Therapeutic aspects of gonococcal and non-gonococcal urethritis

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Therapeutische aspecten van gonorroïsche en niet-gonorroïsche urethritis

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CHAPTER 1

Introduction

1.1. HISTORICAL BACKGROUND

Since the very first historical records, urethral discharge and dysuria have been reported [1,2]. These conditions were already described in ancient Egyptian and Hebrew writings. Hippocrates mentioned urethral discharge in the same breath as other genital diseases. At that time, no distinction was made between various genital diseases. The term gonorrhea which meant 'flow of seed' (gonus, "seed" and rhoea, "flow") was first introduced by Hirsch. Initially, gonorrhea was considered as a benign form of syphilis. In 1554, Fernel, in his book 'Medicina', described gonorrhea as an infection of the urinary bladder and distinguished it as such from syphilis. Sylvius (1614-1672) thought, that the prostate was the organ affected. At the same time, De Graaf (1641-1673) postulated that the disease was located in the prostate and the testes. Cowper (1666-1709) concluded, that the cause was present in the small urethral glands he had discovered. In 1689, Musitanus described gonorrhea as an inflammation of the urethra. In 1765, Hunter inoculated himself with infected material from a patient with urethral discharge in order to prove, that syphilis and gonorrhea had separate clinical presentations. After this experiment, he suffered both from syphilis and gonorrhea. In the years 1831-1837, Ricord conclusively demonstrated, that gonorrhea, chancroid and syphilis were clearly separate diseases on the basis of numerous experimental inoculations in humans. He postulated, that the aetiologic causes were either an irritation of the urethra due to prolonged sexual excitement and/or some substances causing irritation of mucous membranes. In 1879, Neisser identified the agent causing gonorrhea. In 1880, Leistikow successfully cultured the microbe in vitro and it became clear, that there were various forms of urethritis. Gonococcal and non-gonococcal urethritis could be distinguished. It was observed that *N. gonorrhoeae* could not be cultured or demonstrated in Gram-stained preparations from some patients with urethritis. The commonly prescribed anti-gonococcal therapies for that time were also ineffective in these patients. At the beginning of the twentieth century, intracytoplasmic inclusion bodies, which are still today regarded as characteristic for the presence of *Chlamydiae*, were observed in smears of urethral exudates from patients with urethritis. In 1954, *U. urealyticum* was shown to be one of the causative agents of urethritis [3]. In 1965, it became possible to culture *C. trachomatis* in cell cultures [4].

The treatment of urethritis prior to 1935, the year in which sulpha preparations became commercially available, was disappointing. The first therapies of urethritis comprised urethral irrigations and bringing into the urethra pegs and threads (bougies), which were drenched in aluin, zinc sulphate, lead acetate and tannin solutions. These substances had some anti-inflammatory effect on the mucous membranes, but failed to eliminate the cause.

In the nineteenth century, the treatment consisted of santaoil and copaivabalsam given orally. These treatments, however, were not very effective. Once the agent causing gonorrhea had been identified and isolated, the treatment consisted of local

application of antiseptic solutions of silver salts such as silver nitrate and protargol. During a short period prior to the second world war, gonococcal infections were treated with sulpha preparations.

The development of resistance by the gonococci to sulpha preparations soon rendered this therapy ineffective [5]. Once penicillin became available in 1945, gonococcal urethritis could be treated effectively. Non-gonococcal urethritis could be effectively treated only after tetracyclines and macrolides were introduced.

The knowledge about the aetiology of urethritis in men has expanded significantly in recent years. At present, sexually transmitted and not-sexually transmitted urethritis can be distinguished. Sexually transmitted urethritis in turn, can be subdivided into gonococcal and non-gonococcal urethritis. A review of such a classification scheme is provided in Table 1.

1.2 GONOCOCCAL INFECTIONS

Gonorrhea is caused by *N. gonorrhoeae* which belongs to the genus Neisseria of the family Neisseriaceae. Neisseriae are nonmotile, nonspore-forming, Gram-negative diplococci. In Gram-stained preparations, *N. gonorrhoeae* appear as orange-red coffee bean-shaped or kidney-shaped diplococci.

Humans are the only natural host of *N. gonorrhoeae*. A suitable animal model for investigating gonococcal infections is not yet available [67]. To date, all attempts to develop vaccines against *N. gonorrhoeae* have been unsuccessful [68]. The main emphasis of research activities at present is on in vitro and in vivo studies of *N. gonorrhoeae* and on the interactions between *N. gonorrhoeae* and its host.

Gonococcal infections can be divided into symptomatic and asymptomatic, acute and chronic infections with a complicated or an uncomplicated course. The clinical spectrum encompasses on the one hand a full-blown clinical presentation and on the other hand a completely asymptomatic course. Between these two poles many

Table 1. Aetiology of urethritis in males.

sexually transmitted urethritis		not sexually transmitted urethritis
gonococcal:		allergic
	Neisseria gonorrhoeae	iatrogenic
non-gonococcal:		traumatic
	Chlamydia trachomatis (35-60%) *	local proces: like tumors, strictures
	Ureaplasma urealyticum (10-40%) *	psychogenic ?
	Neither (25-50%) *	phimosis
	Trichomonas vaginalis, (rare)	symptom by other disease:
	Yeast, (rare)	Stevens Johnson Syndrome, erythema
	Herpes simplex virus, (rare)	multiforme
	Other bacteria (?)	

* percentages of total number of cases of non-gonococcal urethritis.

variegations are possible. Uncomplicated gonorrhea is defined as a gonococcal infection which is confined to the inoculation site(s).

In men, the clinical presentation may consist of an anterior urethritis, a proctitis and or an infection of the oropharynx.

Acute anterior urethritis is the most common form of gonorrhea in men. The incubation period ranges from 1 to 14 days or longer. In 82% of the individuals, the complaints develop within 2 to 5 days [6]. The predominant symptoms are dysuria and mucopurulent discharge. In most patients, the discharge becomes very profuse within 24 hours after onset and is accompanied by erythema and oedema of the urethral meatus [7]. One-quarter of the patients shows minimum symptoms which are clinically indistinguishable from those of other forms of urethritis [8,9]. There are also an estimated 1 to 2% asymptomatic carriers [10,11]. The usual course of untreated gonococcal urethritis is spontaneous resolution within several weeks and more than 95% of the patients become asymptomatic after six months. It is highly unlikely that spontaneous resolution leads to subsequent asymptomatic carriage of *N. gonorrhoeae*.

Anorectal infections in men are only observed in homosexuals and are attributed to a direct inoculation via rectal sexual intercourse.

In 35 to 50% of the homosexual men with gonococcal urethritis the rectal mucosa is also infected [12]. Solitary rectal infections were observed in 40% of 815 homosexual men that were investigated [12,13,14]. The symptoms ranged from minimal anal pruritis, painless mucopurulent discharge and anal bleeding to symptoms of fulminant proctitis such as tenesmus, severe pain in the rectum and constipation. Often, abnormalities are not externally visible and proctoscopy is required. In 36% to 62% of the men, rectal gonococcal infection follows an asymptomatic course [15,16]. Additional gonococcal infection of the pharynx is also observed in 3% to 7% of heterosexual men and in 10% to 25% of homosexual men suffering from gonorrhea. A solitary pharyngeal gonococcal infection occurs in less than 5% of the patients irrespective of their sexual orientation [16-19]. Gonococcal infection of the pharynx occurs upon orogenital sex and is more efficiently transmitted via fellatio than via cunnilingus [17,20]. More than 90% of the oropharyngeal infections are asymptomatic [17,19,21]. Occasional reports suggest that gonococcal infections of the oropharynx can cause acute pharyngitis or tonsillitis with or without fever and cervical lymphadenopathy [17].

There is a possible increased risk of disseminated (septic) gonococcal infection in patients with pharyngeal gonorrhea, however the natural course resulting in a spontaneous cure in all cases within 12 weeks together with a minimum risk of *N. gonorrhoeae* being transmitted from this site suggest a limited epidemiological importance of this infection site [17,22,23]. The number of rectal and oropharyngeal gonococcal infections has decreased significantly in men due to change(s) in sexual behaviour, reduction in the number of sexual partners and the use of condoms (safe sex) to prevent infection with Human Immunodeficiency Virus [24].

The complications of gonorrhea can be divided into four groups. The first group

consists of additional infections in the structures in the immediate vacinity. Infection of the Tyson's glands belongs to this group. This complication is rarely observed in the industrialized nations. Infection of the Littre's glands is relatively frequent. Another complication is abscess formation. A lymphangitis of the penis, sometimes accompanied by lymphadenitis is rare.

