclusive serogroups. CMRNG are associated with the 1B (WII/WIII) serogroup (*69*). The gonococcus also has a *porA* 'pseudogene' which is not expressed (*205*). In contrast, *N. meningitidis* expresses two porins, PorA and PorB. The combined effect of *penA* mutations and increased expression of *mtr* is to increase the MIC of penicillin by 120-fold (*197*). Gonococci exhibiting these changes are termed chromosomally resistant *N. gonorrhoeae* (CMRNG) (*206*). Reduced susceptibility to cephalosporins (*207*), tetracyclines (*200*) and other agents (*208*) is also mediated by chromosomal mechanisms.

Resistance to penicillins is also mediated by a plasmid-borne, inducible TEM-1 type β-lactamase. This enzyme hydrolyses the β-lactam ring of penicillins, thus inactivating them. In contrast to the slow evolution and incremental increase in resistance associated with chromosomal changes, acquisition of the plasmid confers resistance in a single step. Penicillinase-producing *N. gonorrhoeae* (PPNG) were detected at the same time in the United Kingdom (*209*) and the USA (*210*). The first isolates were imported, 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 (*211*). Thus the Asian strain disseminated more widely and more quickly. Subsequently, a number of PPNG carrying plasmids of different sizes were described, but they all appear to be related (*197*). It is possible that the plasmid was initially acquired from *Haemophilus* species (*212*).

Lactamase production (PPNG) and chromosomal changes (CMRNG) can coexist 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 (*213*) they represent an effective oral therapy for PPNG infections, but more commonly single-dose regimens of penicillin/inhibitor combinations have failed (*214*). 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 (*215*).

Cephalosporin antibiotics

Altered gonococcal susceptibility to cephalosporin antibiotics is chromosomally mediated and is due to the same changes that account for decreased penicillin susceptibility (*197*, *207*). There is cross-resistance between penicillins and early generation cephalosporins such as cefuroxime (*207*, *216*). However, this is not the case for the later generation cephalosporins such as ceftriaxone and cefixime. Not all cephalosporins are hydrolysed 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 Neisseriae. If such an event were to occur it would be devastating for gonorrhoea treatment programmes that rely heavily on the third-generation cephalosporins.

Spectinomycin and aminoglycosides

In *N. gonorrhoeae*, resistance to spectinomycin or to aminoglycosides usually occurs via a single-step,

chromosomal mutation, resulting in high-level resistance. The different ribosomal genes involved in spectinomycin and aminoglycoside resistance are linked (*197*, *217*). However, the somewhat elevated gentamicin MICs reported for some isolates appear to be consistent with porin-related mechanisms. The possibility exists that in the future gonococci will acquire and express plasmid-borne genes encoding enzymes (present in many other bacterial species) that inactivate aminoglycoside antibiotics (*197*).

Quinolone antibiotics

The quinolone antibiotics most widely used for the treatment of gonorrhoea are 'second generation' agents such as ciprofloxacin and ofloxacin. In a manner reminiscent of the development of chromosomal 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. 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. 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 (*160*). The newer quinolones have yet to be assessed for efficacy against gonorrhoea. One of theses agents, trovafloxacin, has been withdrawn from use in many countries because of toxic side-effects.

Quinolone resistance is almost exclusively mediated by chromosomal mutations, which affect either the target sites or access of the antibiotic to the cell. Plasmid-mediated resistance to nalidixic acid in *Shigella dysenteriae* was reported in 1987 but was never confirmed. Plasmid-mediated resistance was recently reported in a clinical isolate of *Klebsiella pneumoniae*. The resistance determinant was carried on a broad host range plasmid and was transferable to other *Enterobacteriaceae* and to *Pseudomonas aeruginosa* (*218*).

Tetracyclines

The tetracyclines are generally not recommended for treatment of gonorrhoea because they must be administered in multiple doses over several days, raising the prospect of decreased compliance and inadequate dosage. However, they are cheap and therefore widely used, particularly in the informal health sector.

Both chromosomal and plasmid-borne resistance mechanisms are found in gonococci, the latter being responsible for high-level resistance. Chromosomal resistance is linked to the *mtr* and *penB* alterations which also reduce susceptibility to the penicillins (*197*). The combination of these and other chromosomal mutations results in clinically significant resistance (*151*). High-level tetracycline resistance in gonococci (TRNG) results from the acquisition of the *tetM* determinant and was first reported in 1986 (*219*). *tetM* in *N. gonorrhoeae* exists as two slightly different 'Dutch' and 'American' types, located on a self-mobilizing plasmid (*220*). A study of the molecular epidemiology of the *tetM* genes by PCR suggests that the Dutch type may have originated in the Far East and the American type on the African continent (*221*). The *tetM* plasmid is widely dispersed in the normal genital tract flora; the mobility of the plasmid and the selective pressure created by the use of tetracyclines to treat other STDs has contributed to the widespread dispersal of the TRNG phenotype (*206*).

Sulfonamide-trimethoprim combinations

Sulfamethoxazole and trimethoprim (co-trimoxazole) have been combined in an oral formulation which is used as a multi-dose treatment for gonorrhoea. As discussed above for the tetracyclines, the need for multiple doses has implications for the development of resistance due to poor compliance.

The two components of the drug produce a sequential block in the synthesis of tetrahydrofolate, which serves as a carrier of methyl groups. In the absence of tetrahydrofolate, several essential functions such as conversion of uridine to thymidine, required for DNA synthesis, are inhibited. Under certain conditions, the lack of thymine metabolites

is critical and bacteria undergo 'thymineless death'. Trimethoprim is not particularly active against gonococci and is in fact used as a selective agent for the growth of gonococci in primary culture plates. This is because of reduced affinity of gonococcal dihydrofolate reductase for trimethoprim. Resistance to the sulfonamides can develop separately by several mechanisms (*197*). Gonococci, in contrast to many other genera, do not appear to have the ability to utilize exogenous thymine or thymidine and thus to bypass the block in synthesis of tetrahydrofolate. However, many strains of gonococci were resistant to the trimethoprim component of the combination and Ho et al (*222*) postulated that this was due to increased production of dihydrofolate reductase by the organism or to decreased cell permeability.

Newer macrolides

A number of newer macrolides have been made available for treatment of *Chlamydia trachomatis* infection, most notably azithromycin. Chromosomal resistance to erythromycin, an earlier macrolide, was dependent on expression of the *mtr* phenotype discussed above (*201*). Slaney et al (*223*) have shown that susceptibility to azithromycin is also affected by *mtr*. It is possible that ribosomal mutations may also determine azithromycin resistance in *N. gonorrhoeae* (*194*). Treatment failures have been reported with low-dose (1 g) azithromycin regimens (*120*, *190*, *192*). Higher doses are not well tolerated (*193*).

B3. Factors contributing to spread of resistance

B3.1 Antibiotic use and misuse

It is no accident that antibiotic-resistant *N. gonorrhoeae* emerged in regions where there is a large informal health sector and the use of antibiotics is not well controlled. (See also section D4.) Resistance to the penicillins, both chromosomally and plasmid-mediated, spread from South and East Asia (*210*, *223*). Spectinomycin resistance appears to be related to the availability of this antibiotic in the region in the 1980s and resistance virtually disappeared after the drug was withdrawn. More recently, quinolone resistance has emerged and spread from the same focus (*106*). The spread of TRNG, in Africa and elsewhere, is also an apparent consequence of the use and probable misuse of the inexpensive, readily available tetracyclines (*133*).

In the above settings there is easy access to antibiotics in the informal health sector (*225*). In one study, about 75% of those attending a STD clinic in Ghana had self-medicated prior to presentation (*226*). The antibiotics had been acquired from a variety of sources and were taken in inappropriate doses, often as mixtures of different agents. Between 70 and 95% of gonococci examined at this clinic were resistant to commonly available antibiotics. Anecdotal reports tell of commercial sex workers in Asia supplying clients with oral quinolones as a means of 'prophylaxis', and self-prescribing of prophylactic antibiotics in CSWs in the Philippines was a factor contributing to the emergence of antimicrobial resistance in that country (*227*). The availability of antibiotics of all varieties in the informal health sector has led to the development, in many bacterial and parasitic genera, of resistance to a wide range of antimicrobials. In the Philippines, gonococci are said to exist in an 'antibiotic soup' (Donovan, 1999, personal communication) and emergence of resistant strains appears to be inevitable. Ironically, some aid agencies that supply free antibiotics (without any support programme to ensure their appropriate use) 'further complicate the problem of resistance development of STD pathogens' (*226*).

Nearly thirty years ago, Willcox (*224*) stated that 'in countries free from social or political turmoil, with limitations on prostitution and widespread clinic and contact-tracing services, where antibiotics are not procurable except on doctor's prescription, where self-treatment or subcurative treatment of undiagnosed gonorrhoea is rare, and where several effective (if more costly) antibiotics are readily available for use against resistant gonococci, the situation (antibiotic resistance) has so far largely been kept in check'. The situation is similar today, with regional antibiotic resistance reflecting differences in the availability of resources between first- and third-world countries. As discussed in section A, the burden of disease is disproportionately high in those countries least able to provide appropriate diagnosis and management, and the levels of antibiotic resistance are also highest in these countries. Many factors contribute both to the high rates of gonorrhoea and the emergence, survival and spread of antibiotic-resistant strains.

