Department of Epidemiology, Swedish Institute for Infectious Disease Control, Solna, Sweden, and
Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Recent trends in the epidemiology of gonorrhoea in Sweden

- the role of importation and core groups

Torsten Berglund

Stockholm 2006

Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 91-7140-692-1

To the patients and
staff at Venhälsan

ABSTRACT

In 1997 the gonorrhoea incidence in Sweden started to rise from a low-prevalent level after some 25 years of steady decline. The rise has continued and coincides with similar trends in other sexual transmitted infections (STIs) and in other Western industrialized countries.

Gonorrhoea is included in the Swedish Communicable Diseases Act and all diagnosed cases should be reported. Additional epidemiological data were collected during 12 months in 1998–99 about all reported cases (n=357), which then were linked to microbiological characterization of the isolated *Neisseria gonorrhoeae (Ng)* strains. Analyses showed that the increase of gonorrhoea is mainly due to an increase of domestic cases: 60% were acquired in Sweden, especially in Stockholm. Heterosexual teenagers and men who have sex with men (MSM) were identified as endemic core groups infected by separate phenotypic *Ng* serovars. In total, 28 different serovars were identified. Among imported cases, Thailand was the most prevalent country of exposure.

Further genotypic characterization by PFGE indicated that one *Ng* clone each of the serovars IB-2 and IB-3 created the majority of the two identified core groups of domestic cases in Sweden 1998–99. When analysing isolates other than those belonging to the two genetically indistinguishable clones, PFGE identified a high genetic diversity within and between the different serovars.

The antibiotic susceptibility varied with the countries where the patients were exposed. When exposed in Asia, 63% of the isolates showed reduced susceptibility to ciprofloxacin (MIC>0.064 mg/l), compared with 0%–8.5% of the isolates from patients exposed in other countries (RR=8.5, $p<0.001$). Ciprofloxacin cannot be recommended as first choice of treatment if the patient was exposed in Asia. All strains were fully susceptible to spectinomycin and ceftriaxone, which are better choices of treatment.

In a long-term study of gonorrhoea among MSM in Stockholm (n=840 cases), a great variation of different serovars (n=66) was seen during a 15-year period, indicating a continuous importation of strains into this core group and possible micro-epidemics caused by only a few persistent serovars. A significant difference ($p=0.001$) was observed in the distribution of serovars correlated to HIV status. The prevalence of HIV among all cases was 10%. Thus, gonorrhoea is a marker for HIV infection in MSM, but the increase in gonorrhoea may be associated with genital–oral sexual practice rather than with high risk sexual practice. Also, a sexual behaviour study in MSM, showed an increase in the reported number of sexual partners ($p<0.001$) and in unprotected oral sex with casual partners ($p<0.05$), findings that are associated in time with the increase of gonorrhoea among MSM.

Further active surveillance of gonorrhoea, including epidemiological data and microbiological characterization, is needed to control the spread and to make it possible to direct intervention efforts to core groups and persons at risk.

SAMMANFATTNING

Efter ca 25 års stadig minskning började gonorréincidensen att öka i Sverige 1997. Ökningen har fortsatt och sammanfaller med liknande trender hos andra sexuellt överförda infektioner (STI) och i andra länder i Västvärlden. Gonorré omfattas av den svenska smittskyddslagen och alla diagnostiserade fall skall därför rapporteras. Kompletterande epidemiologiska data samlades in under 12 månader 1998–99 om alla rapporterade fall (n=357) och kopplades sedan ihop med mikrobiologisk karaktäristik av *Neisseria gonorrhoeae* (*Ng*) isolaten. Analysen visade att ökningen av gonorré framför allt beror på en ökning av inhemska fall; 60% hade smittats i Sverige, främst i Stockholm. Heterosexuella tonåringar och män som har sex med män (MSM) identifierades som inhemska 'kärngrupper' (core groups) infekterade med separata fenotypiska *Ng* serovar. Totalt förekom 28 olika serovar. Bland utlandssmittade patienter var Thailand det vanligaste förekommande smittlandet.

Genotypisk karakterisering med PFGE av *Ng* isolaten indikerade att en genetiskt oskiljaktig klon av vardera serovaren IB-2 och IB-3 stod för flertalet av de inhemska fallen i de två identifierade kärngrupperna i Sverige 1998–99. Vid analys av och jämförelse med övriga isolat, identifierade PFGE stora genetiska skillnader inom och mellan de olika serovaren.

Bakteriernas känslighet för olika antibiotika varierade beroende på var i världen patienten smittats av gonorré. Om patienten smittats i Asien uppvisade 63% av dessa isolat nedsatt känslighet eller resistens mot ciprofloxacin (MIC>0.064 mg/l), jämfört med 0%–8.5% av isolaten från patienter smittade i andra världsdelar (RR=8.5, p<0.001). Ciprofloxacin kan således inte rekommenderas som förstahandsval av behandling om patienten eller hans/hennes partner smittats i Asien. Alla isolat hade full känslighet mot spektinomycin och ceftriaxon, vilka därför är bättre behandlingsval vid gonorré.

I en 15-årsstudie av gonorré bland MSM i Stockholm 1990–2004 (n=840 fall) förekom en stor variation av olika serovar (n=66), vilket indikerar att import av gonorréstammar sker fortlöpande och att endast några få serovar fortlever i denna inhemska kärngrupp och orsakar mikroepidemier. En signifikant skillnad (p=0.001) observerades i förekomsten av olika serovar med hänsyn till patientens HIV-status. HIV-prevalensen bland gonorréfallen var 10%, vilket visar att gonorré är en riskmarkör för HIV-infektion bland MSM. Dock ökade andelen fall med svalggonorré signifikant (p<0.001) under den senare halvan av studien, varför ökningen av gonorré bland MSM under samma tidsperiod snarare kan vara associerad med oralsex än med högrisksex avseende HIV. I en sexvanestudie bland MSM påvisades en ökning av antalet sexualpartners (p<0.001) och av oralsex utan kondom med tillfällig partner (p<0.05), vilket sammanfaller i tiden med ökningen av gonorré.

Fortsatt aktiv epidemiologisk övervakning av gonorré inklusive mikrobiologisk karakterisering är nödvändig för att kontrollera smittspridningen i samhället och göra det möjligt att rikta intervention och preventiva insatser till inhemska kärngrupper och andra personer med riskbeteende.

CONTENTS

ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
C.	Chlamydia
CI	Confidence interval
CMO	County Medical Officer of Communicable Disease Control
DNA	Deoxyribonucleic acid
GS	Genetic Systems
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
LCR	Ligase chain reaction
MAb	Monoclonal antibody
MIC	Minimal inhibitory concentration
MSM	Men who have sex with men
N.	Neisseria
NADT	Nucleic acid detection test
Ng	*Neisseria gonorrhoeae*
n.s.	Not significant
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
Ph	Pharmacia
PID	Pelvic inflammatory disease
PPNG	Penicillinase (β-lactamase) producing *N. gonorrhoeae*
RR	Relative risk
Serogroup	Serological group
Serovar	Serological variant
SFS	Swedish Code of Statutes (Svensk författningssamling)
STD	Sexually transmitted disease
STI	Sexually transmitted infection
SMI	Swedish Institute for Infectious Disease Control
WHO	World Health Organization

LIST OF PUBLICATIONS

The thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Berglund T, Fredlund H, Giesecke J. Epidemiology of the reemergence of gonorrhea in Sweden.
Sexually Transmitted Diseases 2001;28:111–4.

II. Unemo M, Berglund T, Olcén P, Fredlund H. Pulsed-field gel electrophoresis as an epidemiologic tool for *Neisseria gonorrhoeae*.
Sexually Transmitted Diseases 2002;29:25–31.

III. Berglund T, Unemo M, Olcén P, Giesecke J, Fredlund H. One year of *Neisseria gonorrhoeae* isolates in Sweden: the prevalence study of antibiotic susceptibility shows relation to the geographic area of exposure.
International Journal of STD & AIDS 2002;13:109-14.

IV. Berglund T, Asikainen T, Grützmeier S, Rudén A-K, Wretlind B, Sandström E. The epidemiology of gonorrhea among men who have sex with men in Stockholm, Sweden, 1990–2004.
Sexually Transmitted Diseases (Accepted, April 2006).

INTRODUCTION

Gonorrhoea is one of the major sexually transmitted infections in the world. The infection is caused by the bacterium *Neisseria gonorrhoeae*. The disease name gonorrhoea derives etymologically from the Greek language and means "flow of seed". The disease is considered as the oldest known STI and is mentioned in ancient literature as well as in the Old Testament of the Bible [1]. The German physician and bacteriologist Albert Neisser (1855–1916) identified and described the bacterium that causes gonorrhoea in stained smears in 1879 [2]. The *Neisseria* genus and the bacteria family *Neisseriaceae*, to which the species *N. gonorrhoeae* belongs, was named after him. The two primary pathogenic Neisseria species are *N. gonorrhoeae* and *N. meningitidis*. They are genetically and morphologically similar, but colonise mainly different anatomical sites and causes different diseases.

EPIDEMIOLOGY

Global

The incidence of gonococcal infections is estimated to about 60 million cases globally each year [3]. The disease is more frequent in poorer countries with less access to adequate diagnostics and treatment, and in marginalized sub-populations in all countries. An important aspect of gonorrhoea epidemiology is the fact that gonorrhoea increases the risk of sexual transmission of HIV [4, 5] and co-infection with these diseases has been reported in areas with high prevalence of HIV and in sub-groups at higher risk for HIV infection [6-9].

The highest gonorrhoea rates are in South and South-East Asia, sub-Saharan Africa and Latin America. The World Health Organization (WHO) estimates (1999) the prevalence in adults over 15 years of age in some countries in South-East Asia and Western Pacific – such as Cambodia and Papua New Guinea – to >3% [10], and in Vanuatu some 6% of pregnant women have been found to be infected with gonorrhoea [11]. In adolescents who were visiting an urban youth health clinic in Kampala, Uganda, the prevalence of gonorrhoea was 9% in females and 5.7% in males [12]. In men who have sex with men (MSM) attending gay bars in Jiangsu Province in China, the gonorrhoea prevalence in a study from 2003 was 2.7% [13]. In USA gonorrhoea is the second most often reported STI after chlamydia, with a reported incidence of 113.5 cases per 100,000 persons in 2004 [14], but the true rate is estimated to the double, or about 600,000 infected people each year [15].

In Europe, a marked decrease in gonorrhoea incidence was seen in most countries during the 1970s and 1980s [16]. However, a sharp increase occurred in several countries in Eastern Europe from around 1987 with a peak around 1993–1994 [17, 18]. In Russia, the notification rates of gonorrhoea increased from 105 cases per 100,000 people in 1987 to 232 per 100,000 in 1993 [19]. The trend was similar in most other ex-Soviet Union countries, e.g. in Estonia the incidence increased during the early 1990s to 233 cases per 100,000 in 1993 and then declined again to 34 per 100,000 in 2003 [20, 21], and in Lithuania the incidence rose to 152 cases per 100,000 in 1993 and then decreased to only 14 cases per 100,000 in 2004 [21, 22].

In Central and Western Europe no such rise in the gonorrhoea incidence was seen in the early 1990s and the reported rates dropped to below 10 cases per 100,000 people in many countries in the region. However, during the second half of the 1990s and the beginning of the 2000s increase in gonorrhoea rates as well as in other STIs has been reported from several Western European countries. In Great Britain, gonorrhoea more than doubled between 1996 and 2001, in Austria the incidence increased from 2.9 to 5.2 cases per 100,000 between 2000 and 2002, and also in Denmark, Norway and Sweden increases have been seen from very low rates (< 5/100,000) in the middle of the 1990s [18, 23, 24], see Fig. 1.

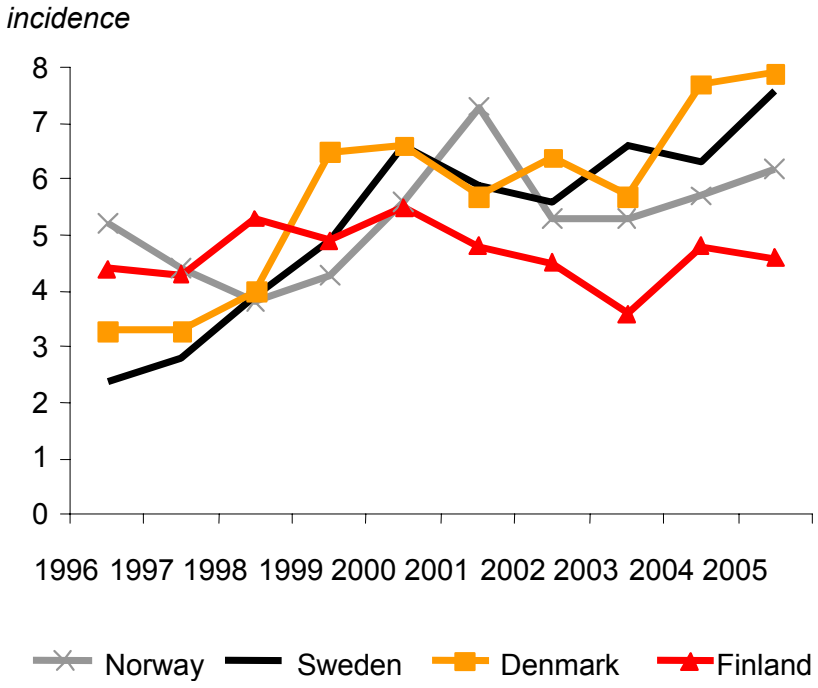

Figure 1. Incidence of gonorrhoea per 100,000 persons in the Nordic countries, 1995–2005. *Source:* National surveillance data.

However, it should be remarked that in many countries, incidence or prevalence data are insufficient and do not give an accurate picture of the distribution of gonorrhoea. Lack of adequate resources for laboratory diagnosis, reluctance to report cases and differences in surveillance systems make it difficult to compare the epidemiological situation in different countries and regions of the world.

Sweden

In Sweden, the gonorrhoea rates can be followed since July 1912, when a regulation was instituted for physicians to report gonorrhoea cases to public health authorities. This obligation was also prescribed by law in "Lex veneris" 1919 [25, 26] and reporting is still obligatory in the present Act of Communicable Diseases (SFS 2004:168), see p. 23. Three main peaks occurred during the 20th century: in the 1910s and the 1940s, coinciding with World War I and II, and in the 1960s culminating in

1970, coinciding with the legalisation of contraceptive pills and the "sexual revolution" (Fig. 2).

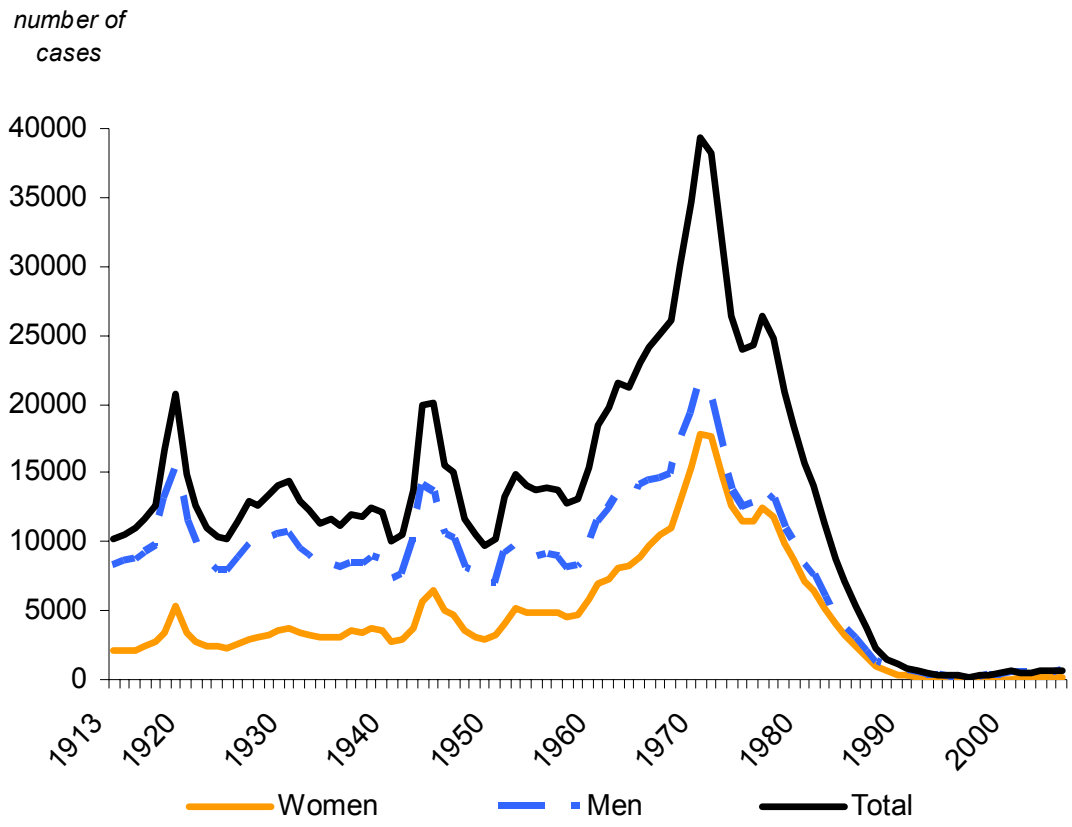

Figure 2. Number of clinically reported cases of gonorrhoea in Sweden, 1913–2005. *Source:* Linglöf (1993) and Department of Epidemiology, Swedish Institute for Infectious Disease Control.

Trends after 1970

In 1970 an all-time high of nearly 40,000 cases were reported, resulting in an incidence of 487 cases per 100,000 persons. The incidence in males was even higher, 531 per 100,000 males, but a bit lower in females, 442 per 100,000. The incidence in males was on the same high level as in the first peak in 1919 (532/100,000) and higher than in 1943 when the second peak was seen (437/100,000 males). However, the difference in the male/female ratio was more pronounced in 1919 (2.8) and in 1943 (2.5) than in 1970 (1.2). The ratio showed a steady fall from the 1910s to the middle of the 1970s when the ratio was close to 1 [25]. Then the male/female ratio started to increase slowly and during the 1990s and 2000s the difference became more accentuated and reached a male/female ratio of 6.0 in 2004.

The other Nordic countries experienced similar increasing rates in the 1960s, but reached their peaks a few years later than Sweden, around 1975, with incidences around 350 cases per 100,000 persons [24]. Then, a remarkably decline was seen in all the Nordic countries. The Swedish gonorrhoea rates rapidly decreased during the 1970s and 1980s, with exception of a minor increase in 1975–1976, and finally reached an all-

time low of only 211 reported cases in 1996 or 2.4 cases per 100.000 inhabitants. Most of the cases, if country of exposure was reported, had contracted their infections abroad and the endemic spread of gonorrhoea was probably almost eradicated at that time [27, 28]. The situation was similar in Norway, Denmark and Finland, where the incidence fell to <5 per 100,000 in the mid 1990s (see Fig. 1). This remarkable decline in the gonorrhoea rates in the Nordic countries has been assumed to be connected with a number of concurrent factors, such as the introduction of better laboratory tests and diagnostics, introduction of per oral treatment, improved routines for partner notification, widespread opportunistic screening in asymptomatic women and increased awareness of STIs among the public due to national prevention campaigns [16, 24, 25]. Also, the awareness of HIV/AIDS and reductions in unsafe sexual behaviours in the 1980s and the early 1990s, may have contributed to the continuous decline of the gonorrhoea incidence during these years, especially in vulnerable sub populations such as men who have sex with men (MSM) [29, 30].