Ascending infections from the urogenital region belong to the second group. Cowperitis, which is an abnormal complication, falls into this group. Symptoms include urinary urgency, pain in the perineum and pain at defaecation. An abscess may also form. An ascending infection may also cause a posterior urethritis and may ascend further, causing epididymitis. In 22% of 51 patients younger than 35 years suffering from epididymitis, *N. gonorrhoeae* was identified as the cause [25]. Epididymitis is generally unilateral. Secondarily, a hydrocéle may develop and leave behind a fibrinous thickening after healing. Bilateral epididymitis occurs infrequently and may result in permanent sterility. Sometimes cystitis can occur, which may very occasionally ascend to the kidneys [26].

The third group consists of gonococcal infections outside the urogenital region, such as the gonococcal conjunctivitis, occurring via digital transmission. In adults, such infections occur via auto-inoculation and are uncommon. Occasionally, such infections may occur in laboratory personnel working with *N. gonorrhoeae* [27]. One eye is usually involved.

Disseminated gonococcal infections (DGI) belong to the fourth group. Approximately 1 to 3% of the patients with mucosal gonorrhea develop DGI [28,29]. Prior to the introduction of antibiotics, DGI were frequently observed in men [30,31]. In the antibiotic era, this frequency has shifted towards women and homosexual men probably as a result of asymptomatic infections in these two groups [28-36]. The clinical manifestations can be sub-divided into an initial bacteremia with accompanying skin lesions followed by a secondary phase in which septic arthritis occurs [32,34]. The clinical symptoms include fever, general malaise, skin lesions, tenosynovitis, polyarthralgies, polyarthritis, hepatitis and myopericarditis. Endocarditis, meningitis, perihepatitis, pneumonia, osteomyelitis and adult respiratory distress syndrome are seldom. More than 90% of the patients have an arthropathy cr arthritis. These may be mild to purulent and sometimes follow a chronic course [37].

N. gonorrhoeae may spread into the general blood circulation from every site of infected mucosa.

N. gonorrhoeae which have been isolated from blood or synovial fluid possess the following characteristics: they are of a transparent colony phenotype, are susceptible to penicillin, require arginine, hypoxanthine and uracil for growth and are resistant to bactericidal action of serum and have a specific type of outer membrane protein I [38].

In some isolated cases, penicillinase producing *N. gonorrhoeae* were reported as the cause of DGI [39]. The host factors which play a role include a deficiency or an abnormality of one of the late-acting complement components (c5, c6, c7, c8),

hormonal factors in women, particularly, since DGI has been observed to occur premenstrually and during pregnancy [40–43]. In patients with DGI, circulating immunocomplexes have been detected which may be the possible cause of skin lesions and arthritis [32–35]. The differential diagnosis includes bacteremias as well as other types of reactive arthritis.

The primary cutaneous infections belong to the particular forms of gonococcal infections. These infections occur mostly in genital ulcers and ulcers on the perineum, the upper legs and/or the fingers [44–46]. Whether these infections are primary or secondary colonizations is not yet clear.

At present, the ratio of gonorrhea in male to female is 1:1.5.

The clinical spectrum of gonorrhea in women includes urethritis, cervicitis, proctitis, acute Bartholinitis, conjunctivitis, endometritis, salpingitis, perihepatitis and DGI. In premenarchal girls, *N. gonorrhoeae* can cause vulvitis and or vaginitis [1]. The symptoms of primary *N. gonorrhoeae* infection in women are less specific than in men and depend on the site of infection. The following symptoms are observed in women in whom gonorrhea has been diagnosed: vaginal discharge (cervicitis), dysuria and urinary urgency (urethritis or skenitis), pain in the vulva and swollen labia (acute Bartholinitis), abnormal uterine bleeding and lower abdominal tenderness with evidence of rebound (pelvic inflammatory disease). An ascending infection from the cervix may lead to endometritis, salpingitis and peritonitis which are collectively also known as pelvic inflammatory disease (PID). Clinically, patients suffering from PID present with lower abdominal pain, mucopurulent cervical discharge and cervical motion tenderness upon vaginal touch. In the United States, the diagnosis of PID is made in 20% of the women with gonorrhea. Conversely, in 40% of the women with PID, *N. gonorrhoeae* was observed to be the cause [1]. Perihepatitis, also called Fitz-Hugh-Curtis syndrome had also been mainly attributed to gonococcal infection, but more recent studies have shown that infection with *C. trachomatis* was frequently associated with this syndrome. The patients with this syndrome presents with an acute or subacute pain in the right upper quadrant of the abdomen, a mucopurulent cervicitis caused by *N. gonorrhoeae* or *C. trachomatis* or PID and/or a normal echography of the bile duct system. The risk of sterility after a single episode of salpingitis varies from 12 to 16%.

The risk of ectopic pregnancy after a single episode of salpingitis is increased seven-fold. A single episode of gonococcal salpingitis resulted in occluded tubae in 24% of the patients.

Ophthalmia gonorrhoica neonatorum occurs more frequently than gonococcal infection of eyes in adults. The exact risk of genito-ocular transmission from a mother with genital gonococcal infection to her child is not known, but is estimated at 2% [47]. The clinical manifestation occurs 1 to 12 days after delivery. If left untreated, blindness can result [48].

The susceptibility-site for antibiotics and the mechanism(s) for developing resistance are generally the same for *N. gonorrhoeae*, *E. coli* and other Gram-negative and many Gram-positive bacteria. After the introduction of sulphonamides

in 1935 to treat gonococcal infections, resistance to sulphonamides developed very quickly. At the beginning, the cure rate was 95% with less than 10% of the isolated strains showing resistance in vitro [5,49]. Within a few years, the cure rate failed by 80% and most of the isolated strains had become resistant to sulphonamides [5]. From 1945 onwards, penicillin became the drug of first choice in the treatment of gonorrhea. Publications about development of resistance and failure of therapy were scarce in the first 10 years of use. In this form of therapy, probenecide should also be given simultaneously. The number of isolated strains that required more than 0.05 units of penicillin per ml for growth inhibition rose from 0.67% in 1955 to 65% in 1968 [50]. In 1945, 100,000 units of procaine penicillin were sufficient to effectively treat gonorrhea. In 1972, treatment required to 4.8 million units. Uptil then there has been talk of chromosomally mediated resistance.

N. gonorrhoeae strains which require 1 μg/ml or more for their growth inhibition and which do not produce β-lactamase are referred to as chromosomally mediated resistant *N. gonorrhoeae* (CMRNG) strains. Chromosomally mediated resistant *N. gonorrhoeae* strains arise from random selection. Mutation induced resistance is generally relative and not absolute and is expressed as a reduced cell permeability for drugs such as tetracycline, penicillin, sulphonamides and erythromycin or via alterations in the bacterial ribosomes as described for aminoglycosides. Chromosomally mediated resistance to various antibiotics occurs frequently in strains isolated from the rectum of homosexual men. These isolates harbour the MTR-gene more frequently, which results in reducing permeability for toxic faecal lipids and antibiotics [51]. Until 1977, there was a decline in the susceptibility for penicillin and tetracycline. In March 1976, the first penicillinase-producing *N. gonorrhoeae* (PPNG) strain was isolated in the United States. The mechanism of resistance was completely different and was caused by a new plasmid with genes coding for a β-lactamase (an enzyme) which is able to split-open the β-lactam ring of penicillin. This circular extra-chromosomal DNA was derived from Haemophilus species. *N. gonorrhoeae* has a TEM-1 type β-lactamase which is typical for many Gram-negative enteric bacilli. In 1983, *N. gonorrhoeae* strains with chromosomal mediated resistance to penicillin and tetracycline were isolated. These strains required 2–4 μg/ml penicillin and 4 μg/ml tetracycline for in vitro growth inhibition. In 1985, plasmid mediated high-level tetracycline resistant *N. gonorrhoeae* strains (TRNG) which required more than 16 μg/ml tetracycline for growth inhibition were isolated in the United States. This plasmid originated from a tetracycline-resistant streptococcus determinant (TetM). In 1985, in The Netherlands twelve *N. gonorrhoeae* strains of type TRNG/PPNG were isolated [52].

Spectinomycin resistance is indicated when more than 64 μg/ml is required for growth inhibition. It is a single-step chromosomally mediated resistance in which the susceptibility of *N. gonorrhoeae* ribosomes for spectinomycin is altered. This alteration is highly drug-specific.