B3.2 Epidemiology of the spread of resistant gonococci

Gonococci are essentially non-clonal organisms; they are highly transformable, acquiring DNA from closely related species, and also undergo antigenic variations that regularly alter their phenotype and genotype. Nevertheless, there are epidemiologically useful strain differentiation techniques. A widely accepted system takes advantage of multiple phenotypic characteristics to define an auxotype/serovar (A/S) class (*70*). Auxotyping of *N. gonorrhoeae*, determination of a strain's growth requirements, was first described by Catlin (*68*). Auxotrophy, the requirement for a specific nutrient, is determined by the inability to grow in otherwise complete culture media lacking that supplement. Gonococcal strains may be auxotrophic for multiple compounds. All gonococci require cysteine for growth. The most familiar association of a gonococcal auxotype with a disease manifestation is that of AHU (arginine, hypoxanthine and uracil) with disseminated gonococcal infection. Serological classification of gonococci, based on reactions to monoclonal antibodies raised against epitopes on the major porin (PI, Por) or outer membrane protein, was developed contemporaneously in Sweden and North America (reviewed by Bygdeman (*69*); Sarafian & Knapp (*70*)). The A/S system provides an extended phenotype and increased discrimination by combining results of two independent, stable systems.

Phenotyping systems have been successfully applied to the study of gonococcal strain populations. A waxing and waning of 'successful' strains can be detected in longitudinal studies. About 30–40% of isolates are represented by a small proportion of A/S classes and persist for some time. Other A/S classes account for small numbers of isolates each and may only appear transiently (*70*). Different A/S classes are at times found only in certain subpopulations or core groups. Factors that may be involved in the persistence of particular A/S classes include their ability to cause asymptomatic infection and their resistance to antibiotics. It has also been suggested that host factors, such as serovar-specific antibodies in vaginal secretions, may produce a level of herd immunity to particular subtypes, resulting in the selection of new subtypes (*228*).

Genotyping of gonococci uses a variety of techniques. In general, the highly discriminatory genotypic methods have not proved as useful in longitudinal studies with gonococci because of the propensity of the organism for horizontal genetic exchange and recombination. One genotyping system that is often applied to antibiotic-resistant strains is pulsed field gel electrophoresis (PFGE). There are a wide variety of PFGE methods, including the choice of restriction enzymes, different pulse times and ramp rates, and different criteria for interpreting the band patterns. Nevertheless, in longitudinal studies using A/S typing and genotyping singly or in combination, it has been possible to track the spread of certain gonococcal subtypes, including those exhibiting antibiotic resistance. Further, outbreaks of antibiotic-resistant gonorrhoea have been demonstrated to be caused by strains that are closely related, phenotypically and/or genotypically (*151*, *166*). The spread of PPNG and TRNG has been monitored using plasmid profiles and other markers (*229*).

The results of these and others studies suggest that there are a number of stages in the establishment of an antibiotic-resistant subtype in a community. At first there is sporadic isolation of multiple subtypes with little or no secondary spread. As the new strain continues to be imported, secondary spread may occur until ultimately there is sustained local transmission of the resistant strain. A resistant strain becomes endemic once there is a critical mass of individuals infected with the strain; this is usually associated with infection of core transmitters, such as CSWs (230). Strains that are not introduced into core transmitters are unlikely to persist. This sequence of events was hypothesized for plasmid-mediated resistance and is supported by data on the appearance of PPNG in Florida (*231*). However, as illustrated in the following example, it appears to apply as well to chromosomally mediated resistance. In Sydney, Australia chromosomally mediated quinolone resistance (QRNG) has been closely monitored for more than a decade. Initially, resistance was low level (in terms of MIC) and of low prevalence (2–3%). There were multiple A/S phenotypes, imported by travellers, and little or no endemicity. This pattern persisted for some years, with little or no secondary spread, although the MICs for the resistant isolates increased substantially. Twelve years after QRNG were first detected, their rate of isolation rose suddenly, manifested as a high rate of endemic infection in CSWs and their clients, caused by a limited number of A/S subtypes (*66*). The CSWs involved were illegal immigrants who used condoms far less frequently than their local counterparts. The QRNG were confined to heterosexual patients and

were not found among a large number of isolates from HAM.

B3.3 Introduction and spread by travellers

The effect of the increasing volume of travel (whether for pleasure, or by migrant workers or refugee populations) on the spread of STDs has been reviewed recently (*62*, *63*). In particular, the current speed of travel facilitates spread of strains from one country to another during the presymptomatic incubation phase of infection (*62*). The importance of strain importation by individual travellers is exemplified by the case of Sweden. As rates of gonorrhoea declined, endemic PPNG disappeared, but continued to be isolated from patients with contacts abroad. Additionally, there are core-transmitters of STDs among travellers: itinerant female CSWs, long-distance truck drivers, sailors and migrant workers (*62*, *63*). Illegal immigrant CSWs were responsible for importation and maintenance of PPNG and QRNG in Sydney, Australia (*65*, *66*). Socioeconomic differences between former Eastern bloc countries and Western Europe have led to increased travel by sex workers and their clients (*62*). Long-distance truck drivers and sailors constitute a significant male core group. There is also a separate group of 'sex tourists' (usually older men) who travel 'specifically for the purpose of sex' (*62*).

Mulhall (*62*) identified travellers as 'important targets for sexual health promotion' and also found that, in contrast to STD clinicians, there was insufficient awareness of risks of STD acquisition among specialists in travel medicine. Most initiatives for travellers have been 'AIDS and mobility' programmes. Mulhall recommended a fresh approach to developing a comprehensive sexual health promotion programme for travellers. An integrated programme of education, diagnosis and treatment is required for refugee and migrant populations if STDs are to be controlled in these groups (*63*).

Section B. Research and implementation needs

In gonorrhoea, there is a close relationship between therapeutic outcome and *in vitro* susceptibility to antibiotics. Monitoring susceptibility, to determine the prevalence of resistance and to detect any change in pattern at an early stage, allows us to devise standardized treatment regimens for use both in developed and developing countries.

At present, there are significant gaps in gonococcal susceptibility data, especially in those regions with the greatest disease burden. Continuous monitoring of susceptibility is needed here, in part as a means of curbing the use of inexpensive but potentially ineffective antibiotics to treat gonorrhoea.

Surveillance is considered in more detail in the following section.

SECTION C

Detection of antibiotic resistance in *N. gonorrhoeae*

Summary of Section C

Since antibiotic resistance affects our ability to treat and control gonorrhoea, continuing surveillance is essential to monitor both its emergence and spread. Population-based surveillance of susceptibility patterns is required in order to establish standardized treatment regimens that cure at least 95% of infections and to modify the regimens when the situation changes. For this purpose, samples of gonococcal isolates must be sufficiently large and representative.

Effective surveillance requires both laboratory facilities and procedures capable of detecting resistance and the infrastructure for handling and analysing data.

Surveillance based on clinical outcome involves issues such as accuracy of diagnosis, compliance with therapy, definition of 'cure' and follow-up assessment, and needs to be supported by valid laboratory data.

Effective surveillance of AMR is limited by the fact that gonorrhoea occurs most frequently in resource-poor settings where facilities are not available for isolation and susceptibility testing of fastidious organisms.

The reference method for susceptibility testing of *N. gonorrhoeae* is the agar dilution MIC. Simplified agar-based methods, such as breakpoint determination, permit economical screening of large numbers of isolates, but these tests can usually be performed only in centralized laboratories which may not always receive a representative sample of strains. Disc diffusion methods have become more reliable with the development of better media and the availability of commercially produced antibiotic discs. The E-test is also reliable, but is more costly than disc methods. While NAA-based systems may ultimately simplify testing, we will continue to rely on conventional methods in the immediate future.

Whatever methods are used, data generated in different laboratories must be comparable in order to assess local, regional and global trends in the emergence and spread of resistance. Attempts to establish a uniform susceptibility test protocol have not been successful and numerical MIC values expressed in mg/l are significantly affected by methodology. Nevertheless, classification/categorisation of isolates (i.e. susceptibility or resistance) determined in different laboratories can be directly compared, other than by use of numerical values as long as suitable criteria and controls are in place. A set of reference cultures has been developed that can be used to define categories of sensitive, intermediate or resistant but whose MIC value will vary with different test methods. In order to ensure consistent results, stringent quality control (internal checks) and quality assurance (external checks) procedures must be in place. This has been achieved in some of the longer established regional surveillance programmes.

The introduction of these procedures has facilitated the development of a number of regionally based surveillance structures which can merge data from various sources on a continuing basis and therefore can track the appearance and dissemination of resistant gonococci. WHO programmes have also sought to build the necessary infrastructure for surveillance within each country in a region. Even where this has not been completed (due to lack of testing facilities) significant data have been generated by intermittently collecting strains for testing elsewhere.

It seems an opportune time to form a network to establish and maintain a database of gonococcal resistance patterns.

C1. Overview and principles of surveillance

The objective of gonococcal susceptibility surveillance is to provide a timely and continuous assessment of the emergence and spread of resistance. These data are needed to devise appropriate treatment regimens and to change them promptly and appropriately, when necessary.

In large parts of the world, many classes of antibiotics are no longer effective against gonorrhoea.

Resistance is not uniform within regions or countries, and can emerge quickly in any locality. It may spread rapidly or slowly, depending on a number of factors, including the mechanism of resistance and whether the strain is being spread by core transmitters. Continual monitoring of antibiotic susceptibility is required to ensure effective treatment. Antibiotic-resistant gonococci may be introduced into new areas by travellers, and in these cases it is recommended that treatment be 'chosen according to international data on sensitivities' (*232*)—a recommendation that assumes that these data are both known and available. Effective treatment regimens are based on knowledge of prevailing resistance patterns. Because local patterns can change due to importation of new strains or development of resistance, it is clear that surveillance must be population based, rather than based on the assessment of individual patients, and that awareness of regional and global, as well as local, trends is of great importance.