Recent trends

In 1997 the incidences of gonorrhoea in Sweden increased for the first time since 1976.

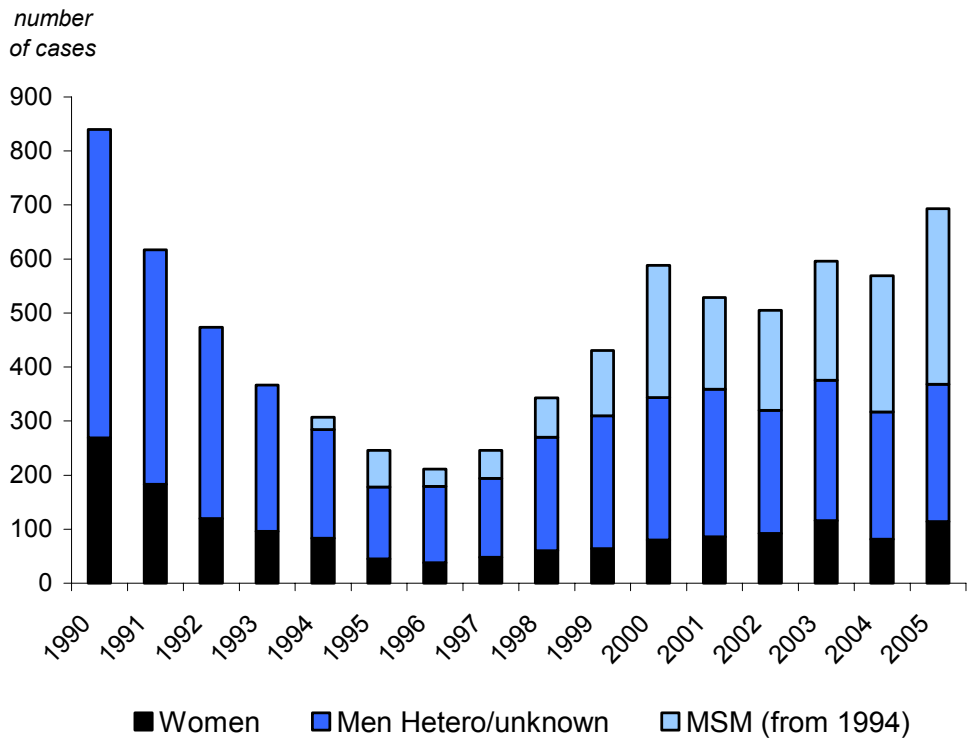

Figure 3. Number of clinically reported cases of gonorrhoea in Sweden by sex and route of transmission, 1990–2005*. *Source:* Department of Epidemiology, Swedish Institute for Infectious Disease Control.

* Since 2005 laboratory reported cases are included in accordance with the new Act of Communicable Diseases (SFS 2004:168).

The increase was mostly due to a rise in domestic cases among heterosexuals as well as in men who have sex with men [31]. The increase continued during the following three years: from 1996 to 2000 the reported number of gonorrhoea cases increased by 180%, from 211 to 590 notified cases. The following four years the curve stabilized on a level around 500–600 cases per year until 2005 when the annual number of reported cases increased to 691 cases or 7.6 cases per 100,000 persons (12.9 cases per 100,000 males and 2.5 cases per 100,000 females). However, it should be remarked that the increase in 2005 partly may be explained by a change in the reporting system for the notifiable STIs in the new Swedish Act of Communicable Diseases (SFS 2004:168), that obliges also laboratories to report all cases positive for *N. gonorrhoeae* (see p. 17).

Sex and route of transmission

Women accounted for 16% of the gonorrhoea cases in 2005, heterosexually exposed men for 34% and MSM for almost half of all cases, 47% (Fig. 3). There were also a few cases in men (3%) without reported route of transmission. There were no reports of gonorrhoea conjunctivitis in newborn children 2005; only three such cases have been reported in Sweden during the last 10 years.

The increase since 1996 has been particularly accentuated in MSM, who account for a disproportionately large burden of gonorrhoea in Sweden as well as in several other Western European countries, e.g. Denmark and the United Kingdom [9, 23, 32]. The number of reported gonorrhoea cases in Swedish MSM has increased ten times since 1996 and also the rates of chlamydia and syphilis, that were rarely seen infections in this sub-population in the 1990s, have increased significantly in MSM in the 2000s [33-35]. These trends are common to most countries in the Western world and have been seen both in HIV-negative and HIV-positive MSM [36].

Age groups

The mean age in heterosexual men as well as MSM has been about 33 years during the last 10 years without any remarkable variations. In women, the mean age has been about 27 years, but it has varied from 24 to 30 years. In 2005 the mean age was 32 years in men (34 years in heterosexually exposed men and 31 years in MSM) and 30 years in women.

In total, the gonorrhoea rates increased by 227% from 1996 to 2005. Increasing curves were seen in all ages from 15 years and older and the rise has been particularly pronounced in the age groups 20–24 years (+406%) and 25–29 years (+334%). There has also been a substantial rise of cases among teenagers (15–19 years) of both sexes; only 11 cases in teenagers were notified in 1996 compared to 42 cases in 2005 (+282%), see Fig. 4. During the whole 10-year period, there was a significant difference in the male/female ratio in all ages (mean ratio 5.1) with exception of the youngest age group. In teenagers the male/female ratio was close to 1 (mean ratio 1.1) 1996–2004, but in 2005 the ratio suddenly increased to 2.5, mainly due to doubled number of cases (from 7 in 2004 to 16 in 2005) in homosexually exposed males in the ages 17–19 years.

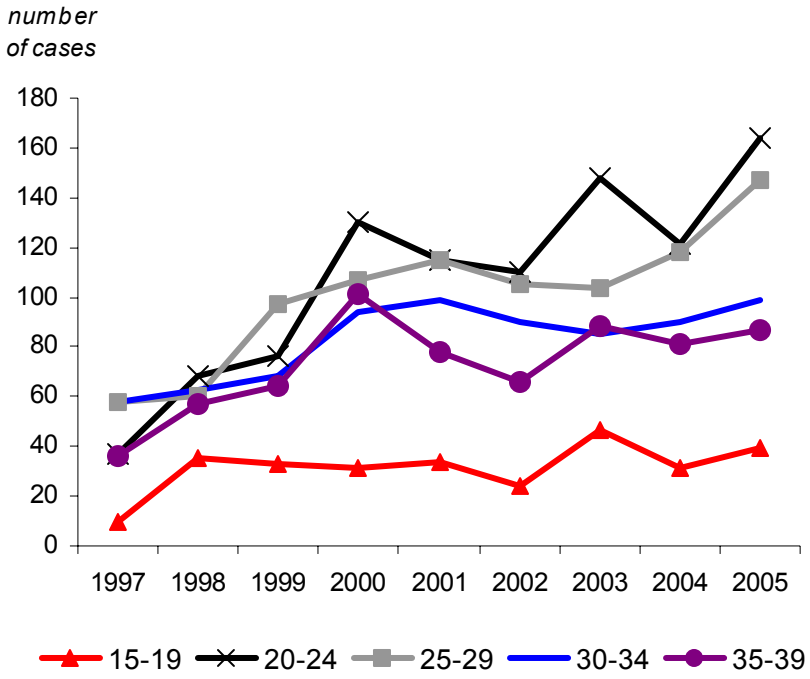

Figure 4. Number of clinically reported cases of gonorrhoea in Sweden by age groups, 1997–2005*. *Source:* Department of Epidemiology, Swedish Institute for Infectious Disease Control.

* Since 2005 laboratory reported cases are included in accordance with the new Act of Communicable Diseases (SFS 2004:168).

Country of exposure

Since the gonorrhoea rates dropped to a low prevalent level in the 1990s, imported cases account for a considerable proportion of all cases in Sweden. In 1997, half of all cases were imported [37]. Even if the proportion of domestic cases has increased since then, international sexual contacts have had a significant role in the Swedish gonorrhoea epidemiology during the last decade, also for the importation and spread of gonococcal strains with antimicrobial resistance.

During the 10-year period 1996–2005 a total of 4,495 cases of gonorrhoea were reported to SMI. Persons exposed in Sweden accounted for 56% of all cases and persons exposed abroad accounted for 37% (unknown country of exposure: 7%). The domestic cases increased tenfold during the 10-year period, while the imported cases increased two and a half times only and levelled out after 2000 (Fig. 5). More than 100 different countries from all continents were represented among the reported countries of exposure.

In 2005, the proportion of persons infected abroad was 31% and in Sweden 64% (5% unknown country of infection). Besides Sweden, 42 different countries in all continents were represented. In women the majority of the cases, 60%, were infected in Sweden, but in heterosexually exposed men, only 48% were infected in Sweden. Thirty five

percent of all imported cases were exposed in Thailand, the by far most frequent country when exposed abroad. More than half of the men infected by a heterosexual contact were exposed in South-East Asia, especially in Thailand. Only a few cases (<5) were imported from the Baltic countries and other ex-Soviet Union countries, which was congruent with the experiences from previous years [31, 38]. Endemic spread of gonorrhoea in heterosexual individuals mainly occurred in the three major city areas in Sweden. In MSM most of the cases, 78%, were infected in Sweden, mainly in Stockholm, but endemic spread did also occur in Gothenburg, Malmö and Uppsala. Most of the MSM who were exposed abroad were exposed in major European cities, e.g. Barcelona, Copenhagen and London.

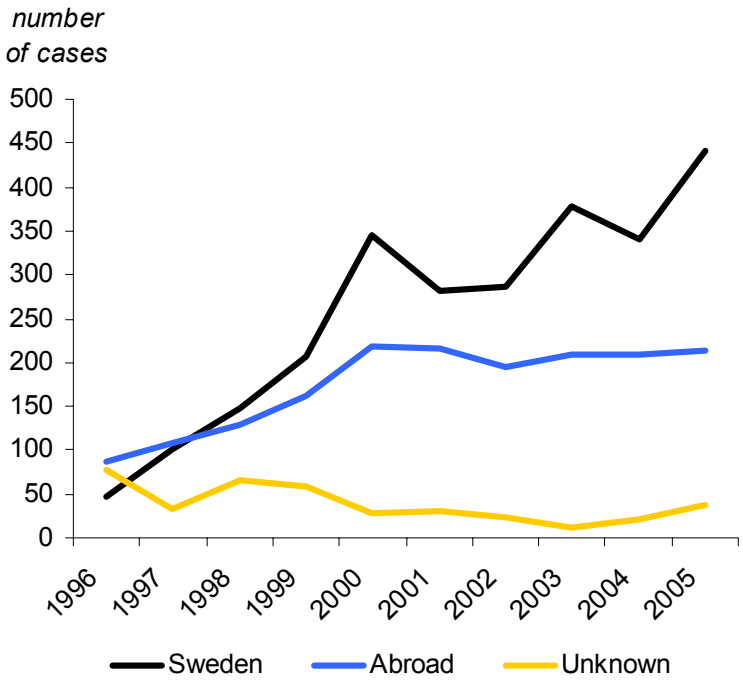

Figure 5. Number of clinically reported cases of gonorrhoea in Sweden by country of exposure, 1997–2005*. *Source:* Department of Epidemiology, Swedish Institute for Infectious Disease Control.

* Since 2005 laboratory reported cases are included in accordance with the new Act of Communicable Diseases (SFS 2004:168).

Laboratory reports

In addition to the notifications by case, twice a year all laboratories in Sweden report the number of analyses performed, and the number of positive samples. Thanks to these numerical reports we know that the clinical surveillance system, based on case reports from the physicians, covered 87%–93% (mean 90%) of the number of positive *N. gonorrhoeae* samples during the 10-year period 1995–2004. That was a high coverage compared with the diverse surveillance systems in most other countries in the European Union [39]. In 2005 the coverage of reported cases was even higher, 97%, because of the changed reporting routines in the new Swedish Act of Communicable diseases (SFS

2004:168) that came into force in July 2004. From that date, not only the physicians in the clinics have to report cases of gonorrhoea, syphilis and chlamydia, but also all laboratories that detect a positive sample are obliged to report each positive case to the County Medical Officer of Communicable Diseases (CMO) and to the Swedish Institute for Infectious Disease Control (SMI).

The number of persons tested for gonorrhoea decreased sharply from >450,000 persons in the mid 1980s to only about 31,000 persons in 1997. Then the number of tested persons began to increase slightly. From 1998 to 2005 the number of people tested increased by 79%. However, during the same 8-year period the number of positive results increased by 152%. Also the proportion of positive results has increased since 1997 from a stable level of 0.6% of all sampled persons to a level about 1.2% (Fig. 6). In 2005 women represented 65% of the persons tested, but only 0.4% of them tested positive compared with 2.9% of the men tested.

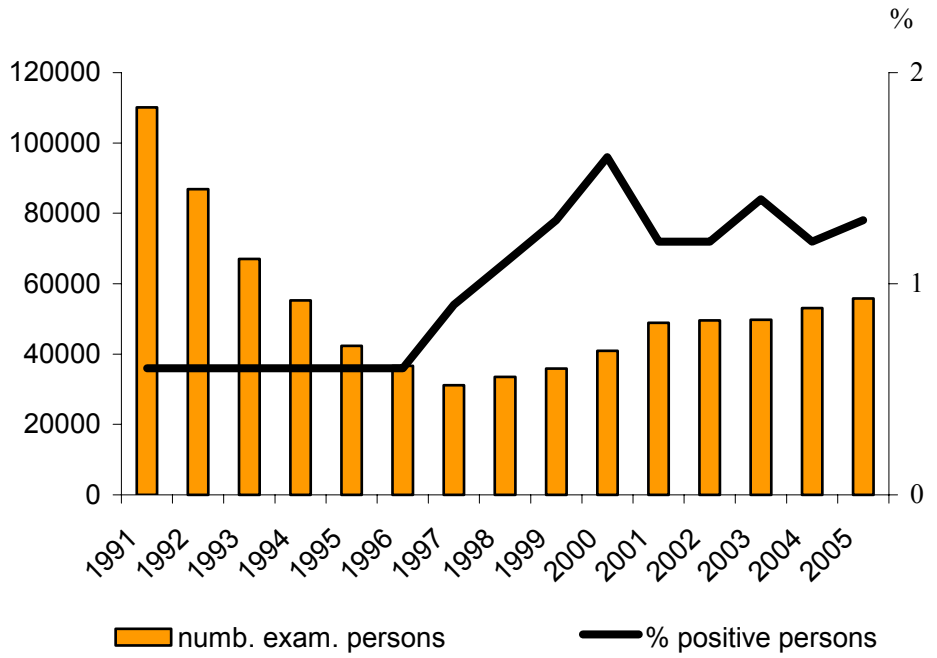

Figure 6. Number of examined persons (bars) for *Neisseria gonorrhoeae* and percentage (%) positive of examined persons (curve) in Sweden, 1991–2005. *Source:* Numerical laboratory reports, Department of epidemiology, Swedish Institute for Infectious Disease Control.

Trends in other reported STIs

The rising trend in gonorrhoea coincides with sharp increases in the reported numbers of chlamydia and syphilis in Sweden. From 1989 to 1994 chlamydia rates rapidly declined to about half and stabilized on a level of some 14,000 cases per year. However, in 1998 that trend was changed and after only a few years the chlamydia rates were more than doubled. In 2005 more then 33,000 cases were reported (369 cases per 100,000 persons). About two-thirds of the cases were detected in young

females and males in the ages 15–24 years, the age group in which the sharpest increase has been noted. The increase of chlamydia can only partially be explained with a change in diagnostic methods during the 2nd half of the 1990s from ELISA or culture methods to more sensitive diagnostic methods, i.e. PCR or ligase chain reaction (LCR) [40].

Also, the reported number of syphilis cases has increased during the 2000s, but from very low rates. In 1999 an all-time low of only 39 cases were reported to SMI. After that the rates increased sharply, mainly due to an increased number of cases in MSM, connected to outbreaks in Stockholm and other major cities in Western Europe [34] [23]. The annual number of cases in Sweden reached 192 in 2004, but then decreased in 2005 to 109 cases (1.2 cases per 100,000 persons).

BIOLOGY

N. gonorrhoeae is a Gram-negative diplococcus, also named gonococcus. Humans are the only natural host and the bacteria survive poorly outside the human body. The organism is aerobic, requires CO_2, enhanced by humidity, and has an optimal growth at 35–37ºC [41]. The outer membrane of the gonococcus contains four components that are involved in the virulence of the bacterium; pili, Opa proteins, porins and lipooligosaccharides [42]. Horizontal genetic exchange frequently occurs between *N. gonorrhoeae* strains and also with other similar species. The genetic exchange in combination with mutations causes a high level of phenotypic and genotypic variability in gonococci, which is important for evasion or adaptation to the immune response of the human host and for the development and spread of resistance to antibiotics [43, 44].

TRANSMISSION

Gonorrhoea is an STI that is transmitted with high efficiency. The gonococci are primarily transmitted by human-to-human contact between the mucosal membranes of the urogenital tract, the rectum or the oropharynx. Transmission via contaminated fingers (digital–genital contact or digital–anal contact) and via sex toys is also possible and has been reported [45, 46]. Gonococci can also infect the eye and cause conjunctivitis in neonatal children when transmitted from mother to child during childbirth, and sometimes in adults when rubbing the eyes with contaminated fingers.

The risk of contracting gonorrhoea depends on factors such as sexual behaviour, condom use and of course the epidemiology, i.e. how common the pathogen is in the community. It also depends on factors such as the virulence of the bacteria and the dose that a susceptible person is exposed to. As there is no known immunity to gonorrhoea [47] and not yet any vaccine, all sexual active persons in a population are susceptible to the infection.

The attack rate, i.e. the proportion of exposed persons that become infected, is high. It has been calculated to 50–70% for a single act of vaginal intercourse [48, 49].

Other factors determining transmission are the incubation period and the duration. The incubation period for gonorrhoea is rather short compared to other STIs and averages from 2 to 7 days. However, 10–20% of men and about 50% of women have an asymptomatic infection [1]; these proportions may vary depending on the anatomic site of infection. The duration of infectiousness, i.e. the time during which an infected person can transmit gonorrhoea, has been estimated at around 4 months if the infection goes un-treated [50], but it can probably vary from a month to at least a year.

In infectious disease epidemiology there is a model for calculating the potential for an infection to spread in a population: $R_0 = \beta \times c \times D$
where β is the risk of transmission per contact (i.e. attack rate), c is the number of contacts that an average person in the population would normally have per time unit and D is the duration period of infectiousness of an infected individual measured in the same time units as c was. R_0 is the basic reproductive rate defined as the average number of individuals directly infected by an infectious person during his or her entire infectious period, when he or she enters a totally susceptible population. If R_0 is greater than 1 the outcome is an *epidemic*, if R_0 is equal to 1 the disease is *endemic*, i.e. we have neither an increase nor a decrease in the number of new cases, and if R_0 is less than 1 the disease will eventually disappear [51].

CORE GROUPS

It is assumed that rather small subsets of the population – core groups of people who are frequently infected and have a large number of partners – contribute disproportionately to the spread of STIs and cause gonorrhoea to remain endemic. These individuals, with a high rate of partner change, form sexual networks from which most of the infections in the community originate [52, 53]. In other studies, depending on the perspectives, the core group term has also been defined as people who infect more than one other person, i.e. $R_0 > 1$, are repeatedly infected, or are prostitutes or their clients, or to geographic areas with clusters of cases [54, 55].