Spectinomycin-resistant gonococci have been isolated mainly in south east Asia and in England. In South Korea, it was observed that 8% of the *N. gonorrhoeae*

strains isolated from the american soldiers in 1985 were resistant to spectinomycin. Ten cases were reported in the United States between 1985 and 1986 [53]. The high incidence of spectinomycin resistance among american soldiers in South Korea was attributed to its widescale use of the drug [54]. Resistance to spectinomycin has not yet been observed in The Netherlands.

Kanamycin-resistant *N. gonorrhoeae* strains have not yet been reported, but in vitro susceptibility is declining [55].

Trimethoprim-sulphamethoxazole (TMP-SXM) is a suitable alternative to treat gonorrhea. Nine tablets of TMP-SMX given for 3 days cured 96% of the men with uncomplicated gonorrhea [57]. Four tablets of TMP-SMX given for 2 days cured 97% of the men with gonococcal urethritis. Chromosomally mediated resistance to trimethoprim-sulphamethoxazole has been reported in the literature.

N. gonorrhoeae is also able to develop non-specific resistance. A single-step alteration which results in reduced susceptibility to many agents such as antibiotics, detergents and colour pigments. Such non-specific resistance involves changes in the cell surface resulting in reduced permeability of the outer membrane or an alteration in the number of peptidoglycan cross-links. The exact mechanism is not known.

Thiamphenicol is also used for treating gonorrhea. In 1984, a large series of clinical and microbiological studies on the efficacy of thiamphenicol for the treatment of gonorrhea was published [56]. A single oral dose of 2.5 g thiamphenicol resulted in a cure rate varying from 59% to 99%. A decreased susceptibility of *N. gonorrhoeae* for thiamphenicol has been reported from south east Asia and from some parts of Africa. Thiamphenicol is unsuitable for treating rectal and oropharyngeal gonococcal infections.

Disappointing results with therapy-failures in 25% of the cases were observed when erythromycin was used to treat gonococcal infections. In vitro, many strains of *N. gonorrhoeae* were resistant to erythromycin [59].

Pharmacokinetic studies and in vitro research on the first generation cephalosporins showed that they were unsuitable for treating gonorrhea [60]. However, certain second generation cephalosporins were found to be suitable for treating gonorrhea. In vitro, the MIC 90 of cefuroxime for PPNG and non-PPNG strains was observed to be 1 µg/ml. A cure rate of more than 96% was obtained with intramuscular cefuroxime at a dose of 1.0 g. Treatment of uncomplicated gonorrhea with cefonicid resulted in cure rates varying from 94% to 96% [61,62]. The use of third generation cephalosporins and quinolones in gonococcal infections is described in chapter 1.2.1.

The present recommendations by WHO and CDC in Atlanta (Georgia, USA), for the treatment of gonococcal infections have all evolved under the influence of factors such as the spread of antibiotic-resistant strains (including PPNG and TRNG strains), the spread of strains with chromosomally mediated multi-antibiotic resistance, the frequent occurrence of coinfection with *C. trachomatis*, the knowledge of possible serious complications of gonococcal and chlamydial infections, and the lack of reliable, rapid and payable tests for detecting *C.*

trachomatis infections. The present recommendations for therapy from CDC in Atlanta are as follows. A single intramuscular (im) injection of 250 mg ceftriaxone followed by oral (po) 100 mg doxycycline twice daily for 7 days. Alternatives are 2.0 g spectinomycin (im), 500 mg ciprofloxacin (po), 800 mg norfloxacin (po), 1.0 g cefuroxime axetil & 1.0 g probenecid (po), 1.0 g cefotaxime (im) and 500 mg ceftizoxime (im). All these anti-gonococcal therapies are given as a single dose and should be supplemented with anti-chlamydial therapy comprising 100 mg doxycycline (po) twice daily for 7 days [63].

The epidemiology of gonococcal infections is complex. Factors that influence the prevalence depend on diverse demographical, sociological, clinical and biological variables. The clinical presentation of gonococcal infections depends on the race, sexe, age and sexual orientation. The study of gonococcal infections should, therefore, be multidisciplinary and falls within the scope and interests of social scientists, epidemiologists, clinicians and microbiologists. The prominent increase in the incidence of gonococcal infections which began in 1960 came to an abrupt end in the middle of the 1980s and reversed into a sharp decline [64]. The number of cases of gonococcal infections reported in The Netherlands in 1985 was 12451. This number declined steadily to 3024 reported cases in 1989. In 1990 there was an increase to 3669 cases. The total number of reported cases since 1981 are shown in Figure 1.

At the University Hospital Rotterdam-Dijkzigt, a steady decline in the number of reported patients with gonococcal infections began in 1984. In 1983, there were still

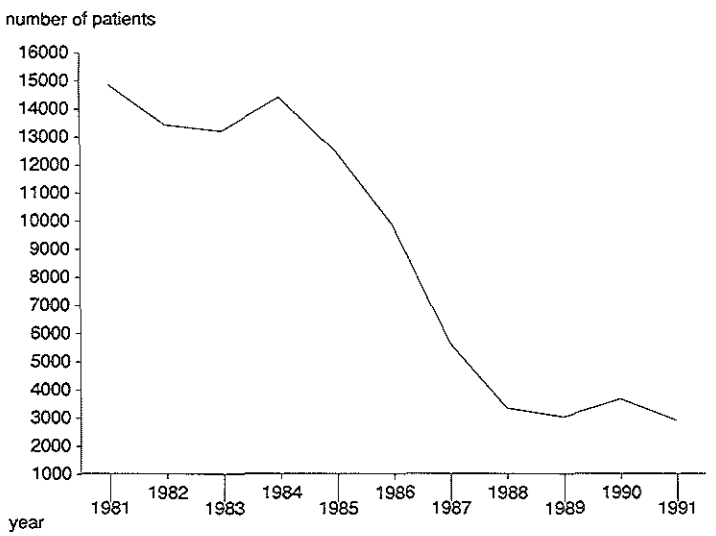

Figure 1. Number of patients suffering from gonococcal infections in The Netherlands. Registrated by the medical head inspector of public health, from 1981 till 1991.

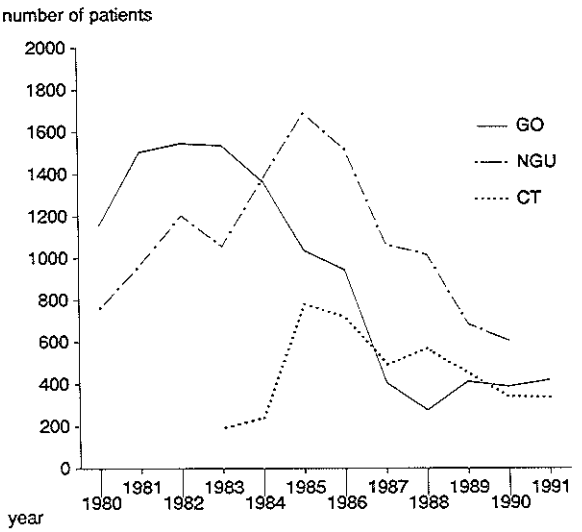

Figure 2. Number of patients suffering from gonococcal (GO), *Chlamydia trachomatis* (Ct) infections and the number of men suffering from non-gonococcal urethritis (NGU) in the period 1980-1991 visiting the out-patient clinic for sexually transmitted diseases at the University Hospital Rotterdam-Dijkzigt.

1539 reported cases which declined to 1367 cases in 1984 and declined further to 287 reported cases in 1989 (Figure 2). This reduction in the total number of reported patients with gonococcal infection was not only observed in The Netherlands but also in other industrial nations and could have been probably due to the changes in sexual behavior coinciding with the arrival of Human Immunodeficiency Virus (HIV) infections [65].

In The Netherlands, data compiled in 1988 within the framework of surveillance for penicillinase-producing gonococcal strains indicated that despite a significant reduction in the incidence of gonococcal infections, the prevalence of PPNG strains was increasing. Fourteen percent of the gonococcal strains isolated were observed to produce penicillinase. In 1986, 9.6% and in 1987 11.4% of the isolated gonococcal strains produced penicillinase. From a total of 416 penicillinase-producing gonococcal strains, 9.1% were also observed to possess, in addition, a plasmid mediated resistance to tetracycline [66]. Figure 3 gives a review of these data in the period 1986-1990.