The relationship between *in vitro* susceptibility determinations and likely treatment outcome is probably stronger in gonorrhoea than in any other disease. This is because the gonococcus at present lacks some of the more elaborate means of inactivating antibiotics such as extended spectrum beta lactamase production, and infections with gonococci are for the most part of mucosal surfaces. Standard treatment regimens, aimed at curing at least 95% of patients, are derived from population-based surveillance of the prevalent susceptibility patterns. (See sections B1.3 and D1.) This strategy is particularly important because the preferred treatment for gonorrhoea consists of a single dose of antibiotic, administered to the patient on presentation (i.e. before any susceptibility test is available). In settings where syndromic treatment is used, knowledge of the local susceptibility pattern is critical. When the prevalence of resistant gonococci rises to a certain level (usually 5%), treatment regimens should be changed. This contrasts with the approach to treatment of many other bacterial infections, in which every strain is isolated and tested for susceptibility and disease is managed on an individual case basis. Thus, the philosophy and laboratory methods used for susceptibility surveillance in gonorrhoea may differ from those used in other infectious diseases.

C2. Laboratory-based methods for detecting resistance

General remarks

Preferably, surveillance of gonococcal susceptibility is population based and continuous. At the same time it is also important to recognize individual patients who are at risk of infection with resistant strains (*116*). Risk factors may include a history of previous ineffective antibiotic therapy and travel to an area where resistant strains are common.

In principle, laboratory methods for susceptibility testing of gonococci are similar to those for other bacteria. However, *N. gonorrhoeae* has specialized growth requirements and efforts to handle this fastidious organism have led to the development of a plethora of tests, with numerous variations in methodology. One obstacle to establishing practical, standardized procedures is the fact that STD microbiology expertise and susceptibility testing expertise have historically resided in different groups of professionals. Recently, there has been a greater appreciation of the complexity of the issues, more adaptability, and the realization that valid and comparable AMR data may be obtained by different laboratory test methods.

Methods in use

Agar dilution (agar incorporation) methods

The agar dilution MIC is the definitive susceptibility test. It is a labour intensive method and is only performed in specialized laboratories, but it is relatively inexpensive when large numbers of strains are tested in batches. Because susceptibility testing of *N. gonorrhoeae* is performed for epidemiological purposes, rather than for individual case management (see D below), delay in treatment is not an issue. A simplified 'breakpoint' method, using a smaller number of antibiotic concentrations (see section B1.3), is useful for screening large numbers of strains when the frequency of resistance is expected to be low. Tests of this type require experienced staff and access to antibiotic powders of known potency. Strains must be stored until they are tested, which involves extra handling, subculture and resources.

The agar dilution methods currently in use are not uniform, and different MIC values expressed in mg/l may be obtained when the same strains are tested in different laboratories (Dillon et al, unpublished data, 1992). Variables include: growth medium (basal medium and supplements), inoculum, incubation conditions and incubation time.

In one report comparing methods used by six different reference laboratories, there were about 50 possible variations if all combinations of media, additives and incubation time were considered (*233*). As in any test method, the one variable most difficult to control is reading the endpoint, which is subjective. However, the extent of variability in endpoint determination can be reduced by including standard strains in each batch of tests for quality control (QC). MICs are generally accepted to be accurate to plus or minus one doubling dilution.

Comparability of MIC data

In analysing the difficulties of comparing susceptibility data from different sources, Willcox (*223*) included type of endpoint (MIC vs. IC_{50}), interpretation of MIC endpoints, use of different units of measurement and strain selection, e.g. from specialized clinics, as important variables. Attempts to standardize test methods began as far back as 1963, and an expert group recommended standardization in 1978 (*103*). The recommendations included: development of disc diffusion susceptibility tests as well as agar dilution methods; making available reference cultures for QC; and continuous surveillance of gonococcal susceptibility. Some of these recommendations have been implemented and have proven to be invaluable. Reference cultures have been developed and are readily available from the Neisseria Department of the State Serum Institute, Copenhagen and other sources (see below). The panel of strains has been periodically revised and there are currently proposals to expand it to include strains resistant to the more recently introduced antibiotics (I. Lind, personal communication). Less progress has been made in standardising test methodology at the international level. However, some national programmes have implemented standardized methods which include internal QC and external QA (*99*, *100*, *233*).

Recently, it has been suggested that data obtained by different methods can be compared if certain test parameters are defined (*157*).

For example, due to the use of different methods, MIC values (in mg/l) for the same strains, obtained in Australia and the USA differ. Thus chromosomal resistance to penicillin in the USA and Canada is defined as an MIC of 2 mg/l or more and in the United Kingdom. and Australia as an MIC of 1 mg/l or more. These differences are ascribed to the different test media employed (GC agar in North America and sensitivity testing agars elsewhere). Thus when the same strains were tested in the different settings, the numerical MIC values obtained were different. However, qualitative classification of the strains (i.e. as sensitive or resistant) was the same in the different countries when the relevant interpretive criteria were applied (Dillon et al, unpublished data, 1992). The value of this approach has been demonstrated in the continuing programme of surveillance in the WHO Western Pacific Region (*106*). To facilitate comparative assessments of resistance patterns it is important to:

- completely describe the methodology used and adhere to it precisely;
- define the test parameters, i.e. what constitutes resistance;
- include as controls reference cultures of known susceptibility;
- and compare only qualitative results and not numerical MIC values if test methods differ.

Disc diffusion methods

Disc diffusion susceptibility tests are widely used (*205*). There are different opinions about their utility and accuracy in assessing gonococcal susceptibility. The method was initially introduced and standardized for rapidly growing organisms, and the rate of bacterial growth and time of incubation greatly affect inhibition zone diameters. Since the introduction of disc susceptibility testing of *N. gonorrhoeae*, the quality of commercial growth media and the reliability of commercially available antibiotic discs have improved substantially. Just as for MIC determination, important variables include the growth medium, inoculum, and incubation conditions. Additional issues are the choice of disc potency and the interpretation of zone sizes. Because the MIC is the reference method, a number of studies have been performed to correlate inhibitory zones with MICs in order to develop interpretive criteria. Although it is unlikely that there will ever be a single internationally accepted disc susceptibility test method, comparable data can still be generated in different laboratories, as long as standardized methods are rigorously followed and QC and QA procedures are in place. As with all susceptibility test methods, the most subjective, and therefore least controllable part of the disc susceptibility test is the determination of the endpoint, in this case the edge of the inhibition zone.

Disc testing is practical because of its low cost

and technical simplicity. A large number of antibiotics may be tested simultaneously at a cost of about US$ .08 per disc. Inaccuracies occur with all test methods, but because of the epidemiological approach for gonococcal susceptibility testing there is a built-in tolerance of occasional errors which would not be permissible in the management of individual patients. Several long-running programmes have demonstrated the utility of disc susceptibility testing in providing reliable data on which to base treatment regimens and for monitoring resistance trends.

E-test

This is a quantitative susceptibility test that uses a strip impregnated with a predefined antibiotic gradient. Reference cultures and controls are needed, as with any susceptibility test. The strip is placed on the surface of an inoculated plate and the endpoint (MIC) is determined by reading the point where the inhibition zone intersects the strip. When performed under reference laboratory conditions, the E-test has compared favourably with the conventional agar dilution MIC. However, results of a study in Malawi, in which the E-test was performed under field conditions and compared with conventional MIC and clinical evaluation, suggested that improvements were needed before reliable data could be generated under these conditions (*29*). MICs obtained with this method in reference laboratories tend to be slightly lower than those obtained by conventional agar dilution methods. Endpoint interpretation poses the same problem as for the disc diffusion test, i.e. determining the precise edge of the inhibition zone.

The ease of use of the E-test makes it attractive as a potential standard method. However, the test strips are very costly (at least US$ 1.50–2.00), particularly when testing susceptibility to multiple antibiotics for epidemiological purposes.

Special test requirements for some antibiotics

Co-trimoxazole, a combination of sulfamethoxazole and trimethoprim, has been used to treat gonorrhoea. Although not generally recommended for this indication, and necessitating multiple-dose treatment, it is still used extensively in some regions because of its availability and low price. Testing susceptibility to this drug requires that the growth medium be free of substances that interfere with its activity. Sulfonamides and trimethoprim both inhibit the folate pathway in bacteria. Folates are methyl carriers that participate in various metabolic pathways, notably the synthesis of thymidine. Under certain growth conditions (in particular, lack of an exogenous source of thymidine for DNA replication) bacteria undergo 'thymineless death' in the presence of folate antagonists. Thymidine is, however, present in many bacteriological media, including sensitivity test media, allowing bacteria that possess a salvage pathway to bypass the metabolic block via 'end product rescue'. Low thymidine test media, such as DST and IsoSensitest agars are used and lysed horse blood is added to further reduce the amount of thymidine present. Horse blood contains thymidine phosphorylase, an enzyme that converts thymidine to thymine, which is 100-fold less active than thymidine in antagonizing the activity of sulfonamides and trimethoprim. The horse blood must be lysed to release the enzyme into the medium.

Azithromycin

Susceptibility testing is more pH-dependent than for some other agents. Since CO_2 (needed for the growth of *N. gonorrhoeae*) can alter the pH of the medium, robust controls must be used when assessing the activity of azithromycin.