In epidemiological research efforts have been made to detect and focus on such core groups to better understand the mechanisms of the transmission of STIs and to refine the prevention strategies and intervention programs to reduce the spread of STIs. Also, the need to analyse the structure and dynamics of the sexual networks has been pointed out as important in order to better understand the transmission of STIs [53, 56, 57]. Data from partner notification combined with socio-demographic data and sexual behaviour studies of groups with high-risk behaviour are essential for the analysis of the complexity of sexual networks [58].

CLINIC, DIAGNOSIS AND TREATMENT

Clinic

The acute clinical manifestations of gonorrhoea are limited to columnar and transitional epithelium and are generally more pronounced than those of chlamydial infections. In males the classical symptoms are acute urethritis with dysuria and purulent discharge. In females gonorrhoea is presented as an uncomplicated cervicitis and/or urethritis with

discharge, dysuria and sometimes vaginal bleeding. The infection in females can progress and cause complications as pelvic inflammatory disease (PID) manifested as endometritis, salpingitis or pelvic peritonitis, and may cause infertility and etopic pregnancy. In males the infection may cause complications as urethral stricture, prostatitis and epididymitits that may lead to infertility. In both females and males, especially men who have sex with men (MSM), pharyngeal and anorectal infections (proctitis) are not uncommon and often asymptomatic. Conjunctivitis, which causes blindness if not treated, occurs in newborn children and rarely in adults. In a few cases (0.5%–1%) septicaemia may occur, with arthritis, skin lesions and in rare cases endocarditis and meningitis [1, 59, 60].

Diagnosis

Microscopic examination of urethral discharge stained with methylene blue or Gram stain is often used in STI clinics as a direct but preliminary method to detect diplococci. However, samples should be sent to laboratory for diagnosis. The culture is still the gold standard for the detection of *N. gonorrhoeae* in Sweden and in most other countries. Samples are sent to the laboratory on swabs in a transport medium and cultured on agar media. The specificity and sensitivity is high, and the method also allows antibiotic susceptibility testing as well as phenotypic and genotypic characterization. A disadvantage is that the gonococci are vulnerable, demand propitious growth conditions and can be destroyed if the transportation time is prolonged [16, 43, 60].

Nucleic acid detection tests (NADTs), detecting genomic parts of the *N. gonorrhoeae*, have been developed recently and are increasingly used in many countries. These tests are fast and easy to perform as urine samples can be used. However, these methods are not suitable for specimens from the rectum and the pharynx, and cannot be used for test-of-cure or for antibiotic susceptibility testing. Furthermore, there are problems with the sensitivity and specificity; false negative results occur because of the genetic variability in the gonococci and there are also problems with false positive results [61-63].

Treatment

Antibiotics can successfully cure gonorrhoea. Not only the patient with confirmed gonococcal infection, but also his or her recent and present sexual partners should always be tested and treated on epidemiological grounds [43, 60].

However, the genetic flexibility of the gonococci has given the organism a remarkable capacity to develop resistance to antibiotics. Chromosomally mediated resistance to a variety of antibiotics are widespread, as well as plasmid mediated resistance to penicillins (PPNG), tetracyclines and early generations of cephalosporins. Drug-resistant strains of gonorrhoea are increasing in many areas of the world, including Sweden, and the choice of treatment of gonorrhoea is becoming more difficult. Thus, antimicrobial susceptibility testing is always required to several antibiotics [43].

In Sweden and many other countries ciprofloxacin has been the recommended first choice of treatment for gonorrhoea since the early 1990s. However, resistance to quinolones, to which ciprofloxacin belongs, has become widespread in Asia during the last decade, and an increase of resistant strains has also been seen in several countries in Western Europe and USA, including Sweden. Thus, spectinomycin, and the third-generation cephalosporins such as ceftriaxone or cefixime often are better choice of treatment [3, 43, 59, 64].

Also to be noted is that gonococcal infections of the pharynx and the rectum can be more difficult to cure than infections of the urethra and cervix. Because of the problematic situation with decreased antibiotic susceptibility, it is important that a test-of-cure is performed about 7–10 days after finished treatment to confirm compliance with therapy and verify that the cure was successful [64, 65].

EPIDEMIOLOGICAL TYPING

An important tool in characterizing outbreaks of gonorrhoea and core groups is epidemiological typing of strains in combination with clinical epidemiological data. There are a number of different typing methods developed for *N. gonorrhoeae*. Serological typing, a phenotypic characterization based on a co-agglutination technique with a panel of monoclonal antibodies (MAbs) [66, 67], is the most commonly used internationally. It is a fast, cost-effective, and easy to perform method, but it has suboptimal discriminatory ability and there are problems concerning reproducibility [44]. Two different panels of MAbs are most widely used, the Genetic Systems (GS) panel [67, 68] and the Pharmacia (Ph) panel [69], which differ in the number of MAbs used in the panels. Thus, the serotypes, called serovars, are not translatable into the other system. In Sweden, about 70% of all notified cases are characterized routinely by either of these two panels, comprising most isolates from Stockholm county and most of the isolates from the other counties in Sweden with exception of Skåne, Halland and Västra Götaland [70].

Another phenotypic characterization method is auxotyping, which is time consuming and has limited discriminatory ability. Also antibiograms are used for detecting epidemiological relationships between gonococci isolates, but antibiograms have low discriminatory ability and are not stable over longer time periods [44].

Recently, several molecular methods for genotypic characterisation of *N. gonorrhoeae* have been developed. These typing methods have a high discriminatory ability compared to the phenotypic methods, and analyse different enzymes or plasmids, genetic loci or the entire genome. Examples of such techniques are pulsed-field gel electrophoresis (PFGE) [71], *porB* gene sequencing (entire or partial) [72-74], *Neisseria gonorrhoeae*-multiantigen sequence typing (NG-MAST) [75] and *opa*-typing [76, 77].

However, these methods are often labour intensive and much more expensive than the phenotypic methods. There are also problems with the reproducibility and standardisation is needed, as well as more knowledge of the time scale of the

evolutionary changes in the *N. gonorrhoeae* genome. For these reasons the molecular methods are more suitable for characterization of strains in suspected outbreaks, transmission chains or sexual networks than in long-term epidemiology [44, 77].

PREVENTION AND SURVEILLANCE

Condoms, when consistently and correctly used, provide protection from gonorrhoea and other STIs including HIV in sexual contacts [78, 79]. Thus, education and information about STIs and safe sex is included in public health and prevention strategies and in school education in Sweden and other countries. Awareness of STIs among the public is fundamental for the reduction and eradication of gonorrhoea and other STIs. Also, opportunistic screening has been successful in regions with previously high prevalence of gonorrhoea, like the Nordic countries [24].

Sensitive diagnostic methods and antibiotic susceptibility tests, as well as adequate treatment and examination of and treatment for partners of patients with gonorrhoea, are all of utmost importance to control gonorrhoea. Easy access to counselling and testing for persons at risk, with prompt treatment and follow-up of infected persons, is also strategic to prevent secondary spread.

To prevent conjunctivitis in newborn children, instillation of silver nitrate aqueous solution stored in individual wax capsules is most widely used. Erythromycin and tetracycline ophthalmic ointments are also shown to be effective [59].

A sensitive epidemiological surveillance system also has an important role in the control of STIs, not only to follow trends in reported cases, but also for identifying risk populations and core groups. Gonorrhoea is, together with chlamydia, syphilis and HIV-infection, included as an infection of public danger in the Swedish Communicable Diseases Act (SFS 2004:168). Consequently, examination and treatment is free of charge. Also, each physician and laboratory that diagnoses a case of gonorrhoea is obliged to report the case to SMI and to the CMO.

Partner notification (contact tracing) is compulsory for the STIs included in the Swedish Communicable Diseases Act, i.e. a physician who diagnoses a case of gonorrhoea is responsible for carrying out partner notification or seeing to it that it occurs. The health care provider contacts the patient's sexual partners, present and previous if there is a risk of exposure, and they are obliged to be examined by a physician. Partner notification in STIs is an established preventive method in many countries, but Sweden and also Norway are among the few countries where partner notification is regulated by law [80].

In most countries in the European Union and in many other countries in the world, gonorrhoea is a mandatory notifiable disease. However, the characteristics of these systems are heterogeneous and the coverage of cases reported in the surveillance systems is very variable. In some European countries, like Spain, Portugal and Belgium, the coverage is very low, and in other, like Sweden, Norway and Finland, it is high (76%–99%). These variations in surveillance systems in different countries make

epidemiological comparison difficult, but still the trends over time are often meaningful to compare [39].

AIMS

The general aim of the present thesis was to describe and analyse the recent trends in the epidemiology of gonorrhoea in Sweden to interpret the increasing gonorrhoea rates since 1997 and better understand the role of risk groups.

The specific aims were:

- To analyse the increase in the gonorrhoea incidence using clinical epidemiological data and microbiological characterisation by serotyping of isolates of *Neisseria gonorrhoeae* (I).

- To evaluate the applicability of pulsed-field gel electrophoresis (PFGE) as an epidemiological tool and to explore the homogeneity/heterogeneity of *Neisseria gonorrhoeae* isolates of different serovars (II).

- To compare epidemiological data with antibiotic susceptibility patterns and characterise the risk of infection with a highly resistant *Neisseria gonorrhoeae* strain, as well as recommend a first choice of antibiotic treatment for gonorrhoea in a national context (III).

- To analyse the spread of gonorrhoea in men who have sex with men (MSM), using serotyping and to compare the distribution of serovars among HIV-positive and HIV-negative MSM (IV).

MATERIALS AND METHODS

EPIDEMIOLOGICAL AND CLINICAL DATA

The epidemiological data in **papers I-III** all proceeded from the same material that was collected from in total 393 gonorrhoea cases diagnosed in Sweden from February 1998 to January 1999. All cases of gonorrhoea diagnosed during the 12-month period and reported to SMI in accordance with the Swedish Communicable Diseases Act were included prospectively. The case reports usual contain information about the patient's sex, birth year and county of residence and if known also probable route of transmission, date and place of exposure and date of diagnosis.

In order to complete – and where appropriate correct – the information in the case reports a separate epidemiological questionnaire was sent consecutively from the Department of Epidemiology at SMI to the physician reporting a case. When a case report was registered at SMI, the questionnaire was sent to the reporting physician together with a copy of the original case report. The questionnaire was filled in by the physician and/or by the counsellor who did the partner notification. A similar questionnaire had been sent in the same way from SMI to the reporting physicians during 1997, which made it possible to compare our data with the results from the previous year.

In the questionnaire patient's birth year and route of transmission (i.e. homosexual, heterosexual) was asked for, as well as where the patient was exposed (i.e. town, country) and the partner's nationality and place of exposure (i.e. town, country), if known. Also the laboratory number of the *N. gonorrhoeae* strain(s) was asked for in order to link the epidemiological data to the isolated *N. gonorrhoeae* strain(s) from each patient.

In **paper IV** clinical and epidemiological data from in total 840 gonorrhoea cases diagnosed during a 15-year period were collected from the medical files at the Department of Venhälsan, Södersjukhuset in Stockholm. 'Venhälsan' (a short form for 'venereological health' in Swedish) is the only STI and HIV clinic in Stockholm primarily serving MSM and the clinic has existed since 1982. Venhälsan offers free counselling, testing and treatment for STIs including HIV. The patients visiting the counselling and testing service are routinely offered tests for HIV, syphilis, hepatitis A and B. In case of symptoms, known exposure, risk for exposure or at patient request tests for gonorrhoea, chlamydia and enteric amoebae are also performed.

All MSM diagnosed with gonorrhoea at Venhälsan during the 15-year period 1990–2004 were included in the study. Those gonorrhoea patients previously not diagnosed with HIV were offered HIV test. Counsellors at Venhälsan performed partner notification and all patients treated for gonorrhoea were routinely followed up with a test-of-cure seven days after the treatment was completed. Also patients who were diagnosed or previously had been diagnosed with HIV were followed up and treated in

the same clinic. About 550 HIV-positive patients (2004) are registered as patients at Venhälsan and followed clinically with visits at least every fourth month. Gonococcal cultures are obtained routinely from these patients when first diagnosed with HIV and later on if there has been a risk of exposure or if symptoms are suggestive of gonococcal disease.

Epidemiological and clinical data about route of transmission, date of diagnosis and site of infection as well as HIV status were collected consecutively from the medical files for all included cases of gonorrhoea.

MICROBIOLOGICAL DATA

Isolation of *Neisseria gonorrhoeae*

In **papers I-III** strains of *N. gonorrhoeae* isolated by routine methods at one of the 29 clinical microbiological laboratories in Sweden from February 1998 to January 1999 were included. During this 12-month period some 33,000 persons were sampled for *N. gonorrhoeae* in Sweden.

The isolated *N. gonorrhoeae* strains were sent for further microbiological characterisation from the primary clinical microbiological laboratories to the Swedish National Reference Laboratory for Pathogenic Neisseria, Department of Clinical Microbiological and Immunology, Örebro University Hospital. At the reference laboratory all isolates were cultured on GCSPP agar for 18 to 20 hours at 37°C in 5% CO_2.

In **paper IV** microbiological data about all isolated *N. gonorrhoeae* strains from patients diagnosed at Venhälsan during the 15-year period 1990 to 2004 were collected consecutively from the laboratory results in the medical files and from annual lists of positive *N. gonorrhoeae* samples from the Clinical Microbiological Laboratory at Karolinska University Hospital in Huddinge.

Swabs were obtained routinely from the urethra, rectum and pharynx for gonococcal culture and sent to the clinical microbiological laboratory where gonococci were cultured on chocolate agar with and without antibiotics (1990–1995: vancomycin, colistin, nystatin and trimethoprim; 1996–2004: polymyxin B and vancomycin) and diagnosed by colony morphology, positive oxidase test, Gram-negative diplococci in the microscope, typical sugar oxidation test and confirmed by co-agglutination (Phadebact Monoclonal GC-kit, Boule, Stockholm, Sweden).

Antibiotic susceptibility testing

The susceptibility to cefuroxime, erythromycin and ciprofloxacin (**paper II**) and to penicillin G, ampicillin, cefuroxime, cefotaxime, ceftriaxone, tetracycline, erythromycin, azithromycin and ciprofloxacin (**paper III**) was tested by the E-test method (AB Biodisk, Solna, Sweden). Suspended bacteria were incubated with test strips on GC II agar, supplemented with 1% hemoglobin and 1% IsoVitaleX for 16 to 18 hours at 35 to 37°C in 5% CO_2 atmosphere and minimum inhibitory concentrations (MIC, mg/l) were determined. The susceptibility to spectinomycin (**paper III**) was

tested by a disc-diffusion method [81] with a disc containing 30 μg spectinomycin (AB Biodisk) on resistance-agar. A chromogenic cephalosporin test with nitrocefin discs (AB Biodisk, Solna, Sweden) was used to analyse ß-lactamase production (**papers II-IV**).

Serological characterisation

To perform phenotypic characterisation of the *N. gonorrhoeae* isolates serogroup and serovar determination of all the isolates was carried out with a co-agglutination technique [66, 67]. The Genetic Systems (GS) panel of monoclonal antibodies (MAbs) [67, 68] was used in **papers I-III**. In **paper IV** the Pharmacia (Ph) panel of monoclonal antibodies [69] was used. The two panels differ in that the GS panel comprised six different MAbs against the *N. gonorrhoeae* outer membrane protein PorB1a and six MAbs against PorB1b, while the Ph panel consisted of five different MAbs against PorB1a and nine MAbs against PorB1b. Thus, the serogroups correspond, but the serovars are not translatable into the other system.

Genetic characterisation by PFGE

In **paper II** pulsed-field gel electrophoresis (PFGE) was performed as a genotypic tool to characterize a subset of the isolated *N. gonorrhoeae* isolates from paper I. The *N. gonorrhoeae* isolates were cultured on GCSPP agar for 18 to 20 hours at 37°C in a 5% CO_2 atmosphere. The bacteria were suspended in sterile NaCl (0.15 m/l) to a final concentration of approximately 2×10^9 cells/ml. From each suspension 300 μL was used for preparation and digestion of the genomic DNA of the bacteria according to the manufacturer's protocol of GenePath Group 3 Reagent Kit (Bio-Rad Laboratories, Hercules, CA, USA). Two different restriction enzymes were separately used: *Spe*I recommended by Bio-Rad and used in previous studies [82-86] and *Bgl*II also used in previous studies [86, 87].

PFGE fingerprints were obtained by separation of the digested DNA on 1% agarose gels (Molecular biology certified agarose, Bio-Rad) in a contour-clamped homogenous electric field apparatus, with a hexagonal electrode array (GenePath™ System, Bio-Rad). The electrophoresis was performed in a 0.5 × TBE buffer (45 mmol/l Tris-borate [45 mmol/l Tris base, 45mmol/l boric acid] and 1 mmol/l EDTA) equilibrated at 14°C using a constant voltage of 6 V/cm with a pulse time ramping of 2 to 17 seconds for 17 hours at a 120° angle. Gels were stained with ethidium bromide after which the separated DNA fragments were visualized under UV-transillumination and digitized using Gel Doc™ 2000 system (Bio-Rad). The fingerprints were normalized, aligned and compared using the software Molecular Analyst Fingerprinting (version 1.6, Bio-Rad) and visual inspection. An international reference strain, WHO A (CCUG 15821), was included in triplicates on each gel for normalization of the gels by the software to reduce inter- and intragel variations.

In accordance with other studies isolates were considered genetically indistinguishable if no band differed and distinguishable when any band difference was documented. Isolates were designated closely related if their fingerprints differed by no more than three bands [88].

HIV serology

In **paper IV,** HIV infection was diagnosed from serum analysed at the Department of Immunology, SMI, by at least two different ELISA tests and confirmed by an immunoblot. Repeat specimens were obtained and subjected to the same procedure.

STATISTICAL METHODS

The Chi-squared test was used in **paper I** for comparison of proportions of domestic cases per year, and the Mann-Whitney U-test was used for comparison of median age of women and men.

In **paper III** relative risks were calculated to assess the difference in antibiotic susceptibility between *N. gonorrhoeae* strains from patients exposed in Asia and in other places. The Chi-squared test or Fischer's exact test was used to calculate the corresponding p-values. Exact binomial confidence limits (95% CI) were calculated for the percentage of multiresistant isolates.

The Chi-squared test or Fisher's exact test was used to calculate the p-values in **paper IV**. Confidence intervals for the proportions of HIV-positive cases were calculated by assuming a binominal distribution. To compare the mean number of cases in different periods a two-sample Wilcoxon sign-test was applied. The analyses were made with R (version 2.2.1) [89].

TWO UNPUBLISHED STUDIES

Some of the results from two unpublished studies are included for the reason that they are of interest for the findings and discussion in the thesis.

The prevalence study of pharyngeal gonorrhoea and chlamydia

The aim of this prospective study was to investigate the prevalence of pharyngeal gonorrhoea and pharyngeal chlamydia in men who have sex with men (MSM) and to identify patients with asymptomatic infection in the pharynx. MSM who visited the Department of Venhälsan, Södersjukhuset in Stockholm for an HIV test or STI tests during the 4-month period October 2000 to January 2001 were offered tests for gonorrhoea and chlamydia from the pharynx. For each included patient it was noted if he had noticed any symptoms and if and if so when the last time was that he had had an oral sexual contact (i.e. oral–genital contact, oral–anal contact, oral–oral contact/deep kisses). Specimens for *Chlamydia trachomatis* were obtained by stroking with an endocervix swab over the posterior pharynx and for *Neisseria gonorrhoeae* specimens were obtained by rolling a cotton swab over the tonsils.