A detailed mapping of which particular *N. gonorrhoeae* strains are in circulation at any given moment is not only important for epidemiologists but also essential for clinical drug research. To date, gonococcal strains are typed using auxotyping, serotyping, lectin agglutination as well as susceptibility testing to a variety of antibiotics.

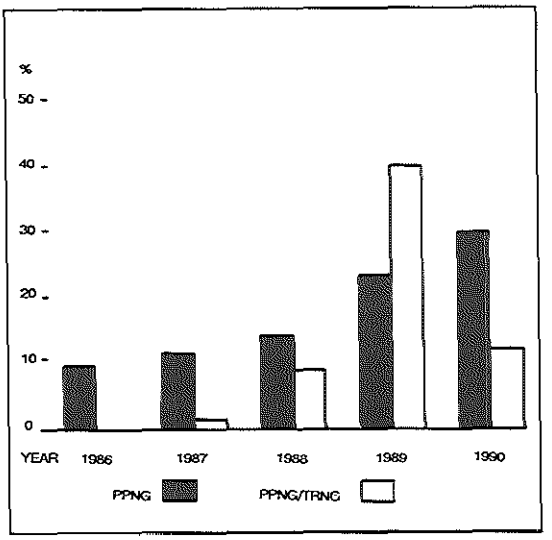

Figure 3. Prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG) and penicillinase-producing tetracycline resistant *N. gonorrhoeae* (PPNG/TRNG) in The Netherlands, from 1986-1990.

1.2.1. New drugs in the treatment of gonococcal infections

An antibiotic comes into consideration for treating gonococcal infections provided it has a low MIC, achieves a high peak serum level and has a long half-life time. There are no exact conditions or criteria which these variables must satisfy. In order to obtain insight into the relationship between the MIC value, the serum concentration and the duration for which this concentration is maintained for a given antibiotic, Jaffe et al [69], conducted investigations in volunteers who were infected with a gonococcal strain of known MIC for penicillin. The results showed that if the peak level of penicillin in the serum was 3 to 4 times as high as MIC of the infecting strain and if this level was maintained for 7 to 10 hours, then this could serve as an indicator for the effectiveness of the therapy. Whether this also applies to other antibiotics in not known.

According to the WHO, a drug for treating gonococcal infections must satisfy the following criteria: it should have a cure rate of 100%, should be usable in both men and women irrespective of age, should be administrable in a single dose, should be non-toxic, should have no cross-reaction with other antibiotics, should be non-allergenic, should invite no resistance formation, should not be the sole drug effective against other serious infections, should be effective against at the same time aquired syphilis and *C. trachomatis* infections, should produce a low incidence of post-gonococcal urethritis and should be payable[70].

The Quinolones

The quinolones are closely related to nalidixic acid (in use since 1962) as far as their structure and mode of action is concerned. Antibacterial activity of quinolones is mediated via the inhibition of bacterial DNA-gyrase which is essential for DNA-transcription and for the generation of messenger RNA. Quinolones are broad spectrum antibiotics with a bactericidal action. In vitro studies have shown that the newer quinolones have much lower MICs than nalidixic acid against *N. gonorrhoeae*. In vitro activity of various quinolones against *N. gonorrhoeae* are depicted in Table 2. The quinolones have also been tested extensively against penicillinase-producing gonococcal strains. Taking into account their unique mode of action, it is not surprising that PPNG strains are highly susceptible to quinolones and that there is no correlation between the MIC values of quinolones and the production of β-lactamase. The quinolones are as effective against chromosomally mediated penicillin resistant as well as tetracycline resistant gonococcal strains. Clinical investigations should demonstrate whether the very promising MIC values except that of nalidixic acid would be effective in treating gonococcal infections and whether the mutual differences in the MIC values of quinolones would be of clinical value and also be reflected in their clinical efficacy. The side-effects of quinolones are dose-dependent and comprise gastrointestinal disorders (3 to 5.6%), central nervous system disorders (0.9 to 4.4%) and allergic reactions (0.5 to 2.2%) [83].

An up-to-date review of clinical studies in men is given in Table 3.

Table 2.In vitro activity of quinolones against *Neisseria gonorrhoeae*

quinolone	(total no. isolates/ no. of PPNG's)	MIC (μg/ml)			references
		range	50%	90%	
difloxacin	(30/3)	<0.0039-0.03	-	-	72
PD 127-391	(30/?)	0.001-0.008	0.001	0.001	71
ciprofloxacin	(1172/128)	0.0005-0.12	0.002	0.008	75
rosoxacin	(2560/515)	<0.001-1.0	0.03	0.06	75
norfloxacin	(955/200)	<0.0015-0.5	0.03	0.06	75
A-56620	(30/?)	0.004-0.008	0.008	0.016	71
fleroxacin	(773/143)	0.004-1.0	0.015	0.03	75
ofloxacin	(601/97)	0.004-0.5	0.015	0.06	75
lomefloxacin	(342/40)	0.004-0.5	0.005-0.015	0.03-0.06	71, 76-78, 79
pefloxacin	(149/10)	0.008-0.12	0.015-0.016	0.03-0.06	71, 80, 82
enoxacin	(496/59)	<0.0125-1.6	0.03	0.06	71
nalidixic acid	(60/30)	0.25-2.0	1.0	1.0	71
sparfloxacin	(250/49)	-	-	0.0063-0.0125	73
sparfloxacin	(56/7)	0.001-0.02	0.004	0.06	74
temafloxacin	(55/?)	0.001-0.12	0.008	0.03	203, 250

Table 3. Quinolones in the treatment of uncomplicated gonorrhea in males.

regimen (single oral dose)		cases cured/cases treated (% cured)			references
		urethra	rectum	pharynx	
ciprofloxacin	100 mg	71/71 (100)	-	-	84-86
	250 mg	334/334 (100)	10/10 (100)	7/18 (88)	84, 86-93
	250 mg	-	26/26 (100)	15/16 (94)	87
	250 mg	83/83 (100)	-	-	88
	500 mg	84/84 (100)	5/5 (100)	-	90, 91
enoxacin	400 mg	99/100 (99)	2/2 (100)	0/3/ (0)	94, 95
	400 mg/800 mg	-	38/38 (100)	10/10 (100)	15
difloxacin	200 mg	29/29 (100)	-	-	72
fleroxacin	400 mg	97/97 (100)	-	3/3 (100)	96-99
	400 mg	15/15 (100)	-	-	101
	200 mg	16/16 (100)	-	-	100
	400 mg	45/45 (100)	-	-	100
temafloxacin	200 mg	31/32 (97)	-	-	203
	400 mg	40/40 (100)	-	-	203
norfloxacin	800 mg	173/173 (100)	-	2/2 (100)	102, 103
ofloxacin	100 mg	26/26 (100)	-	-	104
	200 mg	24/24 (100)	-	-	104
	400 mg	211/215 (98)	19/19 (100)	8/9 (89)	105-108
	600 mg	32/34 (94)	-	-	105
	800 mg	28/29 (96)	-	-	105
pefloxacin	800 mg	35/35 (100)	-	1/1 (100)	80
	800 mg	65/65 (100)	-	-	81
rosoxacin	100 mg	5/18 (28)	-	-	109
	200 mg	15/17 (88)	-	-	109
	300 mg	40/42 (95)	2/2 (100)	-	109-110
	400 mg	16/17 (94)	-	-	109

Ciprofloxacin

Excellent results were obtained at doses of 100 mg, 250 mg and 500 mg. Excellent results were also obtained in rectal gonorrhea. The number of patients with oropharyngeal gonorrhea evaluated was too small to allow any firm conclusions.

Ciprofloxacin was well tolerated and side-effects were minimal and transient.

Enoxacin

Satisfactory results were obtained with 400 mg. In a study, enoxacin at doses of 400 mg and 800 mg was reported to be highly effective in patients with rectal- and or pharyngeal gonorrhea [15]. Enoxacin was well tolerated by all patients who have been treated to date.

Difloxacin

Difloxacin at a dose of 200 mg was highly effective in treating gonococcal infections. To date, only patients with urethral gonococcal infections have been treated. In 4 patients with *C. trachomatis* coinfection, chlamydia could not be

cultured after treatment. This drug was not investigated in further studies since side-effects were observed in 65.5% of the patients who were treated.

Fleroxacin

Fleroxacin at a dose of 400 mg was highly effective in treating gonococcal infections. To date, patients with rectal gonorrhea have not been treated and although the number of patients suffering from oropharyngeal gonorrhea who were treated was too small to allow any firm conclusion, all 3 patients who were treated were cured. No side-effects were observed.