Probe and hybridization techniques for susceptibility determination

Chromosomally mediated resistance in *N. gonorrhoeae* is the result of multiple genetic changes, for which there is no simple probe. For example, high-level resistance to quinolones is determined by mutations in the *gyrA* and *parC* genes, and there may be multiple changes in GyrA alone. Additionally, mutations in *gyrB* may contribute to resistance (*234*). While probes are available that identify known mutations in each of these three genes (*234*, *235*, *236*), even using all of these probes simultaneously will not detect additional resistance determinants that may be present (such as *mtr*). Furthermore, new mutations in *gyrA* and *parC* that affect the level of resistance are being continually discovered. Other mutations may partially reverse resistance; for example, the *env* mutation suppresses expression of the *mtr* phenotype (*197*) (See section B2.).

A probe is available for high-level tetracycline resistance (TRNG) mediated by the *tetM* determinant, which is carried on a plasmid (*237*). However, TRNG is readily detected by simple disc susceptibility methods (*205*).

Thus, despite optimism expressed in some quarters (*41*), it seems likely that, in the immediate future, we will continue to rely on conventional methods to detect resistance in *N. gonorrhoeae*.

Detection of β-lactamase (identification of PPNG)

Penicillinase producing *N. gonorrhoeae* (PPNG) represent a dramatic example of the rapid emergence and spread of resistant strains. PPNG express an inducible TEM-type β-lactamase, which is encoded on plasmids. These enzymes hydrolyse the β-lactam ring of susceptible antibiotics.

Beta-lactamases can be detected by a number of methods, including rapid iodometric and acidometric tests. For convenience, the latter test can be included in a panel of rapid carbohydrate utilization tests for the definitive identification of *N. gonorrhoeae*. One simple test uses a chromogenic cephalosporin; this compound, which is initially yellow, becomes red when the β-lactam ring is disrupted. These tests are easier to perform than MIC determinations or disc susceptibility tests and can provide valuable epidemiological data on the spread of PPNG. There are a number of test formats, including a commercially available chromogenic test. Although these test strips are easy to use and reliable, they are expensive. Since, in most parts of the world, resistance to the penicillins (mediated by β-lactamases and/or chromosomal mechanisms) is now widespread, the utility of and justification for conducting these tests should be reappraised. Further, CMRNG are not detected by this means and are an important cause of treatment failure. Chromosomally mediated resistance can only be reliably detected in PPNG after strains are cured of plasmids.

C3. Gonococcal susceptibility surveillance systems

Existing models of gonococcal susceptibility surveillance

Country-based programmes

There are a number of existing models of susceptibility surveillance networks at regional and national levels. The latter include the Australian Gonococcal Surveillance Programme, the GISP in the USA, and others in China and Malaysia. Some are true networks, with a number of collaborating centres that use standardized methods for continuous surveillance; they have QA/QC programmes and established procedures for data collection and reporting. Other organizations have a central laboratory, which receives and tests strains isolated elsewhere, and still others are hybrids. All of these approaches have had some success, but participatory networks usually survive longer because all of those involved are partners in the programme. In countries lacking resources to support surveillance, externally funded studies have provided important point prevalence data. Additionally, there are instances in which data from many sources have been collected by broadly based surveillance programmes (*109*).

WHO efforts

For some time, WHO has sought to establish a global gonococcal surveillance network. This programme, which has the acronym GASP, was originally envisaged as having a central coordinator and a series of regional networks, each region with its own reference or coordinating laboratory. In addition to the regional reference centre, each country in the region would have one laboratory (or 'focal point'), responsible for surveillance, selected by the participating countries.

Progress and status of the WHO GASP

Unfortunately, insufficient funds were available to establish a central coordinating laboratory. GASP therefore proceeded on the basis of regional offices and a number of regional programmes have developed. The first GASP network was in the WPR, followed by networks in SEAR and PAHO.

WPR and SEAR are using the participatory network model, with member laboratories testing isolates by standard methods and participating in a QA/QC system to ensure validity and reproducibility of results. The regional reference laboratory is responsible for logistics, acts as a reference and advisory centre, supplies reagents, provides training, collates data and produces reports. There are differences between the WPR and SEAR GASP networks, reflecting different capabilities, needs and resources. However, implementation included a number of common elements:

- development of an operational plan
- appointment of a regional reference laboratory coordinator
- consensus building, including method selection
- infrastructure building
- programme start-up
- programme extension
- programme maintenance

Detailed information on each of these phases is available but is not included here.

An extensive network has also been developed in PAHO.

There is a need for a global database for AMR in *N. gonorrhoeae*, for the reasons outlined in section C1. This should include a structure for data analysis and reporting.

C4. Detection of resistance by clinic-based methods

There have been many clinical trials aimed at establishing the efficacy of new antibiotics against gonorrhoea. These include dose-ranging studies, open and blinded trials, randomized and non-randomized comparative studies and single-regimen studies. Well-designed trials can provide valuable information about resistance to existing and newer antimicrobial agents and on the correlation between susceptibility and clinical outcome.

There are several issues of particular importance in assessing efficacy against gonorrhoea.

Diagnosis. As discussed in section A, gonorrhoea can not be reliably diagnosed from clinical symptoms alone. Laboratory procedures (such as culture or NAA) are needed to establish an etiological diagnosis. For practical reasons, clinical trials are likely to be conducted in areas that have suitable laboratory facilities, and the results may not be generally applicable to resource-poor areas where the incidence of gonorrhoea is highest.

Compliance. In order to ensure compliance, single-dose therapy, under direct supervision, is recommended for gonorrhoea.

Endpoint of the study—follow-up and assessment of cure. The number of patients returning for follow-up, the timing of follow-up and the criteria for cure are all important factors. The proportion of assessable patients is often low; in developing countries, many patients are unable rather than unwilling to return for follow-up. The timing of follow-up is critical. There may be an initial response, with partial or even complete resolution of symptoms, followed by reappearance of both the symptoms and the original organism.

In assessing outcome, it may be difficult to distinguish between reinfection and treatment failure, unless the patient has refrained from sexual activity after treatment. Patient history is not always reliable in this regard. Since in many cases strain typing can resolve this issue, isolates must be stored for detailed examination. If the isolates are demonstrably different before and after treatment then reinfection is likely to have occurred; if they are indistinguishable, treatment failure is more probable although reinfection from the same source is possible. Both in clinical trials of new agents and in monitoring the efficacy of standard treatment regimens, it is important to retain isolates for susceptibility testing. Establishing correlations between clinical outcome and MIC is critical for continuing surveillance of susceptibility patterns. Clinical trials are an important source of isolates for this process, in particular isolates from patients who fail treatment.

Treatment with multiple antibiotics. In standard medical practice, a second antibiotic active against *C. trachomatis* is often administered when treating gonorrhoea. A clinical trial protocol may stipulate withholding such treatment, but in practice this may not occur. Administration of a second antibiotic may necessitate changing the timing of follow-up and may complicate both the assessment of outcome and conclusions about the efficacy of the test drug.

Site of infection. Although most currently used antibiotics are efficacious at various sites of infection, there have been some problems in treating pharyngeal gonorrhoea and, with some earlier antibiotics, rectal and endocervical gonorrhoea. Most gonococcal strains will be susceptible to new antibiotics being tested in clinical trials. However, with increasing use, reduced susceptibility to new agents may result in reduced efficacy at certain sites of infection. Only as resistance mechanisms appear and MICs rise do these differences become manifest.

Side-effects. For some antibiotics the necessary effective dose produces an unacceptable rate of side-effects, while lower doses have failure rates that are too high. Thus, data on patient acceptability should be collected during efficacy trials.

Section C. Research and implementation needs

There is a long-acknowledged need to develop programmes for susceptibility surveillance at local, national, regional and international levels.

The WHO programme (GASP) established in 1990 has generated considerable data, from regions with a large burden of gonococcal disease, which have proved useful to local and regional health authorities. Another goal of the programme is to improve the ability of local centres to perform

susceptibility tests in order to enable continuous monitoring on a national basis.

Some networks are already functioning to collect data, either on an intermittent or continuing basis. However, in order to provide a coherent picture of AMR and its spread a structure is needed to collate and analyse the data that are being generated. Implementation of global surveillance needs to be accelerated. GASP has produced successful models which can be used as a blueprint for expansion. Funding mechanisms are required to build and support these activities.

The specific needs of susceptibility surveillance networks for AMR in *N. gonorrhoeae* are:

— A coordinated approach to testing. Test methods should be robust; an inexpensive and reliable test medium is needed for use in developing countries. It is hoped that molecular-based systems for determining antibiotic susceptibility in *N. gonorrhoeae*, combined with etiological diagnosis (using for example NAA), will eventually simplify the task. While these techniques are being developed and validated, conventional methods must be improved and applied in the short and medium term.

— A data bank for global and regional resistance patterns and a structure for analysing, interpreting and distributing data. Sufficient data are already being generated to justify immediate implementation. Issues of data ownership have been successfully resolved in smaller-scale projects.

— Access to isolates that are representative of prevalent gonococci. This is problematic where syndromic management is used and may become more difficult with the introduction of etiological diagnosis based on non-culture methods such as NAA. Susceptibility surveillance in areas with the greatest incidence needs to be addressed urgently. 'Targeted' culture programmes are needed to ensure the availability of isolates from these areas. Additionally, there is a need to define optimal sampling procedures, i.e. the number and source of isolates needed to ensure that treatment regimens are based on reliable data.

— A programme of consistent funding to ensure infrastructure development and maintenance.

SECTION D

Treatment of gonorrhoea

Summary of Section D

Treatment of gonorrhoea is epidemiologically rather than individually based, whether or not an etiological diagnosis is made. Ideally, there are standard treatment protocols, appropriate medication is available, and patients are treated upon presentation. Single- dose regimens increase compliance and oral treatment is preferred. Empirical adjunctive anti-chlamydial therapy is the norm in syndromic management.