The specimens were analysed at the Department of Clinical Microbiology, Karolinska University Hospital in Huddinge; samples for *C. trachomatis* were analysed with the COBAS AMPLICOR *Chlamydia trachomatis* PCR test (Roche Molecular Diagnostics, Basel, Switzerland) and samples for *N. gonorrhoeae* were cultured with routine diagnostic methods (see above). If specimens from the pharynx were positive, a

verification test was taken and tests were also taken from the urethra and the rectum if not already done at the initial consultation (i.e. patients who initially only asked for HIV test but wanted to join the pharyngeal screening study).

The sexual behaviour study

In the sexual behaviour study a questionnaire about sexual lifestyle, behaviour and attitudes to and knowledge of safe sex, HIV and STI was distributed to MSM who visited the Department of Venhälsan, Södersjukhuset in Stockholm for HIV test or STI tests. Most of the approximately 70 questions were closed questions with multiple-choice answers. The patients answered the questionnaire anonymously either immediately at the clinic or afterwards at home and then sent it back in a prepaid envelope. During a 6-month period, from October 1997 to March 1998, 400 questionnaires were distributed and 294 (74%) were filled in and sent back.

The study was repeated at the same clinic during six months, from October 2001 to March 2002. Most of the questions were the same as in the previous questionnaire. 500 questionnaires were distributed to MSM visiting the clinic for HIV test or STI tests, and 322 (64%) questionnaires were filled in and sent back. The results were compared with the results from the previous questionnaire and the Chi-squared test was used to calculate the p-values.

ETHICAL CONSIDERATIONS

Studies **I**, **II** and **III** were primarily based on epidemiological surveillance data collected in accordance with the Swedish Communicable Diseases Act and compared with microbiological characterisation of the *N. gonorrhoeae* strains. The full identities of the patients were not known to the researchers since all cases were reported with a coded identity (”rikskod”) in accordance with the Swedish Communicable Diseases Act. The studies were approved by the ethical committee at Karolinska Institutet North (Dnr 799/03).

Study **IV** was based on epidemiological data and microbiological characterisation of the *N. gonorrhoeae* strains collected from patient files and laboratory test results. All patient identities were omitted before analysing the data. The study was approved by the ethical committee at Karolinska Institutet South (Dnr 325/03).

In the two unpublished studies of pharyngeal gonorrhoea and chlamydia infections and the sexual behaviour study all patients were informed about the studies by written information and by the staff at Venhälsan. Patients who agreed to join the studies were included. In the sexual behaviour study a questionnaire was filled in anonymously by the patient and no identities were made known to the researchers. In the pharyngeal screening study all identities were removed before analysing the data. The studies were approved by the ethical committee at Karolinska Institutet South (Dnr 271/97; 293/00; 302/01).

RESULTS

THE REEMERGENCE OF GONORRHOEA IN SWEDEN (I)

For the purpose of analysing the reemergence of gonorrhoea in Sweden, clinical epidemiological data were collected and microbiological characterisation of isolated *N. gonorrhoeae* strains were performed. During the study period February 1998 to January 1999, 380 cases of gonorrhoea were reported to SMI and 370 *N. gonorrhoeae* strains (one strain from each patient) were sent to the National Reference Laboratory from the clinical laboratories in Sweden. In total, 393 cases of gonorrhoea could be identified by both report and isolated *N. gonorrhoeae* strain (n=357) or either of them (n=36). Thus, in 357 (91%) of the cases both epidemiological data and isolated strain were received and could be linked. The analyses in **paper I** were restricted to these 357 cases (see Fig. 7).

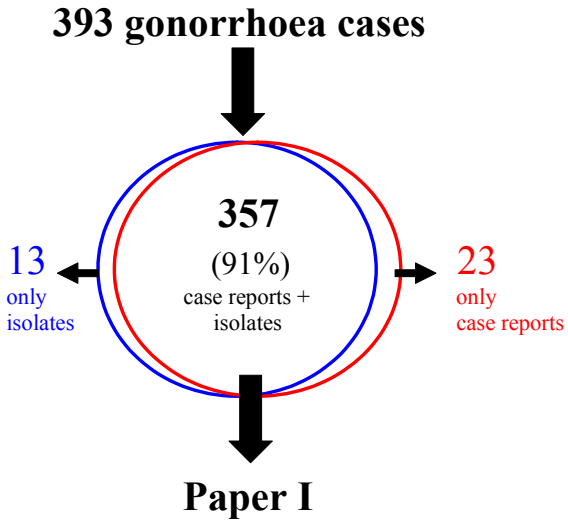

Figure 7. In paper I, 357 out of 393 identified gonorrhoea cases were included.

Serogroups and serovars

Serogroup WII/III (i.e. B serovars) was predominant with 90% of the cases, followed by WI (i.e. A serovars) with 10%. In total, 28 different serovars were identified.

Sex, route of transmission and country of exposure

Of all 357 cases, 60% were domestic which was significantly more than in 1997 (see Paper I, Table 1), with an increase of 76% ($p=0.01$). Only 17% of the cases were in women. Infected women were younger than both heterosexually and homosexually infected men ($p<0.001$), and the majority of the cases in women were domestic (83%). Men who have sex with men (MSM) accounted for 30% of all cases in men. Also in

MSM domestic cases dominated (77%), whereas the majority of heterosexual men were exposed abroad (53%). The increase during the study period compared with 1997 was consequently due to domestic cases of young heterosexuals of both sexes and MSM.

Domestic cases

Among domestic cases in heterosexual teenagers of both sexes, serovar IB-3 was most commonly isolated, independent of where in Sweden the patients were exposed. However, Stockholm County dominated with 66% of these cases. In MSM serovar IB-2 was the most frequent isolates in domestic cases, followed by the serovars IB-5 and IB-6 (see Paper I, Table 2). In all, 21 serovars were identified among domestic cases, 21 among heterosexual and 10 among MSM cases.

For domestic cases Stockholm was dominating as place of exposure (62% of the cases), followed by the Göteborg area (13% of the cases). The Stockholm dominance was seen especially in MSM, 82% of whom were exposed in the Stockholm area. During the study period, mostly single domestic cases were diagnosed in other counties of Sweden.

Imported cases

Among the cases exposed abroad heterosexual route of transmission was most common with 118 of 138 cases (86%). All continents except Australia were represented as areas of exposure. Almost half of the imported cases (47%) were exposed in Asia, the most prevalent country for exposure being Thailand (36 of 65 cases), followed by the Philippines (11 of 65 cases). Europe was reported as place of exposure in 47 cases (34%). Only nine cases (6.5%), all heterosexual, were infected in Eastern Europe including the Baltic States and other ex-Soviet Union areas. Homosexual exposure in men was reported only from Europe (14 cases) and America (4 cases). Among the *N. gonorrhoeae* strains isolated from cases exposed abroad, 25 different serovars were identified. Of imported cases, 7% reported sexual contact with a Swedish partner abroad.

VERIFYING EPIDEMIOLOGICAL CLUSTERS WITH PFGE (II)

In this study the genetic homogeneity/heterogeneity within the predominant domestic *N. gonorrhoeae* serovars, IB-2 and IB-3, which were identified in paper I, were explored by pulsed-field gel electrophoresis (PFGE) and the results were compared to the epidemiological information about the cases. The aim was also to examine the genetic diversity within and between 25 other represented serovars of *N. gonorrhoeae* in paper I.

In **paper II**, isolates of all cases infected with the two predominant serovars IB-2 (n=44) and IB-3 (n=84), as well as one to six isolates of the 25 remaining serovars (n=79) were analysed. In 15 of the cases one to two additional isolates were preserved at the same occasion but from different specimens and in 10 of the cases one to three

additional strains isolated at different occasions were preserved. These multiple isolates (n=30) were also included. Consequently, a total of 237 *N. gonorrhoeae* isolates were analysed (see Fig. 8).

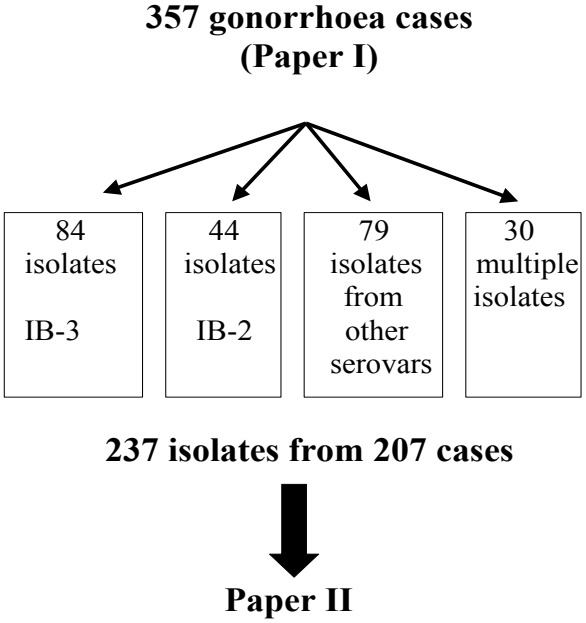

Figure 8. In paper II, 237 isolates from 207 cases analysed in paper I were included.

PFGE of IB-2 isolates

Among the IB-2 isolates (n=44), 12 distinguishable PFGE fingerprints were identified by using the *Spe*I restriction enzyme and 19 by using the *Bgl*II restriction enzyme (see Paper II, Fig. 1). *Spe*I identified one major clone consisting of 25 indistinguishable isolates (see Paper II, Fig. 2). Using *Bgl*II eight of the isolates were further discriminated and the major clone was reduced to 21 isolates (see Paper II, Fig. 2).

Most of these 21 indistinguishable isolates in the major clone were isolated from domestic cases in MSM (n=15). The remaining six IB-2 isolates were either from MSM cases exposed abroad (n=4) or from domestic cases in men reported to be heterosexually infected (n=2). Consequently, in 79% (15/19) of all domestic IB-2 cases in MSM the isolates were indistinguishable (see Fig. 9). The antibiotic susceptibility testing of the 15 indistinguishable isolates from domestic MSM cases showed similar MIC values, with differences within ±1 $\log_2$. Including patterns with one to three bands difference the major clone increased to 26 isolates (five isolates from domestic heterosexual cases were included).

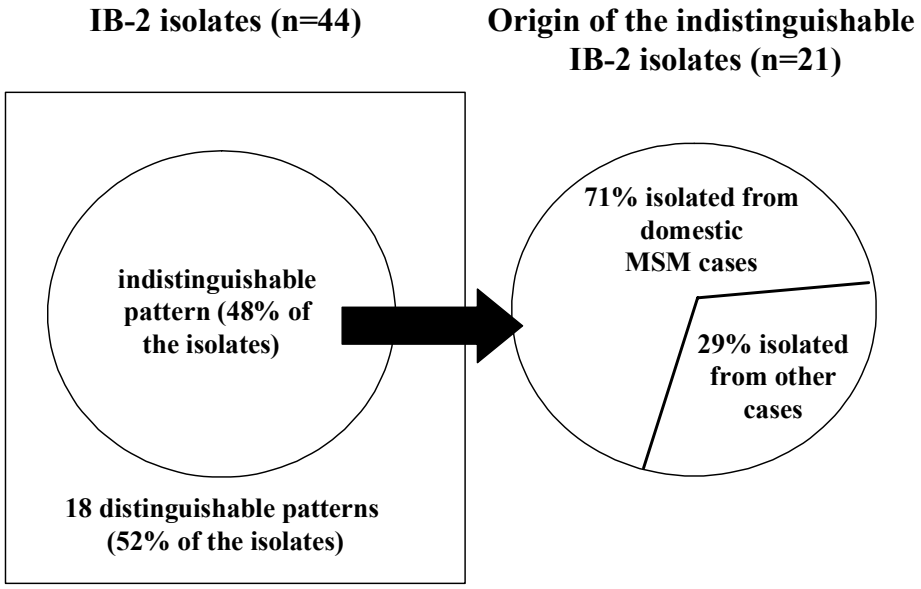

Figure 9. Genetic diversity of the IB-2 isolates (n=44) and origin of the indistinguishable isolates (n=21) belonging to the major clone.

PFGE of IB-3 isolates

The IB-3 isolates (n=84) showed 15 distinguishable fingerprints using either of the restriction enzymes (see Paper II, Fig. 3). *Spe*I identified one major clone of 57 isolates. Using *Bgl*II the major clone was reduced to 52 isolates including one isolate with unique pattern when using *Spe*I (se Paper II, Fig. 4). Consequently, 61% (51/84) of the IB-3 isolates showed the same indistinguishable restriction enzyme patterns when both enzymes were used separately.

Altogether 73% (37/51) of the indistinguishable isolates were isolated from domestic cases in young (<25 years of age) heterosexuals of both sexes. The rest of the major clone (27%) consisted of heterosexual cases exposed abroad (n=3) and domestic cases of heterosexuals (>=25 years of age (n=11)), see Fig. 10. Consequently, of all domestic IB-3 cases of young heterosexuals 66% (37/56) were infected with indistinguishable isolates. The antibiotic susceptibility testing of indistinguishable isolates from the 37 domestic cases of young heterosexuals showed similar MIC values, 32 of the 37 with differences within ±1 $\log_2$. Including patterns with one to three bands difference the major clone increased to 69 isolates and comprised isolates from domestic cases of young heterosexuals to 77%.

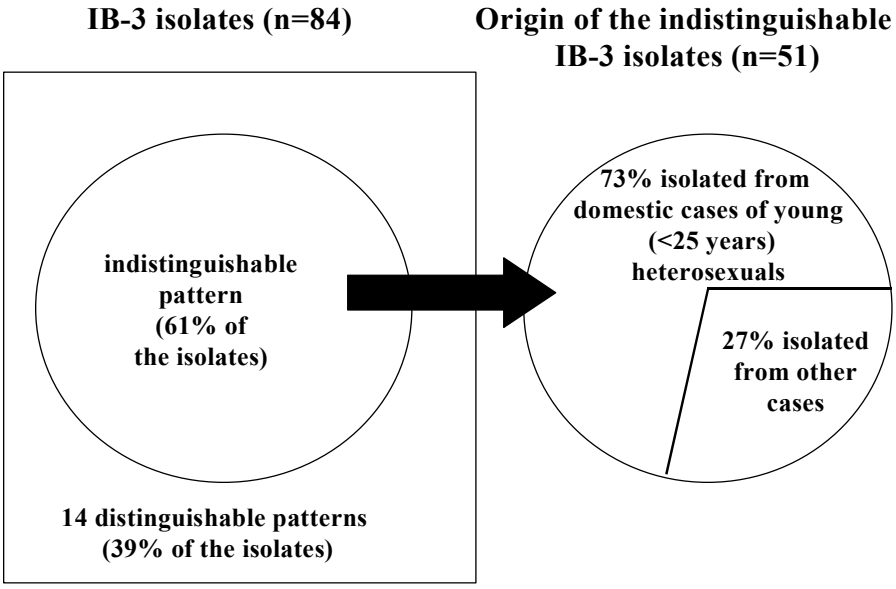

Figure 10. Genetic diversity of the IB-3 isolates (n=84) and origin of the indistinguishable isolates (n=51) belonging to the major clone.

PFGE of isolates of the 25 other represented serovars

A total of 79 isolates of 25 different serovars not belonging to the IB-2 and IB-3 serovars were also analysed with PFGE. With the *Spe*I restriction enzyme 50 distinguishable fingerprints were identified and with the *Bgl*II, 63 distinguishable fingerprints were identified.

However, six of the *Spe*I fingerprint patterns previously identified among the IB-2 isolates and one of the IB-3 isolates were also seen among the isolates of the 25 other represented serovars. Also, four of the *Bgl*II fingerprints of the IB-2 isolates were indistinguishable from some of the fingerprints of the isolates of the 25 other represented serovars.

PFGE of multiple isolates from the same patient

The genetic homogeneity/heterogeneity within multiple isolates preserved from the same patient on the same occasion or another occasion were also examined by PFGE. For 21 of the 25 patients from whom more than one isolate was obtained, all isolates were genetically indistinguishable by the PFGE with either of the two enzymes. This was in congruence with the results in the phenotypic characterization (serotyping and antibiotic susceptibility testing) of the isolates, except in one of the cases in which the phenotypic characterization identified one β-lactamase producing isolate and one β-lactamase negative isolate preserved on the same occasion.

One of the four remaining patients was infected with strains of two different serovars (IB-6 and IB-33), preserved at the same occasion. These strains had different genetic fingerprints by PFGE (more than seven-band differences, independently of enzyme used). From the three other patients, two strains of the same serovar (IB-7, IB-11 and IB-26 respectively) were isolated. In two of the cases they were preserved on the same occasion but from different specimens and in one of the cases the strains were isolated on different occasions. The two isolates of each of the three patients showed separately indistinguishable fingerprints with use of *Bgl*II and similar antibiograms. However, with use of *Spe*I the fingerprints of the two isolates from each patient were only closely related, with two- or three-band differences.

THE ONE-YEAR PREVALENCE STUDY OF ANTIBIOTIC SUSCEPTIBILITY (III)

In **paper III** epidemiological data from the 12-month gonorrhoea study in 1998–99 (papers I-II) were compared with antibiotic susceptibility patterns. For six of the 357 cases analysed in paper I the country of exposure was unknown and for three cases the route of transmission was unknown. Thus, 348 (89%) of all identified gonorrhoea cases during the 12-month period were analysed in this study (see Fig. 11).

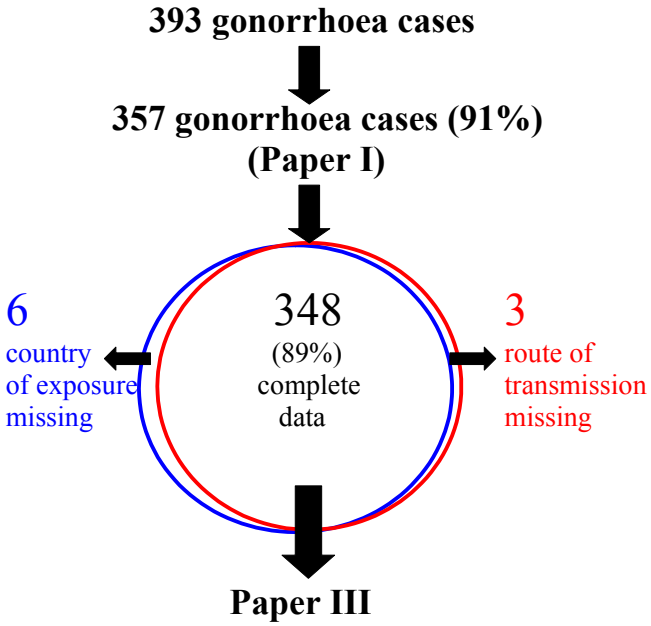

Figure 11. In paper III, 348 out of 357 gonorrhoea cases analysed in Paper I were included.

Country of exposure

The frequency of ß-lactamase producing *N. gonorrhoeae* isolates, i.e. penicillinase producing *N. gonorrhoeae* (PPNG), in the total material was 24% and decreased

susceptibility to ciprofloxacin (MIC>0.064 mg/l) 18%. The antibiotic susceptibility varied with the places where the patients were exposed. Exposure in Asia was dominating among patients with strains with decreased antibiotic susceptibility. The isolates with ß-lactamase production (PPNG) or decreased susceptibility to ampicillin, cefuroxime, erythromycin, tetracykline or ciprofloxacin were statistically correlated with exposure in Asia; relative risk (RR) 1.21 or higher, p=0.008 or lower (see Paper III, Table 1). The risk was especially high for ciprofloxacin resistant (MIC>0.38 mg/l) isolates which represented 34% of the isolates from patients exposed in Asia compared with 4% of the isolates from patients exposed in other places (p<0,001) (see Paper III, Table 1 and Figure 1). The most prevalent countries for exposure in Asia (n=65) were Thailand (n=36) and the Philippines (n=11).