Norfloxacin

Norfloxacin at a dose of 800 mg was highly effective in treating gonococcal infections. To date, no information is available on treatment of rectal gonorrhea. The number of patients with oropharyngeal gonorrhea who were treated was too small to allow any definite conclusion. The results in 2 patients with oropharyngeal gonorrhea who were treated were very promising. Side-effects were mild, although in one study side-effects were observed in 23.7% of the patients [102].

Ofloxacin

At doses of 100 mg, 200 mg, 400 mg, 600 mg and 800 mg ofloxacin, excellent results were obtained. Satisfactory results were also obtained in patients with rectal- and pharyngeal gonorrhea who were treated with 400 mg ofloxacin. The drug was well tolerated.

Pefloxacin

All 100 patients who were treated with 800 mg pefloxacin were completely cured. No patients with rectal gonorrhea have yet been treated, but one patient with oropharyngeal gonorrhea was successfully treated. No side-effects were observed.

Rosoxacin

Rosoxacin at doses of 100 mg and 200 mg was not effective in treating gonococcal infections. At doses of 300 mg and 400 mg, rosoxacin showed lower cure rates of 94% and 95% respectively as compared with other antibiotics in the same group. On the average, side-effects were observed in 51% of the patients who were treated [109,110].

Temafloxacin

At doses of 200 mg and 400 mg excellent results were obtained. No data are available obtained in patients with rectal and pharyngeal gonorrhea. Both treatment regimes were well tolerated.

Conclusion

The results of clinical investigations with these drugs correlated well with the results of in vitro studies except that rosoxacin scored lower in clinical studies. In studies in which *C. trachomatis* was cultured, only difloxacin at the single dose given was effective in eliminating the infection.

All the quinolones except difloxacin were well tolerated. The occurrence of post-gonococcal urethritis was not different from other anti-gonococcal therapies.

The third generation and new oral cephalosporins

Cefotaxime, cefoperazone, ceftriaxone, ceftazidime, cefmenoxime, cefsulodin and cefodizime are among those that belong to this group of antibiotics. All these bactericidal antibiotics have antibacterial activity similar to that of broad spectrum penicillins. These drugs are only suitable for parenteral administration which limits their use. Therefore, cephalosporins for oral use were developed. The first oral cephalosporins were cephalexin and cephardine. The subsequently developed cefaclor is β-lactamase unstable. Cefuroxime axetil is an ester of cefuroxime and belongs to the second generation cephalosporins and is suitable for oral administration. After absorption in the gastrointestinal tract, cefuroxime axetil is unesterified to cefuroxime. Newer drugs are ceftetrame, cefetamet, cefetamet pivoxil and cefixime. A review of in vitro activities of various cephalosporins against *N. gonorrhoeae* is given in Table 4. With the exception of cephalexin and cefadroxil, all the others mentioned in Table 4 are highly effective against *N. gonorrhoeae*.

The therapeutic effectiveness of cephalosporins already in clinical use to treat uncomplicated gonorrhea are depicted in Table 5.

Cefotaxime

Intramuscular cefotaxime at doses of 0.5 g and 1.0 g was highly effective in treating gonococcal infections. Excellent results were also obtained in patients with rectal gonorrhea who were treated with 1.0 g cefotaxime. Although, the number of patients with oropharyngeal gonorrhea who were treated was too small

Table 4. In vitro activity of third generation and new oral cephalosporines against *Neisseira gonorrhoeae*.

compound	(total no. isolates/ no. of PPNG's)	MIC (μg/ml)			references
		range	50%	90%	
cefotaxime	(227/77)	0.001-0.125	0.003-0.03	0.007-0.125	112-115
ceftazidime	(207/77)	0.003-0.12	0.016-0.03	0.032-0.06	112, 114
cefodizime	(123/42)	0.004-0.25	0.004-0.05	0.016-0.25	112, 113, 116
ceftizoxime	(102/2)	0.001-0.004	-	0.004	117
cefpimizole	(46/1)	0.015-1.0	-	-	118
	(11/2)	0.1-1.6	<0.4	<0.8	119
ceftriaxone	(107/35)	<0.001-0.007	0.001	0.003	114
ceftibuten	(50/26)	<0.002-0.12	0.008	0.03	120
cefetamet	(39/5)	0.004-1	0.015-0.12	0.25-1	115, 121
ceftetrame	(39/5)	0.004-0.25	0.008-0.03	0.12	115, 121
cephalexin	(24/5)	0.06-16	1	4	121
cefadroxil	(24/5)	0.12-32	4	8	121
cefetamet pivoxil	(41/7)	0.001-0.12	0.007-0.004	0.015-0.008	122, 132
cefuroxime axetil	(124/0)	0.001-4.0	<0.03	<0.12	123
cefixime	(400/100)	<0.001-0.063	0.004-0.016	<0.05	124, 126

Table 5. Third generation and new oral cephalosporins in the treatment of uncomplicated gonorrhea in males.

cephalosporine	dose	Cases cured/cases treated (% cured)			references
		urethra	rectum	pharynx	
cefotaxime	0.5 g im	95/95 (100)	-	3/4 (75)	127
	1.0 g im	297/300 (99)	20/20 (100)	10/11 (91)	128
ceftizoxime	1.0 g im	49/49 (100)	-	-	117
cefpimizole	0.125 g im	17/24 (71)	0/1 (0)	1/2 (50)	118
	0.25 g im	20/24 (83)	-	-	118
	0.5 g im	19/21 (90)	3/3 (100)	0/1 (0)	118
	1.0 g im	22/22 (100)	1/2 (50)	0/1 (0)	118
ceftriaxone	125 mg im	122/122 (100)	52/52 (100)	16/16 (100)	129, 130
	250 mg im	533/538 (99)	-	58/58 (100)	94, 129, 131
	250 mg im	81/82 (99)	-	-	88
	500 mg im	133/134 (99)	-	-	129
cefetamet pivoxil	0.4 g po	10/10 (100)	-	-	132
	0.8 g po	12/12 (100)	-	-	132
	1.2 g po	10/10 (100)	-	-	132
	1.2 g/				
	1.5 g po	74/74 (100)	-	-	133
cefuroxime axetil	1.0 g po	61/64 (95)	7/9 (78)	0/1 (0)	123, 134
	1.5 g po	106/107 (99)	5/5 (100)	-	135
cefuroxime axetil + probenecid 1 g.	1.0 g po	29/30 (97)	6/6 (100)	-	123
cefixime	800 mg po	96/97 (99)	1/1 (100)	6/8 (75)	124
	800 mg po	63/63 (100)	6/6 (100)	6/7 (86)	249
	400 mg po	56/58 (97)	10/10 (100)	8/8 (100)	249

to allow definite conclusions, treatment with 1.0 g appeared promising. Side-effects were minimal and transient.

Ceftizoxime

In 1984, a small number of patients were treated with ceftizoxime with excellent results. Since at the beginning of the study, a majority of patients experienced pain at the injection-site, mepivacaine was added to the regimen. Additional side-effects were not observed.

Cefpimizole

In a dose-finding study in 1986, patients were treated with cefpimizole at doses of 0.125 g, 0.25 g, 0.5 g, and 1.0 g. Satisfactory results were obtained in the small number of patients using 1.0 g. Since the number of patients with rectal- and pharyngeal gonorrhea was too small, no firm conclusions concerning the therapeutic effectiveness could be drawn. Pain at the injection-site in 59% of the patients was the only observed side-effect. The investigators concluded that cefpimizole offered no additional advantage over the already existing anti-gonococcal therapies.

Ceftriaxone

Excellent results were obtained at doses of 125 mg, 250 mg and 500 mg ceftriaxone used to treat patients with urethral, rectal and pharyngeal gonococcal infections. The drug was well tolerated by all patients.

Cefetamet pivoxil

In a dose-finding study, a small number of patients were treated with cefetamet pivoxil at doses of 0.4 g, 0.8 g and 1.2 g. Excellent results were obtained. No patients with rectal- or pharyngeal gonorrhea were treated [132]. Mild side-effects were observed in a few patients. In an earlier study in 1988 in which a large number of patients with urethral gonorrhea were treated with cefetamet pivoxil, excellent results were obtained. The drug was well tolerated by all patients.