The efficacy of the standard treatment regimen, which can be reliably predicted from *in vitro* susceptibility data, should be >95%. Because susceptibility varies widely among different geographical areas and among subpopulations within an area, different regimens must be available, for example to treat travellers who were infected in regions known to have particular resistance problems or for particular subgroups (e.g. core groups).

The first-line drugs currently recommended for treatment of uncomplicated gonorrhoea are third-generation cephalosporins (cefixime, oral or ceftriaxone, injectable) and oral quinolones (ciprofloxacin or ofloxacin). Cefixime and ceftriaxone have better activity against gonococci than other cephalosporins. Emerging resistance to quinolone antibiotics means that, in a given region, their value as first-line therapy must first be established and then monitored continuously.

Spectinomycin is an injectable agent that has retained its efficacy. Penicillins, usually oral amoxycillin or ampicillin, are still used in some parts of the world, but are ineffective in those regions where the rates of gonorrhoea are highest. Their use must be consistent with susceptibility profiles of local isolates. Susceptibility data are generally insufficient to support the use of co-trimoxazole, chloramphenicol/thiamphenicol and aminoglycosides. Tetracyclines and azithromycin are not recommended for treatment of gonorrhoea.

Standard treatment regimens have been shown to rapidly eliminate gonococci from males with urethritis and effective treatment of gonorrhoea decreases the incidence of complications such as PID and ectopic pregnancy in women, ophthalmia neonatorum in neonates and HIV transmission. However, antibiotic treatment of the infected individual is only one component of the integrated approach required for the control of gonorrhoea. Where comprehensive disease management programmes are in place, principally in developed countries, significant declines in disease rates have been observed.

Follow-up examination to verify cure is not recommended in the USA when standard treatment protocols are used. It is often difficult to distinguish treatment failure from reinfection, but isolates from apparent treatment failures should be examined for susceptibility and, if possible, compared with the original strain.

In some locations, the choice of therapy is often dictated by the cost and availability of antibiotics rather than by a therapeutic rationale. There has been some progress towards the economical purchase and reliable distribution of essential drugs. Unrestricted access to antibiotics (e.g. in the informal health sector) leads to increasing resistance. Additionally, drugs so obtained may be inappropriate, ineffectual or taken in suboptimal doses. Antibiotics used to treat other diseases can also lead to the selection of resistance in gonococci.

Accessible, affordable and effective drugs are a prerequisite for controlling disease and preventing emergence of resistance. Use of ineffective drugs is costly, can have undesirable side-effects, fails to stop disease transmission, and reduces patient confidence in treatment.

D1. Standard treatment guidelines and strategies

The preferred strategy for gonorrhoea is to treat the patient, on first presentation, with a standard single-dose regimen. This applies both to syndromic management and to situations in which etiological diagnosis is available. Compliance is generally poor

when multiple-dose regimens are used to treat STDs. Oral agents are usually preferred, for reasons including patient acceptability and ease of administration. However, in some cultures patients perceive oral agents to be less efficacious than injectable agents. Disposable syringes are sometimes reused, with the potential for transmission of other diseases.

Because treatment is administered on first presentation, the susceptibility of the infecting organism, and in many cases its identity, are not available to guide individual treatment. Standardized treatment protocols, based on resistance patterns of prevalent gonococci, must be used to manage gonorrhoea. The variations in susceptibility patterns that occur among countries or larger geographical regions are discussed above (section B). Susceptibility patterns may also vary greatly between urban and rural areas or, within a single locality, among subpopulations of risk groups. Guidelines are usually available for the treatment of uncomplicated genital gonorrhoea. Pharyngeal and rectal gonorrhoea require different approaches, as do complicated infections (PID, DGI) and gonorrhoea in neonates and pregnant women, who can not be safely treated with all agents.

There are a number of antibiotics of proven efficacy in single-dose treatment; preferences vary with the local situation or the locale where the infection was acquired. Additionally, some multiple dose regimens are still in use (*238*). Efficacious antibiotics available for treatment are third-generation cephalosporins, quinolones and spectinomycin. Penicillins and earlier generation cephalosporins are used in specific situations. Other agents include aminoglycosides, chloramphenicol and thiamphenicol, and co-trimoxazole.

The most comprehensive treatment guidelines are those prepared by the Centers for Disease Control and Prevention in the USA. These have often been adapted for use in other countries, e.g. the guidelines produced by the Venereology Society of Victoria (*239*).

First-line drugs

The third-generation cephalosporins recommended are cefixime (oral, 400 mg single dose) or ceftriaxone (IM, 125 mg single dose). The quinolone agents recommended are ciprofloxacin (oral, 500 mg single dose) or ofloxacin (oral, 400 mg single dose). Spectinomycin (IM, 2 g single dose) is an older antibiotic used almost exclusively for the treatment of gonorrhoea.

Second- and third-line agents.

Some of these are used as first-line agents in some localities, because of proven efficacy or due to cost pressures, but often they continue to be used without clear evidence of efficacy. There are differing opinions as to whether these agents should be included in officially recommended drug lists, such as that compiled by WHO.

Penicillins are most often administered as single-dose amoxycillin (oral, 3 g) or ampicillin (oral, 3 g). Ampicillin is often given together with probenecid (oral, 1 g), which delays renal excretion of penicillins. Amoxycillin-clavulanate combinations are not recommended. There is a general consensus that penicillins should only be used in regions where their efficacy can be demonstrated on a continuing basis.

Earlier-generation cephalosporins are usually not recommended because of cross resistance to them in penicillin-resistant CMRNG (see section B), but their potential use in some situations has been explored (*155*).

Co-trimoxazole is a combination of sulfamethoxazole and trimethoprim (400 mg/80 mg, oral, 3 days). Thiamphenicol is administered as 2.5 g, oral, 2 days. Kanamycin is administered IM (2 g, single dose) (*238*). Gentamicin is administered IM at 240 mg, single dose. A 280 mg dose of gentamicin was found to be superior to the 240 mg regimen in one dose-ranging study (*240*). Data on resistance to these agents should be obtained if their use is contemplated. Reservations have been expressed about the use of multiple-dose regimens. These agents are available as low-cost generic products, and they continue to be used to treat gonorrhoea where the cost of therapy is a significant consideration. In particular, the rationale for using chloramphenicol and thiamphenicol has been questioned. Moran (personal communication of an internal document) recently concluded that 'single-dose thiamphenicol is not reliably effective against gonococcal infections' and that 'multidose thiamphenicol regimens have not been proven to be reliably effective against gonorrhoea'. An analysis of published reports and of laboratory and pharmacokinetic data found that, except in a single study, there was no correlation between MIC and clinical outcome.

Tetracyclines are not recommended for treating gonorrhoea. They require multiple-dose therapy and are contraindicated in pregnancy and for neonates. Additionally, the widespread dissemination of the *tetM* gene, which confers high-level

resistance, has severely compromised their efficacy. However, tetracyclines are readily available in informal health sectors in many countries, so they continue to be used.

Newer macrolides such as azithromycin (oral, 1 g single dose) are not recommended for gonorrhoea, but are used in some localities despite their high cost.

Empirical anti-chlamydial therapy is often prescribed when gonorrhoea is diagnosed or in syndromic management (e.g. of urethral discharge). Treatment is usually with multidose tetracycline or erythromycin; recently, single-dose treatment with newer macrolides such as azithromycin has been used, where affordable. Although there is no single treatment that effectively covers both gonorrhoea and chlamydial infection, the anti-chlamydial agents do have some anti-gonococcal activity which may affect treatment outcome. Simultaneous treatment to cover *C. trachomatis* can mask persistent gonococcal infection.

D2. Definition of cure

Follow-up examination to verify cure is not recommended in the USA when standard treatment protocols are used but, although its value is debatable, follow-up is often performed in other countries. In assessing outcome, it may be difficult to distinguish between reinfection and treatment failure, unless the patient has refrained from sexual activity after treatment. Gonococci isolated after treatment failure should be tested for antibiotic susceptibility. (See section C4 for further discussion.)

D3. Effectiveness of treatment and ethical issues

This review emphasizes the need for a comprehensive approach to the management of gonorrhoea (and other curable STDs). Antibiotic treatment is an essential element, but is most effective as part of a programme of early detection, prevention and behaviour modification, with the goal of reducing the incidence of infection.

In the United Kingdom, national guidelines for the management of gonorrhoea were published in 1996 (*232*). They resulted from a formal consensus process, which included analysis of published data and the convening of workshops. The areas addressed included diagnosis, antibiotic treatment and epidemiological patient management. Standards were set for evaluating the success of disease management; parameters include rates of disease and number of contacts traced. Auditing was suggested (*241*). This approach assumes a level of health care and resources not available in many countries.

The aims of disease management are both the treatment of infected individuals and reduction of the disease incidence in the population. The recent decline in gonorrhoea in developed countries has been ascribed to a combination of decreased transmission (due to lifestyle changes and early diagnosis and treatment) and efficient contact tracing, which reduces the number of undiagnosed cases (*3*). Early detection and treatment break the chain of transmission.

Mass treatment is a possible strategy when clusters or core groups within a community are known to have unacceptably high rates of disease (*50*). However, for success in reducing the rate of gonorrhoea, this must be part of a more complete management programme (*242*). In some cases, failure to continue mass treatment programmes for a sufficient period of time has limited their success.

Treatment of the individual is efficacious.