N. gonorrhoeae isolates from 52 patients were defined as multiresistant (decreased susceptibility to ciprofloxacin MIC >0.064 mg/l, cefuroxime MIC>0.064 mg/l and tetracycline MIC>0.125 mg/l). These multiresistant isolates were PPNG in 46% and they were mostly isolated from patients exposed in Asia (33 of 65 Asian exposed patients, i.e. 51%) (95% CI 38%–63%), or from patients exposed within Sweden (16 of 212 patients exposed in Sweden, i.e. 8%) (95% CI 4%–12%).

Only a few patients were exposed in Africa (n=12). No one of the *N. gonorrhoeae* strains isolated from these patients were resistant to ciprofloxacin, but the isolates showed a high frequency, 67% (95% CI 35%–90%), of resistance to tetracycline.

Route of transmission

When comparing heterosexual women and men (n=262) with MSM (n=86), who all were exposed in Sweden, Europe (excluding Sweden) or America, no difference in antibiotic susceptibility patterns was seen. No MSM was exposed in Asia, so among these men ciprofloxacin resistance or multiresistance was an unusual finding. The differences in antibiotic susceptibility pattern were subsequently dependent on the country of exposure.

Serovars

Decreased antibiotic susceptibility was seen in several serovars exemplified by PPNG in 19 of the 27 different serovars and decreased susceptibility to ciprofloxacin in 17 of the 27 different serovars among the 348 isolates. The 52 identified multiresistant *N. gonorrhoeae* isolates mentioned above, belonged to 16 different serovars. Almost half of these multiresistant isolates (25 of 52) belonged to the serovars IB-1 or IB-4, which were the only serovars with any relation to multiresistance. Most of these patients were exposed in Asia or Sweden (88%). Of the total of 348 studied *N. gonorrhoeae* strains 51 (15%) belonged to the serovars IB-1 or IB-4.

THE 15-YEAR LONG-TERM STUDY (IV)

In this long-term study, 1039 *N. gonorrhoeae* isolates from 840 gonorrhoea episodes representing 721 patients were included. All patients were MSM visiting Venhälsan at

Södersjukhuset 1990–2004. In the text below, each diagnosed gonorrhoea episode is defined as a "case".

Annual variations

During the 15-year study period, the number of gonorrhoea cases increased more than ten times. The number of cases during the 1st half 1990–1997 varied from 13 to 26 cases per year (mean 15). During the 2nd half of the study period 1998–2004 the cases varied from 36 to 158 (mean 102 per year). This was a significant increase ($p= 0.0018$) of the mean number of cases during the 2nd part of the study.

Re-infections

Thirteen percent (93 patients) of all patients were re-infected in the same year (36 patients) or another year during the study period, i.e. they were again positive for *N. gonorrhoeae* the same year or another year after completed treatment and a negative test-of-cure, or they were positive for *N. gonorrhoeae* with another serovar or antibiogram in test-of-cure. The majority of these re-infected patients were only re-infected once during the 15-year period, but 19 patients were re-infected twice to five times. These re-infections were counted as new cases in the study.

Site of infection

The distribution of *N. gonorrhoeae* positive isolates (n=1039) from different sites in all 840 cases was: 48% urethra, 42% rectum and 34% pharynx. In 181 cases (22%) strains were isolated from more than one anatomical site at the same time. Of all 840 cases 34% were only positive in isolates from the urethra, 27% in isolates from the rectum and 17% in isolates from the pharynx.

There was a change over time in the distribution of site of infection. The proportion of cases with positive isolates from the pharynx increased significantly ($p<0.001$) from 15% in the 1st period 1990–1997 to 38% of the cases during the 2nd period 1998–2004. During the same time periods the proportion of cases with positive isolates from the urethra and the rectum decreased, but not significantly (see Table 1).

Table 1. Distribution of site of infection including proportion (%) of cases by site of infection

	cases (isolates)	rectum	pharynx	urethra	eye
1990–1997	123 (142)	57 (46%)	19 (15%)	66 (54%)	0
1998–2004	717 (897)	292 (41%)	270 (38%)	334 (47%)	1
Total	840 (1039)	349 (42%)	289 (34%)	400 (48%)	1

HIV serostatus

The number of HIV-positive men diagnosed with gonorrhoea increased during the 15-year period, from 3 cases in 1990 to 16 cases in 2004. However the proportion of HIV-positive men among all the gonorrhoea cases decreased from as much as 50% (95% CI= 21–79) in 1991 to 11% (95% CI=6–17) in 2004 (see Paper IV, Figure 1).

Ten percent (88/840) of all gonorrhoea cases were seen in HIV-positive patients, of whom 25% (22/88) were diagnosed with HIV and gonorrhoea at the same time. This proportion increased significantly (p=0.038; Fisher's exact test) from 11% during the 1st half of the period (1990–1997) to 32% during the 2nd half of the period (1998–2004).

Sixteen of 22 cases (73%) diagnosed with HIV and gonorrhoea at the same time were positive for gonorrhoea in the rectum, which was a significantly higher proportion (p=0.029; Fisher's exact test) than among the other cases who were HIV-negative or were previously diagnosed with HIV infection. Among the 66 cases with a previously diagnosed HIV infection there was a significantly higher proportion (61%; p=0.015; Fisher's exact test) who were positive for gonorrhoea in the urethra than among the other cases.

Distribution of serovars

When analysing the distribution of serovars, 2 isolates from 2 patients were excluded since the serovars were not determined. Also, 178 isolates from patients diagnosed at one time with multiple identical serovars from different sites were excluded. In 17 cases the patients were diagnosed at one time with 2 different serovars in isolates from different sites and in 2 cases the patients were diagnosed with 3 different serovars from different sites. In total 859 isolates from 838 cases were included in this part of the analysis.

A total of 66 serovars was observed during the 15-year period. Serogroup WII/III (i.e. B serovars) was the most frequent representing 94% of the serovars, while serogroup WI (i.e. A serovars) only represented 6% of the serovars or 1% (9/838) of all cases.

No serovar was present throughout all 15 years, but 5 serovars were present during a period of 11 to 13 years. These 5 serovars represented 62% of all 859 isolates. Almost half (32/66) of the serovars were only present during one single year and most of these (27/32) were only diagnosed in one case each. The number of new serovars that were introduced (not seen before in this study) varied from 0 to 7 serovars per year (mean 4 serovars per year). For the annual distribution and number of serovars, see Paper IV, Table 1.

PPNG strains

Only 30 (2.9%) of the isolates were penicillinase (β-lactamase) producing *N. gonorrhoea* strains (PPNG), representing 26 cases, i.e. 3.1% of all cases. PPNG was seen in 10 different serovars. The serovar Boys was the most frequent in PPNG cases,

represented by 15 cases, i.e. 52% (15/29) of all cases infected with that specific serovar during the 15-year period were PPNG. Fourteen of these 15 cases were diagnosed 1999–2002, indicating an endemic spread of this specific PPNG strain in the MSM population in Stockholm during a limited period. The other 11 cases of PPNG occurred sporadically in 9 different serovars between 1993 and 2001.

THE PREVALENCE STUDY OF PHARYNGEAL GONORRHOEA AND CHLAMYDIA

In total 475 MSM visiting Venhälsan at Södersjukhuset for an HIV test or STI tests during a 4-month period were offered to join the study and 443 (93%) accepted and were included. The median age was 33 years (range 16–69 years). The reason for visiting the clinic was solely to have an HIV test in 34% of the men.

A total of 94% of the 443 men reported oral–genital sex (i.e. giving fellatio), of whom 6% (24/415) had used condom when having that sexual practice, 60% reported oral–oral sex (i.e. deep kisses) and 37% reported oral–anal sexual contact (i.e. giving "rimming"). The median time since the last oral–genital contact (n=409) was 12,5 days (range 0–548 days), for the last oral–oral contact (n=270) the median was 8 days (range 0–365 days) and for the last oral–anal sexual contact (n=169) the median was 17 days (range 0–1460).

Of all men sampled for *N. gonorrhoeae* in the pharynx, 5.2% (23/440) had a positive test result. That was about the same level as in those who were positive for *N. gonorrhoeae* in the urethra (5.3%; 14/265) and the rectum (6.0%; 12/200). However, only 13% (3/23) of those who were positive in the pharynx had symptoms from that site, whereas 25% (3/12) and 86% (12/14) of those who were positive in the rectum respectively the urethra had symptomatic infections. As many as 65% (15/23) of MSM who were positive in the pharynx were positive in isolates from that site only.

The majority (22/23) of the men who were positive for *N. gonorrhoeae* in the pharynx reported oral–genital sex 4–90 days (median 10) before the positive test, 13/23 (57%) reported oral–oral contact 1–18 days (median 10) before and 5/23 (22%) reported oral–anal sexual contact 5–90 days (median 14) before the positive test.

In total 39 men were positive for *N. gonorrhoeae* irrespective of site of infection and six of them (15%) were co-infected with other STIs; four with chlamydia, one with syphilis and one with HIV.

Of the MSM who were sampled for *Chlamydia trachomatis* in the pharynx, 1.4% (6/414) were positive. That was almost similar to the positive findings of *C. trachomatis* in urine (1.1%; 2/187) and the urethra (1.8%, 2/114) but lesser than the positive findings in the rectum (4.2%; 8/192). All six men who were positive for *C. trachomatis* in the pharynx were positive in isolates from that site only. Five out of six men reported oral–genital sexual contact 0–13 (median 7) days before the positive test, 3/6 reported oral–oral contact 0–10 (median 1) days before and 2/6 reported oral–anal

sexual contact 0–1 day before the positive test. In total 17 MSM were positive for *C. trachomatis* irrespective of site of infection and five of them (29%) were co-infected with other STIs; four with gonorrhoea and one with syphilis.

THE SEXUAL BEHAVIOUR STUDY

The median age of the men was 35 years in the 1997–1998 sample (n=293) and 33 years in the 2001–2002 sample (n=321). For more characteristics, see Table 2.

When comparing the results from the two study periods 1997–1998 and 2001–2002, there was a significant increase ($p<0.001$) in the reported number of sexual partners during the last 12 months, from 6 to 10 partners in median. There were also differences regarding where the men usually meet their sexual partners. The proportion of men who had used Internet for getting into contact with a sexual partner increased significantly ($p<0.001$) from 24% to 68%, while other venues decreased significantly such as telephone hotlines ($p<0.05$), personal advertisement ($p<0.001$), public baths ($p<0.001$), restaurants/cafés ($p<0.001$) and other public venues ($p<0.001$). However, there were no significant differences over time when meeting a sexual partner at discotheques, bars and blue movies.*)

Table 2. Age and sexual behaviour in MSM visiting Venhälsan 1997–1998 compared with 2001–2002

	1997–1998	**2001–2002**	**p-value**
Age:	(n=293)	(n=321)	
Median age (range)	35 (18–75)	33 (17–77)	n.s.
Sexual lifestyle at present:	(n=294)	(n=321)	
a) Sex with men only	87%	91%	n.s.
b) Sex with both men and women	13%	9%	n.s.
Type of sexual partner(s) last month:	(n=294)	(n=319)	
a) No partner(s)	9%	7%	n.s.
b) Steady partner only	25%	29%	n.s.
c) Steady partner and casual partner(s)	25%	23%	n.s.
d) Casual partner(s) only	41%	41%	n.s.
Number of sexual partners last 12 months:	(n=275)	(n=316)	
Median number (range)	6 (0–160)	10 (1–150)	p<0.001
Sexual practice with casual partner last month:	(n=194)	(n=203)	
a) Unprotected oral-genital sex	65%	75%	p<0.05
b) Unprotected genital-oral sex	70%	83%	p<0.01
c) Unprotected receptive anal intercourse	8%	10%	n.s.
d) Unprotected penetrating anal intercourse	6%	12%	n.s.

*) Gay saunas were prohibited in Sweden by law (SFS 1987:375) in 1987 with reference to the assumed increased risk of HIV spread in that kind of venue. This law was repealed in July 2004 (SFS 2004:177).

In total 66% of the respondents in 1997–1998 and 63% in 2001–2002 reported sexual contacts with casual partners the last month and 50% respectively 52% reported sexual contact with a steady partner.

The majority of men who reported sex with casual partners the last month had practiced oral sex without condom (83% in 2001–2002). Some men also reported anal intercourse without condom with a casual partner the last month (12% in 2001–2002). There was a significant increase ($p<0.05$) in men having casual partners who reported unprotected oral–genital sex (i.e. giving fellatio) the last month, as well as in men having casual partners who reported unprotected genital–oral sex (i.e. getting fellatio) the last month ($p<0.01$). There were also increases in men reporting unprotected receptive or penetrating anal intercourse with casual partners the last month, but these increases were not statistically significant, see Table 2.

The respondents also had to estimate the risk of HIV transmission in different sexual practices. The majority of the respondents considered that there was a risk when having genital–oral sexual practice (i.e. getting fellatio) without condom. However, the proportion of men who considered this sexual practice as no risk of HIV transmission increased significantly ($p<0.001$) from 17% in 1997–1998 to 34% in 2001–2002. Almost all respondents (98% respectively 97%) considered giving oral–genital sex without condom as a risk if you get semen in the mouth, but if you avoid getting semen in the mouth, there was actually a significant increase ($p<0.001$) from 5% to 14% in men who considered that sexual practice as no risk of HIV transmission. Regarding anal intercourse without condom there were no differences in the two study periods – almost all men (ca. 98%–99%) considered such sexual practice as a risk of HIV transmission irrespective of the receptive or penetrating role.

The proportion of respondents who reported having been diagnosed with one or several STIs the last five years increased significantly ($p<0.01$), from 16% in 1997–1998 to 26% in 2001–2002. The most common STIs in both study periods all together were gonorrhoea (45%) and condyloma (42%) followed by chlamydia (27%).

DISCUSSION

During a quarter of a century the Swedish gonorrhoea incidence was successively reduced from about 40,000 cases in 1970 to an all-time low of only 211 reported cases in 1996. Then the incidence started to increase again and has increased more than 3 times up to 2005, but still the prevalence in the population is low. However, gonorrhoea is a very common STI in many countries in the world. Furthermore, the increasingly high incidence of chlamydia in Sweden, with the highest incidence in young adults (< 25 years), shows the potential risk of increasing gonorrhoea rates to much higher levels than today, which may lead to increasing rates of PID, etopic pregnancy and infertility. Also, the increasing antibiotic resistance in gonococci and the fact that gonorrhoea increases the risk of sexual transmission of HIV [4, 5], makes gonorrhoea a disease of great concern.

ENDEMIC CORE GROUPS AND IMPORTATION

The increase of gonorrhoea in Sweden since 1997 is mostly due to an increase of domestic cases. In **paper I** it was shown that the domestic cases in 1998 could be divided into two main groups: young heterosexual men and women, mainly in their teens, infected by *N. gonorrhoeae* strains of serovar IB-3, and MSM, mostly infected by *N. gonorrhoeae* strains of the serovar IB-2, but also by strains of other serovars. This study also indicated that several *N. gonorrhoeae* clones were endemic among MSM in Sweden at the same time. The clear preponderance of serovar IB-3 among heterosexual teenagers indicated an introduction and spread of one clone of *N. gonorrhoeae* in this young population with no earlier exposure to gonorrhoea. Most cases of gonorrhoea among teenagers were diagnosed in the large cities of Sweden, but during 1998 a spread to several counties was seen. At least in some cases, a sexual contact in Stockholm was identified for the primary case in small towns.

Further studies of these strains with PFGE together with background epidemiological and microbiological data, indicated that the spread of one single *N. gonorrhoeae* clone of the serovar IB-2 and another single clone of IB-3 were responsible for the majority of cases in the two identified endemic core groups. Most of the domestic IB-2 cases (79%) in MSM and the majority of the domestic IB-3 cases (61%) in young heterosexuals belonged to either of these two clones (**paper II**). These findings were also in congruence with the results from sequence analyses of the *porB* gene that were performed in the same isolates [72].

The spread of serovar IB-3 among young heterosexuals stopped in 1999 but during the 2000s the increase of domestic cases in young heterosexuals and in MSM has continued in Sweden, especially in Stockholm. Endemic spread is also seen in Gothenburg, Malmö and sporadically in other areas. For example an outbreak in Gävle-Sandviken area (Gävleborg County) in 2003, which included some 25 heterosexual teenagers and young adults, was caused by one specific serovar resistant to ciprofloxacin. Probably this strain originally was imported from Thailand, where

resistance to quinolones in *N. gonorrhoeae* is high-prevalent [90, 91]. In **paper III**, such resistance was common among *N. gonorrhoeae* isolates from Swedish cases exposed in Thailand (see below).

In **paper I** it was shown that the majority of MSM were exposed in Sweden as well as the majority of the infected women. This was in contrast to heterosexual men, only half of whom were exposed in Sweden. Thailand was the most common country when exposed abroad. This is in concordance with the Swedish surveillance data from the following years. Obviously, some of the heterosexual men infected abroad pass on their infection to partners in Sweden, which in some cases may lead to further endemic transmission. However, the big difference in the male/female ratio – even if MSM are excluded the ratio still is >2.0 – indicates that most imported cases among heterosexuals did not result in further spread of gonorrhoea in Sweden, which is in agreement with earlier experiences from Sweden in the 1990s [28]. A reason for this may be that these men were aware of their risk behaviour – or had symptoms – and early after arrival to Sweden they attended medical examination for diagnostic sampling, effective antibiotic treatment and, if any sexual contacts in Sweden, partner notification.

Homosexual contacts in men were reported only from Sweden, Europe and America, while heterosexual contacts also were reported from Asia and Africa. Only a few cases of gonorrhoea were exposed in Eastern Europe including the Baltic States and other ex-Soviet Union areas **(paper I, III)**. From these regions nearby Sweden, a sharp increase in gonorrhoea and other STIs was reported in the 1990s [17, 19, 92], but this increase has not had any great impact on the Swedish gonorrhoea epidemiology [38]. Most imported gonorrhoea cases were exposed in Asia which may be due to sexual contacts with commercial sex workers in these areas. Sexual contacts abroad can of course also comprise contacts with other Swedish citizens on holidays or living abroad, which is important to observe with regard to partner notification. The nationalities of the sexual contacts were reported in 66% of the cases exposed abroad included in **paper I**, but only 6 of 91 partners were Swedish. In a retrospective study of all cases reported to SMI in 2001 with gonorrhoea or/and chlamydia contracted in Thailand, the majority (77%) of the men infected by a casual partner were exposed to persons from Thailand, while the majority of the women infected by a casual partner were exposed to men from Europe or USA [93]. Sexual risk behaviour in international travellers and in young people visiting international tourist resorts abroad has been pointed out as a substantial conduit for transmission of STIs between countries and continents [94, 95].

In **paper IV**, the spread of gonorrhoea in one of the endemic core groups, MSM in Stockholm, was studied during 1990–2004. Surveillance data from the CMO in Stockholm showed that the majority of the gonorrhoea cases in MSM were domestic. Contrary, the great variation of serovars and the continuing introduction of new serovars during the whole 15-year period indicate an ongoing importation of *N. gonorrhoeae* strains and, in most cases, rapid elimination in line with previously published experiences in Sweden [96]. However, by extending the study period to 15 years, we could demonstrate that several serovars really became endemic in the MSM

group. Four predominant serovars contributed to more than 100 cases each over more than a decade.