Cefuroxime axetil

At doses of 1.0 g and 1.5 g cefuroxime axetil, satisfactory results were obtained. Since only 7 of the 9 patients with rectal gonorrhea who were treated with cefuroxime axetil were cured, in a subsequent study, 1g probenecid was added to the regimen. In six patients with rectal gonorrhea, who were treated with such regimen, *N. gonorrhoeae* could not be cultured from any case after treatment. Treatment with 1.5 g cefuroxime axetil cured 106 of the 107 (99%) patients with urethral gonorrhea and 5 of the 5 (100%) patients with rectal gonorrhea. The drug was well tolerated by all patients. No comment concerning the effectiveness of cefuroxime axetil in patients with oropharyngeal gonorrhea can be made since no patients have yet been treated.

Cefixime

At a dose of 800 mg and 400 mg satisfactory results were obtained. The drug was well tolerated.

Conclusion

Cephalosporins both for oral and parenteral use, with the exception of cefpimizole, have fulfilled the expectations. Ceftriaxone is the most effective of the parenteral drugs for treating urethral-, rectal- and oropharyngeal gonorrhea. Cefotaxime produced not only satisfactory results in the treatment of patients with urethral gonorrhea, but also produced excellent results in the treatment of patients with rectal gonorrhea. No comments can be made concerning the effectiveness of the remaining drugs in treating rectal and pharyngeal gonorrhea because only a limited number of patients have been treated to date.

The new oral cephalosporins are also effective in the treatment of urethral gonorrhea. To date, only a limited number of patients with rectal- and pharyngeal gonorrhea have been treated with these drugs to allow any firm conclusions concerning their therapeutic effectiveness.

The frequency of post-gonococcal urethritis in patients who were treated did not differ from that in patients who were treated with the new quinolones. At the single

dose that was used in the above mentioned studies, cephalosporins failed to effectively eliminate *C. trachomatis* coinfection.

The criteria to which a drug must satisfy to be used for treating gonococcal infections include low MIC values, high peak serum values and a long half-life. In Table 6, a summary of these variables and therapeutic effectiveness is shown. Although quinolones and cephalosporins are highly effective in treating gonococcal infections, the ideal drug as proposed by the WHO still remains elusive. The quinolones are ineffective in treating syphilis, are contraindicated in children and in pregnancy due to cartilage-abnormalities observed in animals treated with quinolones. Given as a single dose, quinolones are also ineffective in treating *C. trachomatis* infections and in reducing post-gonococcal urethritis. At this moment the quinolones are too expensive for the third world. Advantages of the quinolones are: the oral use, no cross resistance with other antibiotics. In the future resistance of *N. gonorrhoeae* for quinolones could form a problem.

Cephalosporins, are ineffective against *C. trachomatis* infections and can mask a coinfection with *T. pallidum*. The development of cephalosporins for oral use can be beneficial. There is a small chance for a cross resistance with penicillin. Chromosomal mediated penicillin resistant gonococci (CMRNG) are also less susceptible to cephalosporins. [149]

Table 6. Pharmacokinetics of the various drugs used in the treatment of uncomplicated gonorrhea.

drug	dose	mean peak serum level (µg/ml)	T 1/2 (hours)	MIC 90 (µg/ml)	efficiency (%)	references
Quinolones:						
ciprofloxacin	500 mg po	2.3	3.9	0.008	100	75, 84-93, 137
difloxacin	200 mg po	2.2	26	0.01	100	72
enoxacin	400 mg po	3.7	6.2	0.06	90-100	15, 71, 94, 95, 138
fleroxacin	400 mg po	5.0	10	0.03	100	75, 96-99, 140
norfloxacin	800 mg po	2.4	4	0.06	100	75, 102, 103, 136
ofloxacin	400 mg po	4.4	6	0.06	94-100	75, 104-108, 139
pefloxacin	400 mg po	5.8	11	0.03	100	71, 80, 82, 141
rosoxacin	300 mg po	6.4	5	0.06	96-100	75, 109, 110
sparfloxacin	200 mg po	0.95	15	0.0125	-	111
temafloxacin	400 mg po	5.3	7.6	0.03	100	203, 204
Cephalosporines:						
cefotaxime	1 g im	20.5	1.3	0.016	99-100	60, 112-115, 127, 128
cefodizime	1 g im	59	3.8	0.008	-	112, 113, 116, 142
ceftizomixe	1 g im	40.9	1.9	0.004	100	117, 143
cefpimizole	1 g im	38.6	2	0.8	100	118, 144
ceftriaxone	500 mg im	49	7.5	0.003	98-100	94, 114, 129-131, 145
ceftetrame	1.2 g po	7.25	1.5	0.12	-	115, 121, 146
cefetamet pivoxil	1.5 g po	7.4	3	0.015	100	122, 132, 133, 147
cefuroxime axetil ester	1 g po	6.3	1.2	<0.12	95-99	123, 134, 135, 148
cefixime	400 mg po	3.85	3.5	<0.05	99	124, 125, 249

1.3. NON-GONOCOCCAL URETHRITIS

Non-gonococcal urethritis (NGU) is at the present, the most frequently occurring sexually transmitted disease (STD) in the industrialized countries including United Kingdom, United States and The Netherlands [150]. In a study of 1492 patients for genital pathogens, *N. gonorrhoeae* was cultured from 9.6% of the men, *C. trachomatis* was cultured from 29.6% of the men and *U. urealyticum* was cultured from 30.2% of the men [151]. The causes of NGU are summarized in Table 1. In 35-60% of the patients, *C. trachomatis* was not only the cause but was also responsible for the subsequent complications. There seems to be a world-wide Chlamydia epidemic [152]. In 25-50% of the cases of NGU, one gropes in the dark as far as the cause is concerned [153].

Genus Chlamydia can be divided into 3 sub-groups, *Chlamydia trachomatis*, *Chlamydia psittaci* and *Taiwan acute respiratory* (TWAR)*Chlamydiae* [154]. They are obligate intracellularly dividing bacteria. To date 15 serotypes of *Chlamydia trachomatis* have been identified using immunofluorescence techniques. Serotypes A, B, Ba and C are associated with trachoma. Serotypes D, E, F, G, H, I, J and K occur in oculogenital infections. Serotypes L1, L2 and L3 occur in lymphogranuloma venereum. An investigation to the serotypes of isolates from urethra and cervix of patients who attended the STD clinic of the University Hospital Rotterdam-Dijkzigt showed that types D, E, F, H and K occurred most frequently [155]. In men, *C. trachomatis* causes NGU, post-gonococcal urethritis, epididymitis, proctitis, conjunctivitis and Reiter's syndrome [156].

There are no clear clinical differences between gonococcal and non-gonococcal urethritis, though generally, the symptoms of non-gonococcal urethritis are less fulminant. The incubation period of a chlamydial urethritis is 21 to 45 days [157]. Chlamydial urethritis occurs more frequently in male younger than 20 years of age [158]. Asymptomatic carriage of *C. trachomatis* in sexually active adolescent boys in some studies was observed to be between 8 and 9% [11,159]. The peak of post-gonococcal urethritis is between 2 and 3 weeks after a successfully treated gonococcal urethritis. In 80 to 100% of the cases, *C. trachomatis* was the cause. The occurrence of *N. gonorrhoeae* and *C. trachomatis* coinfections is estimated at 10 to 50% [160].

Irrespective of the aetiology, about 30% of the patients with NGU either have persisting complaints or relaps within 6 weeks after treatment [150]. The aetiology of persistent or recurrent non-gonococcal urethritis remains unknown. Most cases begin with a typical NGU, without a positive culture. This form of NGU responds poorly to antibiotics generally prescribed for NGU. Infection with antibiotic-resistant microbes such as tetracycline-resistant *U. urealyticum* strains, microbes which cannot be cultured using the routine methods, a reinfection of the urethra from a peripheral focus such as the prostate or a non-infectious cause probably of allergic origin have all been mentioned as possible causes for this type of urethritis.

In addition, poor patient-compliance, a reduced absorption of the drug or a

reinfection via an inadequately treated partner may play a possible role in patients with *C. trachomatis* or *U. urealyticum* infections. In particular, patients with complaints of prostatitis, respond poorly to therapy [150]. In a study on the prevalence of *M. genitalium* in patients with urethritis, it was observed that this microbe played no aetiological role, however this microbe may play a role in persistent and recurrent urethritis [161]. To date, the role of *U. urealyticum* in causing NGU is not clear. *U. urealyticum* was isolated from 20% of the patients younger than 50 years without complaints of urethritis attending a non-venereological out-patient clinic. From between 7 to 10% of the patients older than 50 years, *U. urealyticum* was isolated [162]. In sexually inactive men, the presence of ureaplasmas in the urethra could be demonstrated for a year or longer, but disappeared later.