Early studies with penicillins and tetracyclines demonstrated rapid elimination of gonococci from treated patients (*55*). Haizlip et al (*56*) demonstrated that ceftriaxone, cefixime or ciprofloxacin eliminated susceptible gonococci from the urine, mucosa and semen of men with urethritis within 24 h of single-dose treatment.

Successful treatment of gonorrhoea prevents complications and reduces HIV transmission.

It has been estimated that for every 100 women successfully treated for gonorrhoea (25 of whom will have been pregnant), 25 cases of pelvic inflammatory disease, 7 cases of ophthalmia neonatorum, one ectopic pregnancy and 6 instances of infertility would be averted (*42*). Successful treatment requires appropriate therapy. The use of ineffective drugs is costly, may lead to side-effects, fails to stop disease transmission, and destroys patient confidence in treatment. Over and Piot (cited in (*42*)), estimated that, when considering gonorrhoea in members of a core group, every 100 successful treatments would prevent approximately 425 HIV infections over the following ten-year period.

These projections, which were epidemiologically based, have since been validated by treatment in-

tervention studies. The Mwanza trial in the United Republic of Tanzania, which used pair-matched communities, is an often cited study of the effect of consistently improved STD treatment on the incidence of HIV infection (*242*). HIV incidence was decreased by 38% in the intervention communities. Although education in prevention was part of the programme, behaviour modification (such as increased condom usage) was not a factor. Subsequent cost-benefit analysis of this programme showed that outcomes compared favourably with such public health interventions as childhood vaccination (*98*). Another study, in Uganda, which began at a later stage in the HIV epidemic, employed intermittent rather than continuous treatment intervention and was not restricted to symptomatic patients. The less favourable outcome of this study has been attributed to these differences.

In addition to epidemiological data, there is direct biological evidence of the effect of treating gonorrhoea on the transmission of HIV. In studies conducted in Malawi in HIV-positive men, those with urethritis (which was mainly gonococcal) had eight-fold higher levels of HIV-1 RNA in their semen. Within two weeks after effective treatment of the gonorrhoea, the RNA concentration had declined to the same level as in the men who did not have urethritis (*77*). As with all infectious diseases, transmission of HIV is inoculum dependent. The conclusion drawn from this study is that gonorrhoea leads to increased levels of HIV in the semen of infected men, which is reversed upon resolution of the gonorrhoea. Similar reductions in HIV levels in genital secretions have been found in women successfully treated for bacterial STDs, but not when the treatment was unsuccessful. STDs are also thought to increase the number of cells with receptors for HIV by recruiting them into inflammatory exudates. A recent review (*244*) concluded: 'available data leave little doubt that other STDs facilitate HIV transmission…and that early STD treatment should be part of a high-quality, comprehensive HIV prevention strategy.'

Control of gonorrhoea also prevents gonococcal PID. In Sweden, various initiatives, as well as antibiotic treatment, were employed in an effort to decrease the rate of gonorrhoea. A longitudinal study in that country, comparing the incidence of PID over two five-year periods, demonstrated a significant decrease in gonococcal PID concomitant with a decrease in the incidence of gonorrhoea (*43*). A longitudinal study in Argentina also found that decreasing rates of PID correlated with decreasing trends for gonorrhoea (*31*).

D4. Drug availability

The emergence of AMR in *N. gonorrhoeae* is related both to ready availability of inappropriate antibiotics and lack of access to effective antibiotics.

Uncontrolled availability of antibiotics

This topic has recently been reviewed (*245*). In many developing countries there is unrestricted access to antibiotics. Because of their cost, lower than appropriate dosages may be used, and the course of treatment may not be completed. Individuals may first consult traditional healers (*225*) or self-medicate with antibiotics obtained in the unofficial health sector. In one study at an STD clinic, 70% of patients attending had already received prior medication in the informal health (*225*). Prophylactic self-administration of antibiotics is common among CSWs. One study in the Philippines documented widespread use of antibiotics in CSWs; the agents were inappropriate for the gonococci circulating in that region and were used at subtherapeutic dosages (*246*). Inappropriate antibiotics are often available on the open market, or even from physicians. The potency of these drugs is variable (*247*). They may be counterfeit or of poor quality or may have deteriorated or been adulterated (*248*). The ability to monitor drug quality is extremely limited in developing countries (*245*). In some countries, even where this practice is illegal, antibiotics are readily available from pharmacies. Pharmacists do not have adequate expertise to prescribe antibiotics for STDs, in particular for gonorrhoea (*249, 250*), and individuals seeking to purchase antibiotics often do not disclose their symptoms. It has been suggested that training pharmacists and traditional healers in syndromic management of STD might decrease the amount of inappropriate therapy (Crabbe et al, 1993 cited by Laga (*224*)), and in some countries efforts have been made to improve the competency of pharmacists.

As discussed in section B, the appearance of low-level quinolone resistance in Rwanda may have been related to the widespread use of these drugs to treat diarrhoea. In the Phillipines, uncontrolled availability of rosoxacin, which had been used as a multidose treatment for gonorrhoea in that country, seems to have been a factor in the emergence of quinolone resistance (*251*).

Inadequate supply of effective drugs

While the 'establishment of national treatment guidelines, the improvement of national capacities to monitor antimicrobial susceptibility, and the adequate supply of STD drugs are…key strategies to slow down the spread of resistant strains' (*252*), appropriate drugs are often not available where needed in developing countries. In some of these countries the antibiotics required to treat gonorrhoea account for a substantial proportion of the total drug budget; for example, in Botswana in 1994 20% of total drug expenditure was for ceftriaxone. The cost of a 500 mg dose of ciprofloxacin may be equivalent to the annual per capita health budget. By some estimates, the cost of effectively treating gonorrhoea in Africa is equivalent to half the entire health budget for the continent.

Alternative antibiotics that are available as generics are often used because they are less expensive. However, their effective use requires constant monitoring of susceptibility. Aminoglycoside antibiotics are manufactured as generics in Zimbabwe, and in 1993 gentamicin became the treatment of choice for gonorrhoea in Malawi. Two years later there was a statistically significant trend towards decreased susceptibility and clinical efficacy was only 92%. This was considered to be acceptable because of the limited treatment options available in that country (*29*). In Northern Tanzania, gonococcal resistance to co-trimoxazole, a drug combination used there for first-line treatment, ranged between 9% and 22% in different localities (*132*).

Factors affecting the cost of supplying drugs include source (generic vs. branded), procurement methods, distribution, taxes, and mark-ups in the private sector (*252*). The most economical way to provide effective treatment of STD is through donation programmes, coupled with appropriate management protocols and efficient procurement procedures. However, transportation and other factors increase the cost of supplying remote areas. Analysis of procurement and distribution systems led to the conclusion that drug purchases for STD treatment needed to be integrated into general essential drug programmes. It is important to note that 'offering accessible and affordable care, including effective drugs, can cause a shift of health seeking behaviour to official medical services' (*224*).

Section D. Research and implementation needs

1. Better data on treatment outcome

In many cases, treatment regimens are dictated by cost rather than by proven efficacy. It is difficult to bring about changes in regimens in the absence of supporting data. While clinical efficacy trials would produce the most convincing data, they are complex and require high-quality laboratory support. A more practical approach is to monitor antibiotic susceptibility as described in section C. Other useful data include the effects of appropriate treatment regimens on the overall incidence of gonorrhoea and on long-term complications such as PID.

2. Drug availability issues

Methods for controlling access to and use of antibiotics.

This has been the subject of an international workshop at WHO. In some countries, educational programmes for pharmacists have had a measure of success. While controversial, it has been suggested that pharmacists and traditional healers be trained in syndromic management and permitted to market STD treatment packages (*224*).

Availability of effective drugs

To reduce AMR and control gonorrhoea, national guidelines based on susceptibility, an adequate supply of effective drugs, and an efficient means of distributing them are required. This means that 'the public sector, the private sector, the pharmaceutical industry, and donor agencies need to understand the common interest and seek a consensus for long-term collaboration.' (*252*).

SECTION E

Prevention of gonorrhoea

Summary of Section E

There are many approaches to prevention of gonorrhoea. Interventions that have been proven to affect disease rates include effective treatment with appropriate antibiotics (*240*) and efficient case-finding through improved diagnosis (*60*). The effect of behaviour change can be inferred from changing incidence rates in Western countries, in particular among HAM, since the appearance of HIV/AIDS. The greatest gains are achieved when all elements are included in an integrated strategy.

However, the complexity of the interrelationship of these disparate factors means that a lower rate of disease attained by one means may be offset by decline in compliance in another effector arm. For example, improved case-finding through better diagnostic tests and instigation of proper treatment regimens may provide gains which are offset by deleterious behavioural change, and vice versa. Examples include the recently observed increase in rates of gonorrhoea in homosexually active men seemingly related to adverse behavioural change (*15*).

Mass treatment of core groups has been considered as a means of controlling disease, but this strategy may contribute to increased resistance. A negative impact on STD control was observed when user fees were introduced in public sector STD treatment facilities in Kenya (*253*).

N. gonorrhoeae has the ability to evade host defences. Its antigenic heterogeneity and apparent lack of strong immunogenicity pose difficulties for the development of an effective vaccine, although efforts in this direction continue, supported by studies of the biology of the organism.

E1. General remarks

As previously stated, a comprehensive approach is required for the effective management of gonorrhoea (and of other curable STDs). The equation given in section A is useful in exploring how various factors interact in the dynamics of disease: $R_o = \beta cD$, where R_o is the reproductive rate of the disease, β = 'transmissibility' of the organism, c = the rate of partner exchange and D = the duration of infectiousness. When $R_o = 1$ the disease is endemic and stable. Values greater or less than 1 indicate an increase or decrease in disease, respectively.