The reasons for this variation in serovars over time and greater success for some serovars in becoming endemic are not clear. The existence of serovars-specific immunity has previously been suggested but not found evident by others [47]. The four most common persistent serovars in **paper IV** were present almost every year of the study, but only during the last five years did they occur in clusters of 20–40 cases per year, indicating possible outbreaks of micro-epidemics caused by these serovars. However, it has been shown by more discriminating molecular typing methods that one serovar can represent several different genetic clones as demonstrated in **paper II** and by others [77, 97, 98]. It is not unlikely that these persistent serovars in MSM in Stockholm may have been imported and re-introduced into this core group several times during the 15-year period. Compared to a previous series of isolates, six of the 10 most common serovars in our study belonged to the 10 most common serovars in the general population in Stockholm 1988–89 [99]. Two of the four serovars found to be associated with transmission in MSM in Edinburgh, Scotland, in 1986–90 were also among the four most common in our study [100]. This may indicate that some serovars are more frequent than others in MSM populations, irrespective of time period and country.

TYPING METHODS

By using PFGE we detected two major clones of domestic cases in MSM respectively young heterosexuals, which belonged to seovars IB-2 and IB-3. Apart from these two major clones we identified a high genetic diversity within other isolates of these two preponderant serovars (**paper II)**. This heterogeneity within serovars was also identified among the 25 other included serovars. According to these results PFGE fingerprinting is a sharper and more discriminating epidemiologic tool than the routinely used serological typing. This is in full agreement with previous studies [82, 83, 87].

Overall, the PFGE, independent of restriction enzyme used showed a high reproducibility and typeability (all 237 isolates were typeable). In the comparison between the two restriction enzymes used in the PFGE, *Bgl*II showed a greater discriminatory ability than *Spe*I especially for IB-2 isolates and the majority of the other represented serovars. For IB-3 isolates the major clone was further discriminated using *Bgl*II but overall the two restriction enzymes had an approximately equal discriminatory ability on the IB-3 isolates. Overall, *Bgl*II seems to be the best choice of restriction enzyme to use in PFGE on *N. gonorrhoeae* isolates which is in accordance with the findings in a previous study [88]. However, the *Bgl*II fingerprints contained more bands and were in a few cases more difficult to interpret.

The use of gonococcal serovars is still important as a primary epidemiological marker to detect possible endemic spread in specific core groups or geographical areas (**paper I, IV**). The serovar determination system seemed reliable in that only 19 of 181 patients diagnosed with isolates from more than one location at the same time were infected with isolates of different serovars (**paper IV**). This is also in concordance with earlier studies [101]. Fifteen of the 19 patients had serovars that differed in more than one

reaction with the monoclonal reagents. Thus, it is likely that most of these 19 patients actually were infected by multiple strains which sometimes occur. Similarly, this was also shown in **paper II** by using PFGE in one case diagnosed with multiple isolates at the same time.

However, as shown in **paper II**, one serovar can represent several different genetic clones. PFGE or other molecular methods could, when required, be used to improve the discriminatory ability within serovars. Thus, by PFGE it is possible to confirm presumed epidemiological connections in transmission chains or outbreaks or discriminate isolates of suspected clusters. However, even minor genetic changes of the bacterial chromosome, i.e. point mutations, can cause large differences in the genetic fingerprints of isolates. This makes PFGE, and other genetic typing methods, not suitable as the one and only method for strain characterization and less suitable for long-term epidemiology [44, 77]. More knowledge is needed about genetic relatedness in *N. gonorrhoeae* strain populations in the community, temporal and geographic changes and evolutionary changes over time in the *N. gonorrhoeae* genome.

ANTIMICROBIAL SUSCEPTIBILITY AND CHOICE OF TREATMENT

Culture diagnosis with early and effective antibiotic treatment is a cornerstone in the control of gonorrhoea. For this reason the antibiotic susceptibility pattern has to be studied for individual isolates as well as by surveillance studies [102-104]. The antibiotic susceptibility varies between different parts of the world with, since decades, special focus on antibiotic resistance in South-East Asia. This is in accordance with the results presented in **paper III**. The antibiotic resistant isolates were correlated to place of exposure but not to route of transmission, i.e. homo- or heterosexual contacts, or serovar. Multiresistance (decreased susceptibility to ciprofloxacin, cefuroxime and tetracycline) was seen in 16 different serovars, mostly in patients exposed in Asia.

In Sweden, as well as in several other countries, ciprofloxacin has been the recommended first drug of choice for treatment of gonorrhoea since the early 1990s. However, when exposed in Asia, 63% of the *N. gonorrhoeae* isolates studied in **paper III** were not fully susceptible to ciprofloxacin, compared with 7% when exposed somewhere else. When exposed in Africa, all cases were susceptible to ciprofloxacin which is in concordance with other studies and may reflect that this drug is not commonly used in this part of the world [105]. From a microbiologic point of view, it would be advisable to use another drug as the first drug of choice, if the patient is exposed in Asia, before antibiotic susceptibility pattern is analysed. Even when exposed within Sweden, 8% of the *N. gonorrhoeae* strains showed decreased sensitivity to ciprofloxacin.

In MSM multiresistance and decreased susceptibility to ciprofloxacin was an unusual finding in **paper III**, which could be explained with that fact that no MSM was exposed in Asia. This is in concordance with the experiences from gonorrhoea in MSM diagnosed at Venhälsan during the 1990s; ciprofloxacin resistance was very unusual [64] and PPNG strains did only occur in <3% of the isolates in the long-term study 1990–2004 (see p. 41). However, in 2003 a huge increase of ciprofloxacin resistance isolates was noted at Venhälsan. More than 50% of MSM diagnosed with gonorrhoea were infected with *N. gonorrhoeae* strains resistant to ciprofloxacin. Most of them were domestic cases infected with the same serovar (Bropyst) which indicated an outbreak caused by a resistant strain circulating among MSM in Stockholm [64]. In 2003 an

outbreak of ciprofloxacin resistance gonorrhoea in heterosexual teenagers and young adults also was reported from Gävle-Sandviken [90]. Increased occurrence of ciprofloxacin resistant strains has recently been reported from several other countries in Europe [106-110] as well as Australia [111] and USA [112, 113].

In the annual report of *Neisseria gonorrhoeae* isolates analysed in 2005 at the Swedish National Reference Laboratory for Pathogenic Neisseria, Örebro University Hospital [70], which also included all isolates analysed at Karolinska University Hospital Huddinge, Stockholm, the proportion of isolates with decreased susceptibility to ciprofloxacin was as high as 49% compared with 18% in the national prevalence study from 1998 (**paper III**). Obviously, a shift in the susceptibility to ciprofloxacin has occurred in the 2000s in Sweden and other countries in Europe, which makes ciprofloxacin no longer useful as a first choice treatment.

Also, site of infection should be regarded when choice of treatment is considered. In **paper IV**, 34% of all the cases positive for *N. gonorrhoeae* were infected in the pharynx and as many as 17% of all cases were only positive in isolates from this site. In a screening study at Venhälsan of pharyngeal gonorrhoea and chlamydia in MSM (see p. 42), the prevalence of gonorrhoea in the pharynx was 5.2% which was about on the same level as the prevalence in the urethra and the rectum. It is important findings in consideration of screening, choice of treatment and test-of-cure, since pharyngeal as well as anorectal infections are more difficult to treat and the choice of antibiotics is crucial [65]. Furthermore, pharyngeal gonorrhoea, as well as pharyngeal chlamydia, is most often asymptomatic or subclinical, as shown in the screening study at Venhälsan where only 13% of the patients infected with gonorrhoea in the pharynx had symptoms and 25% of the patients with rectal infection while 86% with urethral infection had symptoms. This underlines that it is not acceptable to only use urine or urethral specimens and DNA amplification methods as diagnostic method in screening in MSM. Almost half of the cases (44%) in our study would have been missed in that case.

In a Swedish context, cefixime (an oral third-generation cephalosporin) and spectinomycin (a parenteral aminocyclitol) are preferable choice of treatment if the patient has an uncomplicated urogenital gonorrhoea. All strains studied in **paper III** were fully susceptible to spectinomycin and during 2000–2005 no resistance to this drug was detected at the Swedish National Reference Laboratory [70]. During the same time, only a few isolates with decreased susceptibility to cefixime were detected at the Reference Laboratory. Nevertheless, resistance to spectinomycin has been reported in some cases from China and the South Pacific Region [104, 114] and cases with decreased susceptibility to cefixime has recently been reported from USA and Japan [115, 116].

Also, the treatment effect on pharyngeal gonorrhoea is not sufficient when using spectinomycin [117] and not evaluated when using cefixime. Therefore ceftriaxone (a parenteral third-generation cephalosporin) should be used for treatment of pharyngeal infections. All strains in **paper III** were fully susceptible to this antibiotic with very low MIC values, and only a few sporadic isolates with decreased susceptibility have been detected 2000–20005 at the National Reference Laboratory [70].

Azithromycin (an oral macrolide) has been suggested as an alternative if a oral treatment is preferred [118, 119]; only two cases in **paper III** were infected with a strain with decreased susceptibility to this drug. However, azithromycin is not recommended in international treatment guidelines for gonorrhoea [60, 120], because the 1-g. dose (recommended for treatment of chlamydia) has low efficacy for gonorrhoea and the 2-g. dose causes gastrointestinal intolerance in many patients [121]. Reduced susceptibility to azithromycin is common in Latin American countries [122-124] and has also been reported from other countries, e.g. USA and Canada [121, 125]. Furthermore, in 2004 the first domestic Swedish transmission chain of an azithromycin-resistant strain was detected in MSM in Stockholm [126].

Due to the great variety of different antibiotic sensitivity patterns among *N. gonorrhoeae* isolates, it is in a low-prevalent country such as Sweden not acceptable to use direct microscopy or DNA amplification methods as the only diagnostic methods for gonorrhoea. These methods are today not able to detect any antibiotic susceptibility patterns or give important epidemiological information about strain populations or emergence and spread of different strains in the community. It is of utmost importance to use a method with high sensitivity for culturing *N. gonorrhoeae*, to use antibiotic susceptibility testing of the isolated *N. gonorrhoeae* strains, to use a first choice of antibiotic with a high rate of susceptible *N. gonorrhoeae* strains and to change antibiotic treatment when indicated by the microbiologic analysis. The fact that resistant strains are domestically transmitted in Sweden [64, 126] underlines the need for continuous surveillance of the antibiotic susceptibility patterns in *N. gonorrhoeae* in order to identify emergence and endemic spread of new resistance, as well as to perform clinical treatment studies, to keep the antibiotic treatment recommendations up to date.

HIV STATUS AND SEXUAL RISK BEHAVIOUR

The decline in gonorrhoea during the AIDS era in the 1980s and early 1990s has been assumed to indicate changed sexual behaviour with a reduced risk of HIV transmission. The increase in gonorrhoea seen in many Western European countries during the 2nd half of the 1990s and the beginning of the 2000s [23], may therefore indicate that guidelines for safer sex have become more difficult to adhere to as the public awareness of the risk for HIV has waned during a post-AIDS era after introduction of highly active antiretroviral therapy (HAART) in the Western industrialized countries. Especially in MSM the introduction of HAART has been assumed to have influenced the increase of STIs and sexual risk behaviour, as reported in several studies in Europe and USA [30, 128-130]. Repeated studies of sexual behaviours and attitudes in the general Swedish population from 1989 and forward show an increase during the 2000s in men and women who reported unprotected intercourse with a new partner already the first night, and also an increase in young men and women (16–19 years of age) who reported more than three sexual partners during the previous three months [127].

The significant increase of gonorrhoea in MSM in Stockholm during the 2nd half of the 15-year study presented in **paper IV** is in concordance with the trends in many other Western countries [23, 36]. In this long-term study, 10% of all gonorrhoea cases were

seen in HIV-positive men of whom one out of four was not previously diagnosed with HIV. This proportion of HIV-positive MSM at Venhälsan was similar to the Gay men's health center in San Francisco where 10% of the gonorrhoea patients in 2003 were HIV-positive, but lesser than San Francisco's municipal STI clinic where 21% of the gonorrhoea cases in MSM were HIV-positive [131]. A high proportion of HIV infection in gonorrhoea patients has also been reported from other Western countries [8, 9]. This suggests that gonorrhoea diagnosis in MSM is an important risk marker for HIV, i.e. HIV test should always be offered to these patients if not already known to be HIV-positive.

We also found that the proportion of gonorrhoea cases that were diagnosed with previously not known HIV infection increased significantly during the 2nd half of the study, whilst the significant difference seen when comparing the distribution of serovars among HIV-positive and HIV-negative cases was not present during the 2nd half of the study. Also, there was a significantly higher proportion of rectal gonorrhoea in cases diagnosed with a previously unknown HIV infection compared to the other cases. It has been shown by others that rectal gonorrhoea is independently associated with risk of HIV seroconversion [132]. These findings may indicate an increased risk of HIV spread in MSM in Stockholm during the later years of the study with consideration of gonorrhoea as a cofactor of HIV transmission.

However, the rates of reported HIV diagnosis in MSM did not increase in Stockholm or in Sweden during the 15-year period according to surveillance data from SMI and the CMO in Stockholm County [133, 134]. The finding that the proportion of pharyngeal infections increased significantly during the 2nd part of the study period while the proportion of anorectal infections decreased indicates that the increase of gonorrhoea in our study rather was associated with unprotected oral sex – with a lesser risk of HIV transmission – than with an increase of high risk sexual behaviour. Similar findings in MSM cohorts have recently been reported from the Netherlands and Germany [135, 136]. A rise in the incidence of gonorrhoea in MSM may not necessarily indicate an increase in high-risk sexual behaviour for HIV transmission. It could also reflect changes in sexual networks and an increase in the number of sexual partners that may also affect the incidence of gonorrhoea in specific core groups. It has also been remarked that rectal gonorrhoea in MSM not always is caused by unprotected anal intercourse; the route of transmission sometimes is oral–anal or digital–anal contact [45, 137].

Results from the study of sexual behaviours and attitudes in MSM who visited Venhälsan for HIV and/or STI tests in 1997–1998 compared with 2001–2002 (p. 43), shows that the proportion of men who considered unprotected genital–oral respectively oral–genital sex (without getting semen in the mouth) as no risk of HIV transmission increased significantly, while there was no such change in the attitudes towards unprotected anal intercourse, which was considered as a risk by almost all the men. Furthermore, there was a significant increase in men who reported unprotected oral–genital sex respectively genital–oral sex with casual partner(s) during the last month, but only a small, not significant, increase in men who reported unprotected anal

intercourse with casual partner(s). These changes in attitudes and sexual practices together with the finding that the reported median number of sexual partners during the last 12 months increased significantly from 6 to 10 partners, might be essential factors for the increasing rates of gonorrhoea in MSM in Sweden. For infections like gonorrhoea, with high transmission probability, the number of sexual partners is more important for the spread in the community than the number of sex acts with each partner.

Furthermore, Internet communities and chat rooms have emerged as new venues for meeting sexual partners which also were shown in the sexual behaviour study from Venhälsan. In another Swedish study it was found that MSM who have experience in visiting chat rooms on the Internet were less likely to be tested for HIV and more likely to have had unprotected anal intercourse [138]. Other studies have reported more previous STIs and more partners in persons seeking sex via the Internet, than those not seeking sex via the Internet [139]. Chat rooms and other Internet communities where people meet sex partners are important media to be used in STI and HIV prevention, especially since it might be possible to reach core groups and key persons in sexual networks with information. Such venues have also been used in contact tracing of STIs to inform anonymous sex partners of their exposure to infection and the need to seek medical consultation [140, 141].

SURVEILLANCE AND INTERVENTION

The incidence of gonorrhoea in Sweden is on the increase. Even though the situation in Sweden today is low-prevalent compared with the high rates in the 1970s, it is important to get detailed epidemiological information about each case to press the incidence of the disease to even lower levels, if possible. A strategy aiming at eradication of a low-prevalent disease may differ from intervention strategies trying to decrease a high incidence in the general population. It may also call for an active collection of additional epidemiological data if the case reports in the surveillance system are not complete enough. In 1996 information about country of exposure was missing in 37% and route of transmission in 34% of all gonorrhoea reports sent to SMI [31]. In the years 1997 and 1998, when questionnaires were sent from SMI to the reporting physicians, missing information about country of exposure was reduced to only 3% and 1%, respectively, and route of transmission was only missing in 2% respectively 1% of the cases (**paper I**). Thus, it is necessary to collect additional epidemiological data about each reported case, if the regular surveillance system is not sensitive enough. As shown in this thesis, adequate epidemiological information combined with microbiological characterization (typing and antimicrobial susceptibility testing) of all isolates makes it possible to identify core groups important to the endemic spread of gonorrhoea and to detect outbreaks and spread of antibiotic resistant strains in specific sub-populations.

To control the spread of gonorrhoea and other STIs in Sweden both broad and targeted intervention is needed. It is important to direct adequate information adapted to core groups and other persons at risk, e.g. people who have sexual contacts when travelling abroad, so that the use of condoms can be addressed. Also, it is urgent to increase the knowledge and awareness about signs and symptoms of gonorrhoea, transmission risks

and the connection to HIV, shown in **paper IV**. In a Swedish retrospective study of all cases reported to SMI in 2001 with gonorrhoea or/and chlamydia infection contracted in Thailand, as many as 41% of the patients (n=221) were not tested for HIV, neither directly when they were diagnosed with gonorrhoea and/or chlamydia, or three months later as would have been appropriate [93]. This underlines the need of increased awareness also in health care staff who meet these patients, not only in the core groups and other persons at risk.

It should also be stressed that several of the gonorrhoea cases in the present studies were co-infected with chlamydia. The general practitioners have to be aware of gonorrhoea and not only perform diagnostic samples for chlamydia, which is the most prevalent STI in Sweden, and treat the patients with adequate antibiotics or preferably send the patients with gonorrhoea to STI clinics for treatment, test-of-cure and partner notification [142].

Easily accessible clinics directed to specific core groups, such as Venhälsan in Stockholm [143] and Gayhälsan in Gothenburg [144], which are directed to MSM, as well as youth health clinics directed at teenagers and young adults, have an important role to play in the control of gonorrhoea and other STIs. Also, adequate guidelines and routines for testing and choice of treatment is crucial in consideration of the variation of sexual practices, site of infections and antimicrobial resistant patterns, as shown in this thesis. An example of guidelines directed towards a specific core group is the recently published Norwegian recommendations about HIV and STI testing and consultation in MSM aimed at general practitioners [145], similar to guidelines produced by public health authorities in USA and Australia [120, 146].

Partner notification (contact tracing) is also an important tool in the control of gonorrhoea and should be performed by trained staff. It has previously been shown in Sweden that contact tracing of patients with genital chlamydia infection performed by special trained staff is more effective than contact tracing by physicians [147]. Such specialists perform partner notification at STI clinics in Sweden, where the majority of gonorrhoea patients are treated. However, guidelines for partner notification should be developed by the health authorities and brought up to date. Also, knowledge about the impact of sexual networks and new sexual venues, e.g. Internet communities, is necessary if contact tracing will be successful, especially in specific core groups such as MSM where partner notification can be difficult to perform due to anonymous partners.

All this efforts together may keep the gonorrhoea incidence at a low level in Sweden, which may in turn contribute to maintaining the low rate of PID [148] and keep down the endemic spread of HIV in MSM.