B. ureolyticus, an anaerobic microbe is observed more frequently in patients with NGU than in patients without urethritis. In a double blind placebo controlled study with metronidazole in patients with *C. trachomatis* negative urethritis, it was observed that 7 of the 9 patients with a positive anaerobe culture responded to metronidazole therapy, whereas, only 1 of the 4 patients in the placebo group responded. Six of the 26 men with a negative anaerobe culture responded to metronidazole [163]. *B. ureolyticus* probably plays a minor role in the aetiology of NGU [163,164].

Trichomonas vaginalis and herpes simplex virus are responsible in less than 5% of the NGU cases.

Clinically, patients with epididymitis caused by *C. trachomatis* present with unilateral testicular pain, swelling and fever. In a study of 51 men with acute epididymitis, it was observed that in 19 of the 42 (46%) heterosexual men and in none of the 9 homosexual men, *C. trachomatis* was the cause [25].

E. coli was the cause in 67% of the homosexual men and in none of the heterosexual men. In addition, bacteriuria occurred frequently in homosexual men. There are only case reports on *U. urealyticum* as the cause of acute epididymitis [165].

The incidence of *C. trachomatis* infections is lower in homosexual men than in heterosexual men.

Proctitis in homosexual men, besides many other causes can also be caused by *C. trachomatis*. In a study in homosexual men with proctitis, it was shown that *C. trachomatis* and *N. gonorrhoeae* could be cultured from respectively 10% and 31% of the patients [166]. *C. trachomatis* play no role in prostatitis [167,168].

Aseptic arthritis occurs in approximately 3% of the cases as complication of STD and intestinal infections with certain bacteria [169]. The term "sexually acquired reactive arthritis" (SARA) should not be used here since only a proportion is caused via a sexually transmitted disease and stigmatization of the patients must be avoided. Urethritis, arthritis and conjunctivitis form the classic triad in Reiter's syndrome.

About one third of the patients with a reactive arthritis have the classic Reiter's Syndrome.

C. trachomatis could be isolated from the urethra of one-third of the patients and in one-half of the patients elevated titres of IgG antibodies to *C. trachomatis* were present [170, 171]. The natural course of Reiter's syndrome is that two-thirds of the patients heal within 6 months. Healing is generally quicker in patients who are HLA-B27 negative, in whom only a single or a few joints are affected and who have no extra articular lesions. In 15% of the cases, the disease follows an aggressive course with the destruction of affected joints [172]. Psoriasis and Reiter's syndrome are regarded by some as variants of the same cutaneous disease [173,174].

Chlamydial conjunctivitis occurs via accidental digital transfer from the urogenital region. In adults it progresses as an acute follicular conjunctivitis. The course of the disease is self-limiting. Permanent damage of the eye is very rare. Sometimes the course is chronic [175].

In the 1950s, tetracyclines, erythromycin and sulphonamid-aminocyclitol were the most effective therapeutic agents for treating NGU. More recent studies have shown that tetracyclines and erythromycin are effective against *C. trachomatis* and most of the *U. urealyticum* strains [176]. Sulphonamids are effective against *C. trachomatis* but not against *U. urealyticum*. For years, tetracyclines and erythromycin have been the drugs of first choice for treating NGU. Although these drugs generally produce satisfactory results, they are by no means ideal. Tetracyclines have numerous side-effects such as gastrointestinal disorders and overgrowth of candida which presents as vulvovaginitis in women and as balanoposthitis in men. In addition, tetracyclines cannot be used in pregnancy, in lactating mothers and in children. Erythromycin is a suitable alternative for treating above mentioned groups. Besides their gastrointestinal side effects, different erythromycin preparations are unreliable because of bioavailability problems.

Tetracyclines and erythromycin have the same in vitro activity of 0.06 μg/ml against *C. trachomatis* [176]. The MIC 90 of tetracycline against *U. urealyticum* is 1 μg/ml and that of erythromycin is 0.5 μg/ml.

A clinical cure in 80% of the patients with NGU is the maximum obtained with these drugs despite a seeming elimination of *C. trachomatis* [177]. An explanation for this may be the possible polymicrobial aetiology of NGU. Other problems in clinical studies that may be encountered in this field are the duration of therapy, the patient-compliance, recurrence or reinfection, possible ineffective treatment of the partner(s), the follow-up and the reduced bioavailability of tetracycline if taken with milk. Arya et al [178] used six different treatment schedules in order to investigate the dose, the length of therapy and the type of tetracycline preparation that was required to obtain the maximum therapeutic effectiveness in patients with NGU. The patients were divided into three treatment groups and were examined for 12 weeks after the start of the therapy. The first group of patients were treated with a single dose of 300 mg doxycycline. Within 2 weeks after treatment, 28 of the 45 (62%) patients had to be retreated due to therapy-failures. The second group of patients were treated with 3 different tetracycline preparations for 7 days.

Oxytetracycline at a dose of 250 mg four times daily for 7 days cured 99 of the

127 (78%) patients. Triple tetracycline at a dose of 300 mg twice daily for 7 days cured 84 of the 120 (70%) patients. Doxycycline at a dose of 200 mg on the first day followed by a dose of 100 mg for 6 days cured 83 of the 113 (74%) patients. The third group of patients were treated for 21 days with triple tetracycline and oxytetracycline using the same doses that were used to treat the patients in the second group. Oxytetracycline treatment cured 66 of the 85 (78%) patients, whereas, triple tetracycline cured 61 of the 72 (85%) patients. The conclusion of this study was that a single 300 mg dose of doxycycline was ineffective in treating patients with NGU. Oxytetracycline treatment for 7 days produced the best results and cured 78% of the patients. The therapeutic effectiveness after 21 days of therapy was not superior to the therapeutic effectiveness after 7 days of therapy and was, therefore, of little clinical value in the routine treatment of patients with NGU. In this study a clear association between renewed therapy and sexual contact during the investigation period was observed. In a study conducted by Juvakoski et al [179], patients with *C. trachomatis* positive urethritis were treated with 200 mg doxycycline on day 1 and subsequently with 100 mg doxycycline for 6 days or with 1.0 g tetracycline chloride for 7 days. Patients were examined 7 and 14 days after therapy. Fifty-five of the 62 (89%) patients who were treated with doxycycline were bacteriologically cured after 7 days, whereas, 45 of the 55 (82%) patients who were treated with tetracycline were bacteriologically cured. Five patients (8%) who were treated with doxycycline and 7 patients (13%) who were treated with tetracycline relapsed. When the number of leukocytes were examined in the urine sediment after 7 days of therapy, all 62 (100%) patients who were treated with doxycycline and 53 of 55 (96%) patients who were treated with tetracycline were cured. At 14 days after therapy, *C. trachomatis* could not be cultured either from any of the returning 54 patients who were treated with doxycycline or from any of the returning 42 patients who were treated with tetracycline. Evaluation of urine sediments 14 days after therapy showed no abnormalities in patients who were treated with doxycycline, but abnormalities in the urine sediment were observed in 2 patients who were treated with tetracycline.

It can be concluded that neither of the two tetracyclines at the particular doses used was suitable for treating *C. trachomatis* positive urethritis. Absence of abnormalities in urine sediment is no guarantee that chlamydial infection is over and examination later than 14 days after treatment is not meaningful. Bowie [180] compared the effectiveness of 1.0 g and 2.0 g tetracycline given for 7 days in 200 men with urethritis. *C. trachomatis* was isolated from 40% of these patients and *U. urealyticum* was isolated from 48% of these patients. *U. urealyticum* was isolated more frequently from patients with chlamydia negative urethritis and from men with 10 or fewer sexual partners during their life-time. Patients were examined 3, 14 and 35 days after therapy. Therapy-failures were observed in 28 of the 76 (37%) patients who were treated with 250 mg tetracycline four times daily and in 22 of the 67 (33%) patients who were treated with 500 mg tetracycline four times daily. When tissue culture results were taken into account, therapy-failures were observed in 13

of the 59 (22%) patients with chlamydia positive urethritis and in 37 of the 84 (44%) patients with chlamydia negative urethritis. When treatment schedule was taken into account, therapy-failures were observed in 5 of 29 (17%) chlamydia positive patients who were treated with 1.0 g tetracycline and in 8 of the 30 (27%) chlamydia positive patients who were treated with 2.0 g tetracycline. Therapy-failures were observed in 17 of the 42 (40%) patients with *U. urealyticum* positive urethritis who were treated with 1.0 g tetracycline and in 9 of the 30 (30%) patients with *U. urealyticum* positive urethritis who were treated with 2.0 g tetracycline. It can be concluded that at both doses, tetracyclines were equally effective in treating patients with *C. trachomatis* and *U. urealyticum* positive urethritis.