β and D are affected by availability of appropriate treatment. Both β and c may be affected by behavioural interventions such as the use of physical barriers to transmission, 'safe sex' programmes or lifestyle changes. D may be reduced by improving diagnostic facilities so as to identify asymptomatic patients (case-finding). These approaches for control of STDs have been thoughtfully considered by expert committees (*254*).

E2. Mass treatment for gonorrhoea

The duration of infectiousness, D, and also β, the transmissibility, are reduced by antibiotic treatment, both prophylactic (*255*) and curative. Since gonococci are no longer cultivable, patients are presumably no longer infectious a short time after treatment with appropriate antibiotics (*55, 56*). As discussed in section D3, this has led to consideration of mass antibiotic treatment of core groups or of particular patient clusters known to have unacceptably high rates of infection. In the case of gonorrhoea, this must be part of a more complete management programme in order to succeed in reducing the rate of disease (*55*). In some cases, failure to continue mass treatment programmes for a sufficient period of time has limited their success. A pitfall of mass treatment is the potential for antibiotic overuse and the consequent emergence of resistance. This must be balanced against the potential benefits, which include prevention of the complications and sequelae of gonorrhoea and reduction in HIV transmission.

E3. Improved diagnosis

The ability to find, diagnose and treat asymptomatic infected individuals affects D, the duration of infectiousness. The recent decline in gonorrhoea

in developed countries has been ascribed to decreased transmissibility, resulting from lifestyle changes, and from early diagnosis and treatment. (*3*). The Swedish experience of the 1970s clearly demonstrated the benefits of efficient contact-tracing (4).

The new technologies based on NAA facilitate the identification of asymptomatic patients. There is not yet a cost-benefit analysis of the use of these methods in case-finding (*41*). However, reductions in disease rates have been demonstrated when improved diagnostic facilities were introduced, even in the absence of any changes in therapeutic regimen or in behaviour (*60*).

E4. Evasion of host defences by *N. gonorrhoeae* and prospects for a vaccine

E4.1 How gonococci evade host defences

The survival of *Neisseria gonorrhoeae in vivo* involves attachment and adherence of bacteria to the mucosal surface, invasion of epithelial cells, avoidance of non-specific and specific host defences, and multiplication within the host. Cohen & Sparling (*256*) have reviewed these processes, which occur principally at the mucosal surface.

There appears to be no effective natural or acquired immunity to gonococcal infection (*4*). Additionally, the organism adapts to changing environments through phenotypic and genotypic alteration. These are rapid events within the bacterium, which induce a host response over a longer time frame. This 'pathogen-host coevolution…a Darwinian process that involves both the generation of genetic diversity in the pathogen and the operation of immune selection at the molecular level by the host' (*67*) results in the survival of a highly adapted but still very adaptable pathogen.

N. gonorrhoeae colonizes and infects the columnar epithelial surfaces of the genital tract and also the mucosal surfaces of the anorectum, pharynx and eye. It is a Gram-negative organism closely related to and probably evolved from *Neisseria meningitidis.* However, unlike meningococci, gonococci do not possess a virulence-associated polysaccharide capsule. Because it is strictly a human pathogen, animal models are not available for the study of gonococcal pathogenesis. Human volunteer studies of gonococcal infection (*257*) and *in vitro* studies using cell and organ cultures have elucidated many aspects of the pathogenesis of *N. gonorrhoeae* at the molecular level (*258*). However, care must be taken when extrapolating from *in vitro* studies to the *in vivo* situation (*259*).

Gonococci attach and adhere to, then invade and enter epithelial cells, passing through the cell in vacuoles to the subepithelial matrix where they usually remain localized and elicit an often intense inflammatory reaction (*260, 261*). The separate processes involve different virulence factors expressed by the organism at different stages of the infection cycle. These include initial attachment of the organism to the epithelium by pili, followed by adhesion by means of opacity (Opa) outer membrane proteins (previously called outer membrane protein II) and lipooligosaccharide (LOS) all of which are surface structures of the gonococcus. Expression of pili, opacity proteins and LOS is highly variable and antigenically heterogeneous. Antigenic variation of the major subunit of the pilus occurs by recombination of genes at a silent locus with those at an expression locus (*262*).

Porin proteins are also involved early in the invasive process. The porin expressed in *N. gonorrhoeae* is porin B; the *porA* gene is suppressed in this organism (*263*). *porB* exists as two alleles PIA and PIB (WI and WII/III in the Swedish nomenclature), which are closely related to the porins of some other Neisseria species. The gonococcal porin has the ability to translocate into host cell membranes where it functions as a GTP/ATP regulated pore. It induces rapid calcium influx into target cells, resulting in accelerated apoptosis (programmed cell death) and enhanced invasion by the bacteria (*264*).

Galactose residues in LOS can be modified by sialylation, using sialic acid from the host. By this means, gonococci mimic epitopes present on host cells, thereby avoiding some host responses such as serum killing, complement activation and antibody formation. Gonococci may also evade serum killing by expressing a reduction modifiable protein (Rmp, formerly known as protein III). This protein is highly immunogenic and is similar in structure to proteins of other species of *Neisseria* and of enteric bacteria. It is likely that antibodies formed to cross-reacting homologues in normal hosts react with Rmp to neutralize the killing effects of otherwise bactericidal antibodies (*4*).

The role of factors other than cell surface structures, such as IgA protease, is also being clarified. Secretory IgA1 has a number of protective functions, including the inhibition of adhesion of organisms to epithelial cells. Gonococci produce IgA1 protease; it has been suggested that the abil-

ity to cleave IgA1 is a virulence factor. Recent studies, however, suggest that the protease plays no role in the pathogenesis of gonococci in the female lower genital tract (*265*). It has now been proposed that IgA1 protease plays an indirect role in the intracellular survival of gonococci, by altering lysosomal proteins (*266, 267*).

Hypervariable expression and heterogeneity of gonococcal pili, Opa and LOS are among the tools used by *N. gonorrhoeae* to evade host defences. Gonococci 'use phase and antigenic variation to change expression of many of their virulence determinants, …modulate their virulence and adapt to their changing environment'. This 'high-frequency, apparently random antigenic switching …controls the progression of subpopulations of the invading bacteria through the host and the ultimate survival of the bacteria' (*258*). In addition, external or environmental influences also regulate some survival factors. One example is iron utilization, in which different methods of iron acquisition are employed under different conditions (*268*).

It has been suggested that serovar-specific antibodies are produced to epitopes of the primary or outer membrane Por (porin) protein (formerly protein I) and it is postulated that the bacteria respond with changes in the outer membrane structure. This may result in circulation of particular subtypes of gonococci within a core group of infected individuals (*227, 269*). However, other studies in men and women with recurrent urethral infection demonstrated that reinfection with the same serovar occurs, suggesting that there was no serovar-specific immunity (*270*). Hedges et al (*271*) also found that gonococci failed to elicit a strong humoral immune response during uncomplicated genital infections; although levels of IgA1 specific to homologous gonococci increased in cervical mucous, the responses were 'weak at best'. Thus, while the local antibody response may be too weak to protect against repeat infection it may be sufficient to induce changes in porins over time. Lack of induction of a strong antibody response by gonococci has implications for the success of vaccine development.

Gonococcal infection usually triggers an intense inflammatory reaction with a purulent exudate from infected membranes, especially in the male urethra. (Asymptomatic infections, which evoke little if any inflammatory response, are less frequent.) (*272*). Numerous polymorph neutrophils are present in the exudate and there are Gram-negative diplococci within some of the cells, a feature which is of considerable diagnostic value. Observations on opsonization, phagocytosis and intracellular killing 'suggest that gonococci have evolved mechanisms by which they can evade the host's defence system and have developed ways to evade these defence systems once encountered' (*273*). At least some of the bacteria found within neutrophils appear to be viable (*274*), although this is still a matter for debate. Rest and Frangipane (*251*) point out that 'most studies of the interactions of gonococci with human neutrophils have been performed *in vitro*, with gonococci grown *in vitro*, by using isolated peripheral blood neutrophils.' In these studies, the survival time of intracellular gonococci was short (*272*). However, investigations using urethral exudates from men with gonorrhoea found that there was survival of 'a substantial proportion of *in vivo* grown gonococci …after exposure to exudate neutrophils' (cited by Rest & Frangipane (*259*)). When the LOS of bacteria grown *in vitro* was sialylated, so as to simulate the *in vivo* state, the non-opsonic interaction with neutrophils was greatly reduced. Thus, sialylation may help gonococci resist killing by human neutrophils.

Once opsonized and internalized in neutrophils, gonococci reside in phagolysosomes where they are subjected to oxygen-dependent and oxygen-independent killing mechanisms. The latter are thought to be more important (*272*). *In vitro*, the porin proteins of gonococci inhibit a number of functions of human neutrophils, including actin polymerization, degranulation and phagocytosis; but they prime the neutrophils to increase their oxidative burst (*275*). Damage to the bacteria within phagolysosomes includes degradation or modification of gonococcal components, preventing cell division (*272, 276*). Naids and Rest (*270*) reported that the stimulation of oxidative activity by gonococci occurs only within the phagolysosome and not externally at the plasma membrane. This may be why extracellular gonococci can persist for long periods in vivo, even in the presence of large numbers of neutrophils. It is difficult to determine, and thus reproduce, the microenvironment in which these events occur in order to deduce the mechanisms of killing or survival (*259*). However, Mosleh et al (*277*) have demonstrated that the porin proteins arrest phagosome maturation in macrophages.