CONCLUSIONS

– The increase of gonorrhoea in Sweden is mainly due to an increase in domestic cases. Heterosexual teenagers and men who have sex with men (MSM) were identified as core groups, but they were infected by different phenotypic serovars of *N. gonorrhoeae*. Among the imported cases Thailand was the most prevalent country of exposure.

– Genotypic characterization by PFGE indicated that one single *N. gonorrhoeae* clone of the serovar IB-2 and another single clone of IB-3 were responsible for the majority of cases in the two identified core groups of domestic cases in Sweden 1998–99. When analysing isolates which did not belong to one of these two genetically indistinguishable clones, PFGE identified a high genetic diversity within and between the different serovars.

– The antibiotic susceptibility varied with the countries where the patients were exposed. For patients exposed in Asia, 63% of their isolates showed reduced susceptibility to ciprofloxacin, compared with 0%–8.5% of the isolates from patients exposed in other countries. Ciprofloxacin cannot be recommended as the first choice of treatment if the place of exposure was in Asia. All strains were fully susceptible to spectinomycin and ceftriaxone, which are better choices of treatment.

– Due to the great variety of different antibiotic sensitivity patterns among *N. gonorrhoeae* isolates, it is not acceptable to use direct microscopy or DNA amplification methods as the only diagnostic methods for gonorrhoea.

– A great variation of serovars was seen among MSM in Stockholm during a 15-year period, indicating a continuous importation of strains into this core group and micro-epidemics caused by a few persistent serovars. There was a significant difference in the distribution of serovars correlated to HIV status. Gonorrhoea is a marker for HIV infection in MSM, but the increase in gonorrhoea may be associated with genital–oral sexual practice rather than with high risk sexual practice.

– An increase in the number of sexual partners and in unprotected oral sex with casual partners is associated in time with the increase of gonorrhoea among MSM. Internet has emerged as the most common way to meet sexual partners.

– Active surveillance of gonorrhoea, including epidemiological data and micro-biological characterization is needed to control the spread and to make it possible to direct intervention efforts and prevention campaigns to core groups and persons at risk.

ACKNOWLEDGEMENTS

The present thesis would not have been possible with out all people who have contributed direct or indirect to my work. I wish to express my sincere thanks to all of you. Special warm thanks to all patients, counsellors, physicians and laboratory staff who have contributed to the project with data and samples.

My three supervisors for their advice, support and friendship:
Professor **Johan Giesecke**, my former head of the Department of Epidemiology at Smittskyddsinstitutet, for being my main supervisor and giving me the trust and opportunity to start my doctoral studies and for inspiring guidance and advice in the field of infectious disease epidemiology; Associate professor **Hans Fredlund** for guidance and advice in the research field of *N. gonorrhoeae*, for his encouragement and exceptional enthusiasm, for inspiring discussions and always giving of his time for answering my questions; Professor **Eric Sandström**, my former boss and head of Venhälsan, Södersjukhuset, for first encourage my interests in scientific research and giving me the opportunity to develop them, and for guidance and advice in the research field of *N. gonorrhoeae*.

Associate professor **Karl Ekdahl** for being my main supervisor during my first year as a doctoral student when Johan Giesecke was working for WHO in Geneva.

My co-author **Magnus Unemo** for friendship and skilful guidance in microbiological issues and introduction in genetic typing methods in the research field of *N. gonorrhoeae*.

My co-authors **Per Olcén**, **Sven Grützmeier**, **Ann-Kerstin Rudén** and **Bengt Wretlind** for their valuable contributions and friendly advice.

Johan Lindbäck and **Tommi Asikainen** for helpful statistical advice and discussions.

Angela Steen, **Margaretha Jurstrand** and **Annika Hellberg** at Department of Clinical Microbiology, Örebro University Hospital, for assistance in serovars determination and **Karin Bäsén**, **Birgitta Sjöberg** and **Helena Eriksson** for assistance in antibiotic susceptibility testing.

Betina Colucci, **Kirsti Jalakas Pörnull** and **Ingrid Arremark**, Department of Clinical Microbiology, Karolinska University Hospital, Hudding, for assistance in serovars determination and **Lotta Fransson** for collecting *N. gonorrhoeae* isolates.

Lisbeth Henriksson at the County Medical Officer in Stockholm for epidemiological data and for her kind helpfulness.

Lars Eriksson for his long friendship, for assistance in the analysis of the sexual behaviour study and pleasant cooperation in other projects during the years.

Kristina Ramstedt for her friendship and enthusiasm and for giving me the trust and opportunity to start working with STI epidemiology at Smittskyddsinstitutet.

Hans Blystad, my good friend at the Norwegian Institute of Public Health, and **Kristy Delisle**, **Anders Milton** and **Johan Carlson**, my friends and former colleagues in the Committee of inquiry into HIV/Aids for many inspiring discussions on STI/HIV epidemiology and prevention and other essential topics of life.

All my colleagues at the Department of Epidemiology, Smittskyddsinstitutet, for friendship and support. Special thanks to **Malin Arneborn**, **Margareta Löfdahl** and **Anders Blaxhult** for covering for me during my research leaves, **Louise Magnesten** for support in administrative matters, and professor **Annika Linde**, the new head of the Department, for her understanding and support during the last months when I was completing my thesis.

All my friends and former colleagues at Venhälsan, Södersjukhuset, for support in different projects during the previous years. Special thanks to **Göran Bratt**, whose friendship, good advice and enthusiasm always have been a great support and inspiration, to **Bernt Lund** for his value assistance in data collection in the 15-year study of gonorrhoea and to **Bo Hejdeman** och **Stefan Ekroth** for their assistance in the pharyngeal screening study.

My doctoral student colleagues in the field of infectious disease epidemiology **Lotta Högberg**, **Birgitta de Jong** and **Eva Dannetun** for encouragement and exchange of experiences during the PhD studies and research process.

The staff and colleagues at the Institution of Medical Epidemiology and Biostatistics, Karolinska Institutet, for letting me be a part of their research program and for support.

My friends **Daniel Pashley** and **Ian Fraser** for kindly help with linguistic review and revision of the present thesis.

My dear family and all other dear friends that have contribute with friendship, positive attitudes and welcome pauses in the writing process with dinners and stimulating social company; special thanks to **Nedo Entenza** and his family for hospitality and love during my visits to Cuba; my parents **Ingalill** and **Lars** for all the love and support they always have given me in my life; my brother **Erik** and sister-in-law **Monica** for their love and support; **Anders** for being there for me with love and for his patience during the writing process, and not to forget our four-footed family member **Frida** for her never ending love and faithfulness and for doing me company during many late hours when I was writing on my thesis.

The present work was supported by grants from Karolinska Institutet, Stockholm, the Swedish National Institute for Public Health, the Research Committee of Örebro County and the Örebro University Hospital Research Foundation.

REFERENCES

1. Rudén AK, Danielsson D. Gonorré. In: Sandström E, editor. Sexuellt överförbara sjukdomar. Lund: Studentlitteratur; 1994. p. 137-53.
2. Neisser A. Ueber eine der Gonorrhoe eigentümliche Micrococcusform. Vorläufige Mitteilung. Zentralbl Med Wissenschaften. 1879;17(28):497-500.
3. Tapsall JW. What management is there for gonorrhea in the postquinolone era? Sex Transm Dis. 2006;33(1):8-10.
4. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sex Transm Infect. 1999 Feb;75(1):3-17.
5. Beck EJ, Mandalia S, Leonard K, Griffith RJ, Harris JR, Miller DL. Case-control study of sexually transmitted diseases as cofactors for HIV-1 transmission. Int J STD AIDS. 1996 Jan-Feb;7(1):34-8.
6. Kehinde AO, Lawoyin TO. STI/HIV co-infections in UCH, Ibadan, Nigeria. Afr J Reprod Health. 2005 Apr;9(1):42-8.
7. AIDS epidemic update. December 2005. Geneva: UNAIDS/WHO; 2005.
8. Torian LV, Makki HA, Menzies IB, Murrill CS, Benson DA, Schween FW, et al. High HIV seroprevalence associated with gonorrhea: New York City Department of Health, sexually transmitted disease clinics, 1990-1997. Aids. 2000 Jan 28;14(2):189-95.
9. Johansen JD, Smith E. Gonorrhoea in Denmark: high incidence among HIV-infected men who have sex with men. Acta Derm Venereol. 2002;82(5):365-8.
10. Global prevalence and incidence of selected curable sexually transmitted infections overview and estimates. Geneva: World Health Organization; 2001.
11. Sullivan EA, Abel M, Tabrizi S, Garland SM, Grice A, Poumerol G, et al. Prevalence of sexually transmitted infections among antenatal women in Vanuatu, 1999-2000. Sex Transm Dis. 2003 Apr;30(4):362-6.
12. Rassjö EB, Kambugu F, Tumwesigye MN, Tenywa T, Darj E. Prevalence of sexually transmitted infections among adolescents in Kampala, Uganda, and theoretical models for improving syndromic management. J Adolesc Health. 2006 Mar;38(3):213-21.
13. Jiang J, Cao N, Zhang J, Xia Q, Gong X, Xue H, et al. High prevalence of sexually transmitted diseases among men who have sex with men in Jiangsu Province, China. Sex Transm Dis. 2006 Feb;33(2):118-23.
14. Sexually Transitted Diseases Surveillance 2004 Supplement: Gonococcal Isolates Surveillance Project (GISP) Annual Report 2004. Atlanta, GA, USA: Division of STD Prevention, Department of Health and Human Services, Centers for Disease Control and Prevention (CDC). 2005.
15. Little JW. Gonorrhea: update. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006 Feb;101(2):137-43.
16. van Duynhoven YT. The epidemiology of *Neisseria gonorrhoeae* in Europe. Microbes Infect. 1999 May;1(6):455-64.
17. Linglöf T. Rapid increase of syphilis and gonorrhea in parts of the former USSR. Sex Transm Dis. 1995 May-Jun;22(3):160-1.
18. Laukamm-Josten U, Klavs I, Renton A, Fenton K. Sexually transmitted infections in Europe: no impact on HIV - yet. In: Matic S, Lazarus JV, Donoghoe MC, editor. HIV/AIDS in Europe Moving from death sentence to chronic disease management. Copenhagen: WHO Regional Office for Europe; 2006. p. 171-87.
19. Borisenko KK, Tichonova LI, Renton AM. Syphilis and other sexually transmitted infections in the Russian Federation. Int J STD AIDS. 1999 Oct;10(10):665-8.
20. Uusküla A, Plank T, Lassus A, Bingham JS. Sexually transmitted infections in Estonia - syndromic management of urethritis in a European country? Int J STD AIDS. 2001 Aug;12(8):493-8.

21. Blystad H, Blad L, Tulisov A, Aavitsland P. Status on some important infectious diseases in North-west Russia and the Nordic and Baltic Countries 1999-2003. EpiNorth. 2004/2005;5/6(4/1).
22. Lapinskaite GS, Bingham JS. Sexually transmitted diseases in Lithuania: some epidemiological and social aspects. Int J STD AIDS. 1999 Oct;10(10):673-6.
23. Fenton KA, Lowndes CM, and the ESSTI network. Recent trends in the epidemiology of sexually transmitted infections in the European Union. Sex Transm Infect. 2004 Aug;80(4):255-63.
24. Moi H. Care of sexually transmitted infections in the Nordic countries. Int J STD AIDS. 2001 Dec;12(12):819-23.
25. Danielsson D. Gonorrhoea and syphilis in Sweden - past and present. Scand J Infect Dis Suppl. 1990;69:69-76.
26. Linglöf T. Tabeller och diagram över epidemiska sjukdomar i Sverige under 1900-talet. Smittskyddsinstitutet, Solna; 1993.
27. Berglund T, Giesecke G. Uppföljning av gonorré. Smittskydd. 1997;3(4):31.
28. Fredlund H, Garpenholt Ö, Danielsson D. Endemic gonorrhea eradicated in certain areas of Sweden but imported infections show a constancy. Sex Transm Dis. 1994;21 (suppl 2):136.
29. Fox KK, del Rio C, Holmes KK, Hook EW 3rd, Judson FN, Knapp JS, et al. Gonorrhea in the HIV era: a reversal in trends among men who have sex with men. Am J Public Health. 2001 Jun;91(6):959-64.
30. Ciesielski CA. Sexually Transmitted Diseases in Men Who Have Sex with Men: An Epidemiologic Review. Curr Infect Dis Rep. 2003 Apr;5(2):145-52.
31. Berglund T, Fredlund H, Ramstedt K. Reemergence of gonorrhea in Sweden. Sex Transm Dis. 1999 Aug;26(7):390-1.
32. Macdonald N, Dougan S, McGarrigle CA, Baster K, Rice BD, Evans BG, et al. Recent trends in diagnoses of HIV and other sexually transmitted infections in England and Wales among men who have sex with men. Sex Transm Infect. 2004 Dec;80(6):492-7.
33. Berglund T, Blystad H. Increase in genital chlamydia infections in Sweden. Eurosurveillance weekly. 2002;6(26)(27 June 2002), available from: http://eurosurveillance.org/ew/2002/020627.asp.
34. Blystad H, Nilsen Ö, Berglund T, Blaxhult A, Aavitsland P, Giesecke J. Syphilis outbreak in Norway and Sweden among men who have sex with men 1998-2002. Eurosurveillance weekly. 2003;7(24) (12 June 2003), available from: http://eurosurveillance.org/ew/2003/030612.asp.
35. Payne L, Berglund T, Henriksson L, Berggren-Palme I. Re-emergence of syphilis in Sweden: results from a surveillance study for 2004. Eurosurveillance weekly. 2005;10(11) (10 Nov 2005), available from: http://www.eurosurveillance.org/ew/2005/051110.asp.
36. Fenton KA, Imrie J. Increasing rates of sexually transmitted diseases in homosexual men in Western europe and the United States: why? Infect Dis Clin North Am. 2005 Jun;19(2):311-31.
37. Berglund T, Fredlund H, Giesecke J. Epidemiology of the reemergence of gonorrhea in Sweden. Sex Transm Dis. 2001 Feb;28(2):111-4.
38. Blystad H, Berglund T, Aavitsland P, Rosvold Gundersen V. Spread of gonorrhoea from the Barents and Baltic Sea regions to the Nordic countries? Eurosurveillance weekly. 2002;6(32) (8 Aug 2002), available from: http://www.eurosurveillance.org/ew/2002/020808.asp.
39. Lowndes CM, Fenton KA, and the ESSTI network. Surveillance systems for STIs in the European Union: facing a changing epidemiology. Sex Transm Infect. 2004 Aug;80(4):264-71.
40. Götz H, Lindbäck J, Ripa T, Arneborn M, Ramstedt K, Ekdahl K. Is the increase in notifications of *Chlamydia trachomatis* infections in Sweden the result of changes in prevalence, sampling frequency or diagnostic methods? Scand J Infect Dis. 2002;34:28-34.

41. Knapp JS, Koumans KH. Neisseria and Branhamella. In: Murray PR, Baron, E.J., Pfaller MA, Tenover FC, Yolken RH, editor. Manual of Clinical Microbiology. 7th ed. Washington DC: American Society for Microbiolgoy; 1999.
42. Merz AJ, So M. Interactions of pathogenic neisseriae with epithelial cell membranes. Annu Rev Cell Dev Biol. 2000;16:423-57.
43. Tapsall JW. Antimicrobial resistance in *Neisseria gonorrhoeae*. [Geneva]: World Health Organization; 2001.
44. Fredlund H, Falk L, Jurstrand M, Unemo M. Molecular genetic methods for diagnosis and characterisation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: impact on epidemiological surveillance and interventions. Apmis. 2004 Nov-Dec;112(11-12):771-84.
45. Tomlinson DR, French PD, Harris JRW, Mercey DE. Does rectal gonorrhoea reflect unsafe sex? Lancet. 1991;337:501-2.
46. Kleist E, Moi H. Transmission of gonorrhoea through an inflatable doll. Genitourin Med. 1993;69(4):322.
47. Fox KK, Thomas JC, Weiner DH, Davis RH, Sparling PF, Cohen MS. Longitudinal evaluation of serovar-specific immunity to *Neisseria gonorrhoeae*. Am J Epidemiol. 1999 Feb 15;149(4):353-8.
48. McCutchan JA. Epidemiology of venereal urethritis: comparison of gonorrhea and nongonococcal urethritis. Rev Infect Dis. 1984;6:669-88.
49. Hooper RR, Reynolds GH, Jones OG, et al. Cohort study of venereal disease. I. The risk of gonorrhea transmission from infected women to men. Am J Epidemiol. 1978;108(2):136-44.
50. Korenromp EL, Sudaroyo MK, de Vlas SJ, et al. What proportion of episodes of gonorrhoea and chlamydia becomes symptomatic? Int J STD AIDS. 2002;13:91-101.
51. Giesecke J. Modern Infectious disease epidemiology. 2nd ed. London: Arnold; 2002.
52. Brunham RC. Core group theory: a central concept in STD epidemiology. Venereology. 1997;10(1):34-9.
53. Liljeros F. Sexual networks in contemporary Western societies. Physica A. 2004;338:238-45.
54. Thomas JC, Tucker MJ. The development and use of the concept of a sexually transmitted disease core. J Infect Dis. 1996;174 Suppl 2:S134-43.
55. Law DC, Serre ML, Christakos G, Leone PA, Miller WC. Spatial analysis and mapping of sexually transmitted diseases to optimise intervention and prevention strategies. Sex Transm Infect. 2004 Aug;80(4):294-9.
56. Rothenberg R, Narramore J. The relevance of social network concepts to sexually transmitted disease control. Sex Transm Dis. 1996 Jan-Feb;23(1):24-9.
57. De P, Singh AE, Wong T, Yacoub W, Jolly AM. Sexual network analysis of a gonorrhoea outbreak. Sex Transm Infect. 2004 Aug;80(4):280-5.
58. Liljeros F, Edling C, Nunes Amaral LA. Sexual networks: implications for the transmission of sexually transmitted infections. Microbes Infect. 2003;5:189-96.
59. Heymann DL, editor. Control of communicable diseases manual. 18th ed. Washington DC: American Public Health Association; 2004.
60. Bignell CJ. European guideline for the management of gonorrhoea. Int J STD AIDS. 2001 Oct;12 Suppl 3:27-9.
61. Lum G, Freeman K, Nguyen NL, Limnios EA, Tabrizi SN, Carter I, et al. A cluster of culture positive gonococcal infections but with false negative cppB gene based PCR. Sex Transm Infect. 2005 Oct;81(5):400-2.
62. Palmer HM, Mallinson H, Wood RL, Herring AJ. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae*. J Clin Microbiol. 2003 Feb;41(2):835-7.
63. Airell Å, Lindbäck E, Ataker F, Pörnull KJ, Wretlind B. Verification of clinical samples, positive in AMPLICOR *Neisseria gonorrhoeae* polymerase chain reaction, by 16S rRNA and *gyrA* compared with culture. Int J STD AIDS. 2005 Jun;16(6):415-9.