The ability of the gonococcus to cause asymptomatic infections creates reservoirs for transmission and is critical to its long-term survival in the

human host. Infections in women frequently produce no or mild symptoms. The asymptomatic but infectious state may persist for long periods. This is a feature of the 'co-evolutionary' survival described by Brunham et al (*67*). In addition to being able to adapt to the physiology of the host, gonococci adapt to antibiotic selection pressures by developing resistance. In some instances, virulent subtypes have emerged that are actually more susceptible to antibiotics (*45*). That is to say that antibiotic resistance is subordinate to other factors affecting transmission and survival in the gonococcus.

E4.2 Vaccines for gonorrhoea

The long search for an effective gonococcal vaccine has not yet borne fruit. A number of different components of the gonococcal surface structure have been investigated as possible vaccine candidates. The antigenic heterogeneity described above and variable expression, sometimes dependent on the phase of the infective process, make this approach difficult. Efforts are continuing to produce a vaccine based on outer membrane protein 1 components (Por) free of contaminating Rmp (protein III).

There is some epidemiological evidence from studies of prostitutes in Nairobi that immunity, when it appears, may be strain–specific; this may contribute to the emergence of strain diversity (*278*). Recent data (*271*) suggest that, in addition to evading host defences, gonococci fail either to elicit a strong antibody response or to induce a memory response, using an 'as yet undefined mechanism of protection which may subvert the natural immune response'. For this reason, approaches such as mucosal immunization, alternative adjuvants, or different mechanisms of antigen presentation (such as DNA-based vaccines) have been suggested.

Should a vaccine be developed, cost considerations may limit widespread use in regions with the greatest incidence of gonorrhoea (*279*). If partial immunity actually does exist in individuals who have recurrent infections (i.e. core groups) (*270*), then 'immunization will need to be targeted to a population larger than core groups to be maximally effective' (*278*).

In theory, a vaccine would decrease transmissibility (β). Thus, as seen with antibiotic treatment, even a vaccine with less than 100% efficacy could be beneficial if sufficient inoculum reduction is attained or if the bacteria are attenuated so that the requisite infective inoculum for transmission is increased.

Section E. Research and implementation needs

Advances in clinical management ultimately stem from basic research into the biology of disease. The development of an effective vaccine has long been a strategy for the control of gonorrhoea. To this end, there is a continuing need for basic research on the biology of the gonococcus and its interaction with the host. If this effort is successful, the next task will be to solve the problems of appropriate distribution.

Research and implementation needs for reduction in antimicrobial resistance in the gonococcus and for control of gonococcal disease

1. General remarks

There can be no control of gonorrhoea without effective antibiotic treatment. The continuing emergence and spread of antibiotic-resistant *Neisseria gonorrhoeae* compromise effective treatment. Other factors that affect transmission (such as long-term changes in behaviour) are equally important. These factors also pertain to other STDs.

1.1 Factors common to the control of all sexually transmissible diseases

Programmes to control gonorrhoea must be integrated with plans for other STDs, including HIV. Comprehensive health policy decisions and interventions to control disease are required. The initiatives required to control STDs have been thoughtfully considered by expert committees, who have made recommendations (*254*).

1.2 Factors specific to the control of antimicrobial resistance in gonococci

Gonorrhoea differs from most other curable STDs because of the extent to which antibiotic resistance limits our ability to provide effective treatment and thus control the disease. Among other STD organisms, this situation is found to some extent only in *Haemophilus ducreyi*. In contrast, for other curable STDs, such as syphilis and chlamydial infection, treatment outcome is predictable and the therapeutic options have remained effective.

Currently, the primary approach to treating STDs is syndromic management. Providing appropriate and effective treatment requires up-to-date information about susceptibility patterns. Antimicrobial resistance in *N. gonorrhoeae* is already a major obstacle in disease management, whether it is syndromic or based on etiological diagnosis. The continued emergence of resistance in this organism threatens to further complicate management strategies.

2. Specific requirements

2.1 Better data on incidence and prevalence

Gonorrhoea is grossly underreported, particularly in areas where the incidence is high. Reliable incidence and prevalence data are essential to understanding the true scope of the problem, monitoring the success of intervention programmes and providing a basis for informed advocacy. Obtaining such data will require concerted efforts by international agencies, national health services, private companies, and the research community.

A practical goal in the short term is to conduct sentinel surveys, which target particular population subgroups.

Cost-benefit analyses are needed to justify both disease management initiatives and the introduction of new diagnostic methods.

2.2 Better diagnostic capabilities

There is a great need for low-cost, accurate, simple near-patient tests for diagnosis in symptomatic patients and for screening (case-finding). Newer nucleic acid-based amplification assays (NAA) have the potential to improve diagnosis, and thus patient treatment and disease management, and to facilitate epidemiological studies, but at present they are expensive and require sophisticated equipment. Particular advantages of these tests include non-invasive specimen collection, stability of specimens/less stringent transport requirements and sensitivity of the assays.

In the short term, these tests should be used selectively in developed countries for diagnosis and to help define patient risk markers. Subject to cost-benefit analysis, their use might be extended in the future. In developing countries, NAA tests can be used to estimate the prevalence of gonorrhoea in sample populations (e.g. women with vaginal discharge) and thus to improve syndromic management. These tests also have potential in screening programmes aimed at determining prevalence and treating asymptomatic and oligosymptomatic individuals.

There is a need for continued development of NAA tests (e.g. using cycling probe technology) that have fewer resource and personnel constraints and can be performed near-patient at low cost.

2.3 More complete AMR data

Because of the close correlation between therapeutic outcome and in *vitro* susceptibility to antibiotics, surveillance programmes are of great importance, both in developed and developing countries. By establishing the prevalence of resistance and providing an early warning of any change in susceptibility patterns, data from susceptibility surveillance programmes can be used to establish reliable treatment regimens and to modify them when necessary.

At present, there are significant gaps in gonococcal susceptibility data, especially in those regions with the greatest disease burden and the fewest resources. Continuous monitoring of susceptibility is needed here, in part as a means of curbing the use of inexpensive but ineffective antibiotics to treat gonorrhoea.

Data on gonococcal AMR are not just of local relevance, because resistance rarely remains localized for very long. It is clearly in their best interest for developed countries to be aware of AMR emergence and trends in other parts of the world, and thus to continue collaborating in and supporting international programmes.

The WHO programme (GASP) established in 1990 has generated considerable data from regions that have a large burden of gonococcal disease. An important goal of the programme is improving the ability of local centres to perform susceptibility tests, in order to enable continuous monitoring of AMR on a national basis. Some networks are already functioning to collect data, either on an intermittent or a continuing basis. Implementation of global surveillance of gonococcal susceptibility needs to be accelerated. In particular, a structure is needed to collate and analyse the data that are being generated. Funding mechanisms are required to support these activities.

2.4 Specific needs of AMR susceptibility surveillance networks

Coordinated approach to testing

Test methods must be robust and, in developing countries, must use inexpensive and reliable materials.

Data bank

Sufficient data are already being generated to justify immediate implementation of a data bank of global and regional resistance patterns and a structure for analysing, interpreting and distributing the data.

Sampling procedures

Access to isolates that are representative of circulating strains is needed for accurate survey of AMR. This is problematic where syndromic management is used and may become more difficult with the introduction of non-culture-based diagnostic methods such as NAA. Targeted culture programmes are needed to ensure the availability of isolates from geographical areas that have the greatest incidence of gonorrhoea. Additionally, there is a need to define optimal sampling procedures, i.e. the number and source of isolates required to ensure that treatment regimens are based on reliable data.

Funding

Consistent funding is needed to ensure infrastructure development and maintenance.

Extension of surveillance networks

It is particularly urgent to extend AMR surveillance to those geographical areas that have the greatest need.

Assay development

DNA-based assays for diagnosis and susceptibility determination hold promise for the future. Development of cost-effective tests should be encouraged.

3. Other measures

3.1 Better data on treatment outcomes

In many cases, treatment regimens are dictated by cost rather than by proven efficacy. While clinical efficacy trials produce the most convincing data, a more practical approach is to utilize antibiotic susceptibility data, as well as data on the success of appropriate treatment in reducing the incidence of gonorrhoea and of PID, to bring about changes in regimens.

3.2 Drug availability

Controlling access to and use of antibiotics has been the subject of a WHO international workshop. In some countries, educational programmes for pharmacists have had a measure of success. While controversial, it has been suggested that pharmacists and traditional healers be trained in syndromic management and permitted to market STD treatment packages.

Reducing AMR and controlling gonorrhoea require the existence of national treatment guidelines based on susceptibility, an adequate supply of effective drugs, and an efficient mechanism for distributing them. Informed consensus among the public, the private sector, the pharmaceutical industry and donor agencies is needed to ensure that established treatment policies are followed.

3.3 Biology of *N. gonorrhoeae*

Advances in clinical management ultimately stem from basic research into the biology of disease. Understanding the mechanisms of antibiotic resistance is crucial to the development of molecular susceptibility tests.

3.4 Vaccine development

The development of an effective vaccine has long been a strategy for the control of gonorrhoea. To this end, there is a continuing need for basic research on the biology of the gonococcus and its interaction with the host.

3.5 Spread of resistant gonococci by refugees, migrants and travellers

An integrated programme of education, diagnosis and treatment of refugee and migrant populations is required to control STDs in these groups. There is insufficient awareness of the risks of STD infection among clinicians working in travel medicine. Most programmes for travellers are directed at AIDS. A fresh approach is needed to promote sexual health in travellers, through education about safe behaviour.

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