64. Berglund T, Colucci B, Lund B, Qvarnström I, Sandström E. [Increasing incidence of ciprofloxacin resistant gonorrhea in Sweden. Choose a correct antibiotic and follow up the treatment!]. Läkartidningen. 2004 Jul 8;101(28-29):2332-5.
65. Manavi K, Young H, McMillan A. The outcome of oropharyngeal gonorrhoea treatment with different regimens. Int J STD AIDS. 2005 Jan;16(1):68-70.
66. Sandström E, Danielsson D. Serology of *Neisseria gonorrhoeae*. Classification by co-agglutination. Acta Pathol Microbiol Scand [B]. 1980 Feb;88(1):27-38.
67. Knapp JS, Tam MR, Nowinski RC, Holmes KK, Sandstrom EG. Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein I. J Infect Dis. 1984 Jul;150(1):44-8.
68. Tam MR, Buchanan TM, Sandström EG, Holmes KK, Knapp JS, Siadak AW, et al. Serological classification of *Neisseria gonorrhoeae* with monoclonal antibodies. Infect Immun. 1982 Jun;36(3):1042-53.
69. Sandström E, Lindell P, Härfast B, Blomberg F, Rydén A-C, Bygdeman S. Evaluation of a new set of *Neisseria gonorrhoeae* serogroup W-specific monoclonal antibodies for serovar determination. In: Schoolnik, GK, editor. The pathogenic Neisseria. Proceedings of the fourth international symposium. Washington DC: American society for Microbiology; 1985. p. 26-30.
70. Unemo M. *Neisseria gonorrhoeae* 2005. Annual report regarding serological charcterisation and antibiotic susceptibility of Swedish *Neisseria gonorrhoeae* strains. Örebro: National Reference Laboratory for Patogenic Neisseria, Department of Clinical Microbiology, Örebro University Hospital; 2006.
71. Unemo M, Berglund T, Olcén P, Fredlund H. Pulsed-field gel electrophoresis as an epidemiologic tool for *Neisseria gonorrhoeae*: identification of clusters within serovars. Sex Transm Dis. 2002 Jan;29(1):25-31.
72. Unemo M, Olcén P, Berglund T, Albert J, Fredlund H. Molecular epidemiology of *Neisseria gonorrhoeae*: sequence analysis of the *porB* gene confirms presence of two circulating strains. J Clin Microbiol. 2002 Oct;40(10):3741-9.
73. Unemo M, Olcén P, Albert J, Fredlund H. Comparison of serologic and genetic *porB*-based typing of *Neisseria gonorrhoeae*: consequences for future characterization. J Clin Microbiol. 2003 Sep;41(9):4141-7.
74. Unemo M, Olcén P, Jonasson J, Fredlund H. Molecular typing of *Neisseria gonorrhoeae* isolates by pyrosequencing of highly polymorphic segments of the *porB* gene. J Clin Microbiol. 2004 Jul;42(7):2926-34.
75. Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J Infect Dis. 2004 Apr 15;189(8):1497-505.
76. O'Rourke M, Ison CA, Renton AM, Spratt BG. Opa-typing: a high-resolution tool for studying the epidemiology of gonorrhoea. Mol Microbiol. 1995 Sep;17(5):865-75.
77. Howie F, Young H, McMillan A. The diversity of the opa gene in gonococcal isolates from men who have sex with men. Sex Transm Infect. 2004 Aug;80(4):286-8.
78. Holmes KK, Levine R, Weaver M. Effectiveness of condoms in preventing sexually transmitted infections. Bull World Health Organ. 2004 Jun;82(6):454-61.
79. Warner L, Stone KM, Macaluso M, Buehler JW, Austin HD. Condom use and risk of gonorrhea and Chlamydia: a systematic review of design and measurement factors assessed in epidemiologic studies. Sex Transm Dis. 2006 Jan;33(1):36-51.
80. Arthur G, Lowndes CM, Blackham J, Fenton KA, and the ESSTI network. Divergent approaches to partner notification for sexually transmitted infections across the European union. Sex Transm Dis. 2005 Dec;32(12):734-41.
81. Ericsson HM, Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol Microbiol Scand [B] Microbiol Immunol. 1971;217:Suppl 217:1+.

82. Ng LK, Carballo M, Dillon JA. Differentiation of *Neisseria gonorrhoeae* isolates requiring proline, citrulline, and uracil by plasmid content, serotyping, and pulsed-field gel electrophoresis. J Clin Microbiol. 1995 Apr;33(4):1039-41.
83. Poh CL, Lau QC. Subtyping of *Neisseria gonorrhoeae* auxotype-serovar groups by pulsed-field gel electrophoresis. J Med Microbiol. 1993 May;38(5):366-70.
84. Xia M, Whittington WL, Shafer WM, Holmes KK. Gonorrhea among men who have sex with men: outbreak caused by a single genotype of erythromycin-resistant *Neisseria gonorrhoeae* with a single-base pair deletion in the mtrR promoter region. J Infect Dis. 2000 Jun;181(6):2080-2.
85. Cooke SJ, de la Paz H, La Poh C, Ison CA, Heckels JE. Variation within serovars of *Neisseria gonorrhoeae* detected by structural analysis of outer-membrane protein PIB and by pulsed-field gel electrophoresis. Microbiology. 1997 Apr;143 (Pt 4):1415-22.
86. Poh CL, Loh GK, Tapsall JW. Resolution of clonal subgroups among *Neisseria gonorrhoeae* IB-2 and IB-6 serovars by pulsed-field gel electrophoresis. Genitourin Med. 1995 Jun;71(3):145-9.
87. Van Looveren M, Ison CA, Ieven M, Vandamme P, Martin IM, Vermeulen K, et al. Evaluation of the discriminatory power of typing methods for *Neisseria gonorrhoeae*. J Clin Microbiol. 1999 Jul;37(7):2183-8.
88. Tenover FC, Arbeit RD, Goering RV. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. Infect Control Hosp Epidemiol. 1997 Jun;18(6):426-39.
89. R [computor program]. Version 2.2.1. Vienna, Austria; 2005. R Development Core Team, R Foundation for Statistical Computing, available from: http://www.r-projcet.org.
90. Berglund T, Fredlund H, Hedin G. Inhemsk spridning av ciprofloxacinresistent gonorré bland unga heterosexuella. EPI-aktuellt. 2003;2(27):2 (3 July 2003), available from: http://www.smittskyddsinstitutet.se/upload/EPI-Aktuellt/EA-0327.pdf
91. Trees DL, Sirivongrangson P, Schultz AJ, Buatiang A, Neal SW, Knapp JS, et al. Multiclonal increase in ciprofloxacin-resistant Neisseria gonorrhoeae, Thailand, 1998-1999. Sex Transm Dis. 2002 Nov;29(11):668-73.
92. Poder A, Bingham JS. Sexually transmitted infections in Estonia. Int J STD AIDS. 1999 Oct;10(10):669-72.
93. Azerkan F. "Han kom aldrig tillbaks...!". HIV-uppföljningsstudie av hemvändande turister med gonorré och/eller klamydia smittade i Thailand år 2001. Smittskyddsintitutets rapportserie nr 1. Solna: Smittskyddsinstitutet; 2002.
94. Carter S, Horn K, Hart G, Dunbar M, Scoular A, MacIntyre S. The sexual behaviour of international travellers at two Glasgow GUM clinics. Glasgow genitourinary medicine. Int J STD AIDS. 1997 May;8(5):336-8.
95. Bellis MA, Hughes K, Thomson R, Bennett A. Sexual behaviour of young people in international tourist resorts. Sex Transm Infect. 2004 Feb;80(1):43-7.
96. Rudén AK. Temporal changes in the gonococcal serovar patterns in Stockholm during two years with special reference to PPNG strains. Genitourin Med. 1994 Aug;70(4):256-61.
97. Palmer HM, Young H, Martin IM, Ison CA, Spratt BG. The epidemiology of ciprofloxacin resistant isolates of *Neisseria gonorrhoeae* in Scotland 2002: a comparison of phenotypic and genotypic analysis. Sex Transm Infect. 2005 Oct;81(5):403-7.
98. Aydin D, Koksalan K, Komec S, Aktas G. Auxo-, sero-, and opa-typing of Neisseria gonorrhoeae strains isolated in Istanbul, Turkey. Sex Transm Dis. 2004 Oct;31(10):628-30.
99. Bygdeman SM, Rudén AK, Jonsson A, Lidbrink P, Olofsson MB, Bäckman M, et al. Antibiotic susceptibility, serovars and auxotypes of gonococcal isolates in Stockholm. Relation to geographical origin of the infection. Int J STD AIDS. 1993 Jan-Feb;4(1):33-40.

100. Young H, Moyes A, Ross JD, McMillan A. Patterns of homosexually acquired gonococcal serovars in Edinburgh 1986-90. Genitourin Med. 1991 Aug;67(4):312-6.
101. Bäckman M, Rudén AK, Bygdeman SM, Jonsson A, Ringertz O, Sandström EG. Gonococcal serovar distribution in Stockholm, with special reference to multiple infections and infected partners. Acta Pathol Microbiol Immunol Scand [B]. 1985 Jun;93(3):225-32.
102. Lind I. Antimicrobial resistance in *Neisseria gonorrhoeae*. Clin Infect Dis. 1997 Jan;24 Suppl 1:S93-7.
103. Beattie T, Moyes A, Patrizio C, Young H. Subtyping of high-level plasmid-mediated tetracycline resistant *Neisseria gonorrhoeae* isolated in Scotland between 1992 and 1998. Int J STD AIDS. 1999 Oct;10(10):646-51.
104. Guoming L, Qun C, Shengchun W. Resistance of *Neisseria gonorrhoeae* epidemic strains to antibiotics: report of resistant isolates and surveillance in Zhanjiang, China: 1998 to 1999. Sex Transm Dis. 2000 Feb;27(2):115-8.
105. van Dyck E, Alary M, Guedou A, Abdellati S, Lafia E, Anagonou S. Antimicrobial susceptibilities and plasmid patterns of *Neisseria gonorrhoeae* in Benin. Int J STD AIDS. 2001 Feb;12(2):89-93.
106. Fenton KA, Ison C, Johnson AP, Rudd E, Soltani M, Martin I, et al. Ciprofloxacin resistance in *Neisseria gonorrhoeae* in England and Wales in 2002. Lancet. 2003 May 31;361(9372):1867-9.
107. Borgen K, van Loo I, Koedijk F, van der Laar M. Increase of gonococcal quinolone resistance in the Netherlands from 2002-2004. Eurosurveillance weekly. 2005;10(11) (17 Nov 2005), available from: http://www.eurosurveillance.org/ew/2005/051117.asp.
108. Martin IMC, Hoffman S, Fenton KA, et al. *Neisseria gonorrhoeae* antimicrobial resistance in Europe: ESSTI, the first European surveillance data. Abstract WP-002. 16th Biennial Meeting of the International Society for Sexually Transmitted Disease Research. Amsterdam; 2005.
109. Herida M, Desenclos JC, Martin IM, Goulet V, Laurent E, Sednaoui P. Increase of *Neisseria gonorrhoeae* ciprofloxacin resistance in France in 2001-2003. Sex Transm Dis. 2006 Jan;33(1):6-7.
110. Enders M, Turnwald-Maschler A, Regnath T. Antimicrobial resistance of *Neisseria gonorrhoeae* isolates from the Stuttgart and Heidelberg areas of southern Germany. Eur J Clin Microbiol Infect Dis. 2006 Apr 29.
111. Tapsall JW. Antibiotic resistance in *Neisseria gonorrhoeae*. Clin Infect Dis. 2005 Aug 15;41 Suppl 4:S263-8.
112. Bauer HM, Mark KE, Samuel M, Wang SA, Weismuller P, Moore D, et al. Prevalence of and associated risk factors for fluoroquinolone-resistant *Neisseria gonorrhoeae* in California, 2000-2003. Clin Infect Dis. 2005 Sep 15;41(6):795-803.
113. Ghanem KG, Giles JA, Zenilman JM. Fluoroquinolone-resistant *Neisseria gonorrhoeae*: the inevitable epidemic. Infect Dis Clin North Am. 2005 Jun;19(2):351-65.
114. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* in the WHO Western Pacific Region, 2001. World Health Organization. Commun Dis Intell. 2002;26(4):541-5.
115. Wang SA, Lee MV, O'Connor N, Iverson CJ, Ohye RG, Whiticar PM, et al. Multidrug-resistant *Neisseria gonorrhoeae* with decreased susceptibility to cefixime-Hawaii, 2001. Clin Infect Dis. 2003 Sep 15;37(6):849-52.
116. Shigemura K, Okada H, Shirakawa T, Tanaka K, Arakawa S, Kinoshita S, et al. Susceptibilities of *Neisseria gonorrhoeae* to fluoroquinolones and other antimicrobial agents in Hyogo and Osaka, Japan. Sex Transm Infect. 2004 Apr;80(2):105-7.
117. Lindberg M, Ringertz O, Sandström E. Treatment of pharyngeal gonorrhea due to beta-lactamase-producing gonococci. Br J Vener Dis. 1982 Apr;58(2):101-4.
118. Bignell C. Antibiotic treatment of gonorrhoea - clinical evidence for choice. Genitourin Med. 1996 Oct;72(5):315-20.

119. Dan M, Poch F, Amitai Z, Gefen D, Shohat T. Pharyngeal Gonorrhea in Female Sex Workers: Response to a Single 2-g Dose of Azithromycin. Sex Transm Dis. 2006 Mar 27.
120. Sexually transmitted diseases treatment guidelines 2002. Centers for Disease Control and Prevention. Morb Mortal Wkly Rep MMWR. 2002;51(No. RR-6):1-80.
121. McLean CA, Wang SA, Hoff GL, Dennis LY, Trees DL, Knapp JS, et al. The emergence of *Neisseria gonorrhoeae* with decreased susceptibility to Azithromycin in Kansas City, Missouri, 1999 to 2000. Sex Transm Dis. 2004 Feb;31(2):73-8.
122. Dillon JA, Li H, Sealy J, Ruben M, Prabhakar P. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from three Caribbean countries: Trinidad, Guyana, and St. Vincent. Sex Transm Dis. 2001 Sep;28(9):508-14.
123. Dillon JA, Rubabaza JP, Benzaken AS, Sardinha JC, Li H, Bandeira MG, et al. Reduced susceptibility to azithromycin and high percentages of penicillin and tetracycline resistance in *Neisseria gonorrhoeae* isolates from Manaus, Brazil, 1998. Sex Transm Dis. 2001 Sep;28(9):521-6.
124. Sosa J, Ramirez-Arcos S, Ruben M, Li H, Llanes R, Llop A, et al. High percentages of resistance to tetracycline and penicillin and reduced susceptibility to azithromycin characterize the majority of strain types of *Neisseria gonorrhoeae* isolates in Cuba, 1995-1998. Sex Transm Dis. 2003 May;30(5):443-8.
125. Ng LK, Martin I, Lau A. Trends of chromosomally mediated antimicrobial resistance in *Neisseria gonorrhoeae* in Canada: 1994-1999. Sex Transm Dis. 2003 Dec;30(12):896-900.
126. Lundbäck D, Fredlund H, Berglund T, Wretlind B, Unemo M. Molecular epidemiology of *Neisseria gonorrhoeae*: identification of the first presumed Swedish transmission chain of an azithromycin-resistant strain. Apmis. 2006 Jan;114(1):67-71.
127. Herlitz C. Allmänheten och hiv/aids: kunskap, attityder, beteenden 1987-2003. Rapportserien R 2004:7. Stockholm: Statens folkhälsoinstitut; 2003.
128. Stolte IG, Dukers NHTM, de Wit JBF, Fennema H, Coutinho RA. A summary report from Amsterdam: increase in sexual transmitted diseases and risky sexual behaviour among homosexual men in relation to the introduction of new anti-HIV drugs. Eurosurveillance. 2002;7(2):19-22.
129. van der Snoek EM, de Wit JB, Mulder PG, van der Meijden WI. Incidence of sexually transmitted diseases and HIV infection related to perceived HIV/AIDS threat since highly active antiretroviral therapy availability in men who have sex with men. Sex Transm Dis. 2005 Mar;32(3):170-5.
130. Miller M, Meyer L, Boufassa F, Persoz A, Sarr A, Robain M, et al. Sexual behavior changes and protease inhibitor therapy. SEROCO Study Group. Aids. 2000 Mar 10;14(4):F33-9.
131. Kent CK, Chaw JK, Wong W, Liska S, Gibson S, Hubbard G, et al. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. Clin Infect Dis. 2005 Jul 1;41(1):67-74.
132. Craib KJ, Meddings DR, Strathdee SA, Hogg RS, Montaner JS, O'Shaughnessy MV, et al. Rectal gonorrhoea as an independent risk factor for HIV infection in a cohort of homosexual men. Genitourin Med. 1995 Jun;71(3):150-4.
133. Swedish Institute for Infectious Diseases, available from: http://www.smittskyddsinstitutet.se/SMItemplates/Article.aspx?id=2834. [cited: 1 May 2006].
134. Smittskyddsenheten i Stockholms läns landsting, available from: http://www.sll.se/w_sme/138569.cs?dirid=9421. [cited: 1 May 2006].
135. van der Bij AK, Stolte IG, Coutinho RA, Dukers NH. Increase of sexually transmitted infections, but not HIV, among young homosexual men in Amsterdam: are STIs still reliable markers for HIV transmission? Sex Transm Infect. 2005 Feb;81(1):34-7.

136. Marcus U, Bremer V, Hamouda O, Kramer MH, Freiwald M, Jessen H, et al. Understanding recent increases in the incidence of sexually transmitted infections in men having sex with men: changes in risk behavior from risk avoidance to risk reduction. Sex Transm Dis. 2006 Jan;33(1):11-7.
137. McMillan A, Young H, Moyes A. Rectal gonorrhoea in homosexual men: source of infection. Int J STD AIDS. 2000 May;11(5):284-7.
138. Tikkanen R, Ross MW. Looking for sexual compatibility: experiences among Swedish men in visiting Internet gay chat rooms. CyberPsychology & Behavior. 2000;3(4):605-16.
139. McFarlane M, Bull SS, Rietmeijer CA. The Internet as a newly emerging risk environment for sexually transmitted diseases. Jama. 2000 Jul 26;284(4):443-6.
140. Klausner JD, Wolf W, Fischer-Ponce L, Zolt I, Katz MH. Tracing a syphilis outbreak through cyberspace. Jama. 2000 Jul 26;284(4):447-9.
141. Pioquinto RM, Tupas EA, Kerndt PR, Taylor MM, Holmberg SD, Patel PA. Using the Internet for partner notification of sexually transmitted diseases - Los Angeles County, California, 2003. MMWR Weekly. 2004;53(6):129-31, available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5306a4.htm.
142. Fredlund H, Berglund T, Giesecke J. [Gonorrhea occurs even in young people--treat it correctly]. Läkartidningen. 1999 Jan 27;96(4):304.
143. Karlsson A, Hejdeman B, Pernetun T, Sandström E. [HIV, gonorrhea, chlamydia and syphilis are increasing among homosexual men]. Läkartidningen. 2001 Apr 11;98(15):1793-5.
144. Christiansen MA, Löwhagen GB. Sexually transmitted diseases and sexual behavior in men attending an outpatients' clinic for gay men in Gothenburg, Sweden. Acta Derm Venereol. 2000 Mar-Apr;80(2):136-9.
145. Blystad H, Klouman, E. Recommendation for annual HIV and STI testing in MSM introduced in Norway. Eurosurveillance weekly. 2005;10(7) (7 July 2005), available from: http://www.eurosurveillance.org/ew/2005/050707.asp.
146. Sexually transmitted infections in gay men. South Eastern Sydney Area Health Services, available from: http://www.sesahs.nsw.gov.au/publichealth/InfectiousDiseases/STIguidelines.asp
147. Eitrem R, Erenius M, Meeuwisse A. Contact tracing for genital Chlamydia trachomatis in a Swedish county. Sex Transm Dis. 1998 Sep;25(8):433-6.
148. Kamwendo F, Forslin L, Bodin L, Danielsson D. Programmes to reduce pelvic inflammatory disease - the Swedish experience. Lancet. 1998;351 Suppl 3:25-8.