Therapy-failures were identical in both groups. Therapy-failures were frequenter in patients with chlamydia negative urethritis and in men in whom *U. urealyticum* persisted after treatment. Tjiam et al [181] compared two different dose schedules of doxycycline to treat NGU. Patients were examined 1 day after treatment. The first group of patients was treated with 200 mg doxycycline on the first day followed by 100 mg doxycyline for 6 days. The second group of patients was treated with 200 mg doxycycline for 7 days. *C. trachomatis* was isolated from 40 of the 117 (34%) patients. In the first group, 51 of the 59 (87%) patients were cured and in the second group 49 of the 58 (84%) patients were cured. When the *C. trachomatis* culture results were taken into account, in the first group 20 of the 23 (87%) patients with chlamydia positive urethritis were cured and in the second group 13 of the 17 (76%) patients with chlamydia positive urethritis were cured. In the first group 31 of the 36 (86%) patients with chlamydia negative urethritis were cured and in the second group 36 of the 41 (88%) patients with chlamydia negative urethritis were cured. Chlamydia was cultured from 3 patients who were considered clinically cured. There were no differences in the cure rates of chlamydia positive urethritis and chlamydia negative urethritis.

Scheibel et al [182] compared the effectiveness of tetracycline and erythromycin in patients with chlamydia positive urethritis. Patients were examined 1 and 7 days after therapy.

Bacteriological cure was observed in 34 of the 36 (94%) patients who were treated with 250 mg erythromycin four times daily for 7 days and in 33 of the 35 (94%) patients treated with 250 mg tetracycline four times daily for 7 days. No abnormalities in the urine sediments were observed after treatment in any of the patients. One patient who was treated with erythromycin and 3 patients who were treated with tetracycline still had dysuria. Both drugs were equally effective in treating chlamydia positive patients with NGU.

At the present time, 100 mg doxycycline twice daily for 7 days or 500 mg tetracycline four times daily are recommended for treating patients with NGU [63]. As alternative therapies, the following are recommended: erythromycin base 500 mg four times daily for 7 days, 800 mg erythromycin ethylsuccinate four times daily for 7 days, 400 mg erythromycin ethylsuccinate four times daily for 14 days or 250 mg erythromycin base four times daily for 14 days.

In order to obtain insight into the compliance of patients with NGU who were treated for 3 weeks with 250 mg tetracycline four times daily, the tetracycline tablets were marked with phenobarbitone [183]. Patients were requested to return for examination after 2 weeks. Thirty-nine of the 62 (63%) patients failed to return for examination. Nine of the 33 (27%) evaluable patients had not complied with the therapy schedule and was also admitted as such by these patients. The phenobarbitone serum levels were too low in these patients. Five patients assured having complied with the therapy schedule but had too low phenobarbitone serum levels. Only 18 patients had phenobarbitone levels which were identical to the serum levels in volunteers who had been treated using the same therapy schedule. In these patients there was no evidence that they had not taken the medication properly. Poor therapy compliance was the reason for therapy-failure only in 1 patient.

No reliable data are available in The Netherlands on the correlation between the occurrence of NGU and *C. trachomatis* infections. The number of patients with NGU at the STD clinic of the University Hospital Rotterdam-Dijkzigt began to decrease after 1985. This decrease was less than that of patients with gonococcal infections (Figure 2). At present, the number of patients with NGU is three times as high as that of patients with gonorrhea. In Figure 2, a survey of the numbers of patients since 1980 is given. The number of *C. trachomatis* infections in men and women at the STD clinic of the University Hospital Rotterdam-Dijkzigt had increased from 191 positive cultures in 1983 to 357 positive cultures in 1989. This increase was attributed to an improved accessibility for culturing *C. trachomatis*. The percentage of cultures that were positive during this period remained approximately the same (± 10%) with the exception of 1984 during which 5.2% of the cultures were positive. In an investigation into the occurrence of *C. trachomatis* infections at the STD clinic of the Groenburgwal in Amsterdam, it was observed that 14.3% of the men had a *C. trachomatis* infection and 11.5% of the men had gonorrhea. The number of *C. trachomatis* infections in bi- and homosexual men was extremely low [184].

A possible cause for the less prominent reduction in NGU as compared to gonorrhea may lie in the fact that NGU affects heterosexual men who may feel less at risk for infections with HIV and did not change their sexual behavior. In the beginning, the anti-AIDS campaign was mainly directed at the high risk groups such as homosexual men and intravenous drug users. This campaign received a broader basis later on. In the United States also, the number of chlamydial infections is twice as high as the number of gonococcal infections [185].

Untill recently, *C. trachomatis* has not developed resistance to tetracyclines [186]. A decreased in vitro susceptibility of *C. trachomatis* for erythromycin is suspected [187].

Jones et al [188] isolated *C. trachomatis* strains resistant to tetracycline from five patients. These isolates were also resistant to doxycycline, erythromycin, sulfamethoxazole and clindamycin. The clinical significance of this resistance is not known. Tetracycline resistance of *U. urealyticum* has been known for some time and

defined as MIC > 64 µg/ml. The incidence of in vivo resistance of *U. urealyticum* to tetracycline in patients with NGU was estimated at 6 to 10% [189].

Investigations by Robertson et al [190], showed that in tetracycline-resistant *U. urealyticum* strains, no plasmid was present, but a tetM sequence was detected. Twenty-six tetracycline-resistant strains were tested using various antibiotics. All strains were susceptible to rosaramycin but were resistant to 2 mg/l erythromycin.

1.3.1. New drugs in the treatment of non-gonococcal urethritis

At present, 200 mg doxycycline for 7 days is the drug of first choice for treating patients with NGU. As second choice, 500 mg tetracycline four times daily for 7 days is recommended. Doxycycline is chosen in the hope of increasing patient-compliance and reducing the risk of side-effects. Similar to tetracycline, the bioavailability of doxycycline is also reduced when taken with milk [191].

Erythromycin is the drug of first choice in patients who are allergic to tetracycline, in pregnancy and in children. The development of new therapies for NGU is necessary taking into account the therapeutic effectiveness of the currently used drugs, the polymicrobial aetiology of NGU and the large number of patients who fail to return for examination after therapy. A single oral gift which would also cure the gonococcal and luetic coinfections would be preferable. The recently developed new quinolones and new macrolides are currently being tested. Kojima et al [192] reported the first successful one minute therapy in men with chlamydia positive urethritis. All 98 patients with *C. trachomatis* positive urethritis who were treated orally with a single dose of sulphamethopyrazine (a long working sulphonamide) were cured when examined 14 and 21-35 days after treatment. No side-effects were observed.

The quinolones

A review of the in vitro activity of quinolones against *C. trachomatis* is given in Table 7. The in vitro activity of nalidixic acid, rosoxacin and norfloxacin is poor. Ofloxacin, sparfloxacin and temafloxacin closely followed by ciprofloxacin are the most active against *C. trachomatis*. Good in vitro activity against *C. trachomatis* was also observed with fleroxacin, lomefloxacin and pefloxacin.

A review of the in vitro activity of quinolones against *U. urealyticum* is given in Table 8. Nalidixic acid and cinoxacin have very high MIC values against *U. urealyticum* and are, therefore, unsuitable as therapeutics. The remaining quinolones shown in Table 8 do not differ significantly from each other with respect to their activities against *U. urealyticum*. These in vitro studies form a good base for clinical evaluations.

A review of clinical investigations in patients with NGU is given in Table 9. The diagnosis of NGU is made provided that more than 10 leukocytes are observed in the urine sediment using a magnification of 250 x and the Gram-stained preparation

Table 7. In vitro activity of quinolones against *Chlamydia trachomatis*

Quinolone (no. of isolates)		MIC (μg/ml)			references
		range	50%	90%	
ciprofloxacin	(18)	1.0-4.0	-	-	194
	(73)	0.39-5.0	0.39-5.0	1.0-5.0	75
	(45)	0.78-1.56	-	-	193
	(11)	-	-	1.0	197
enoxacin	(21)	2.0-40.0	2.0-40.0	8.0-40.0	75
fleroxacin	(2)	4.0-2.0	-	3.0	205
	(6)	1.5-2.5	-	-	75
	(18)	-	-	8.0	194
lomefloxacin	(10)	2.0	-	-	79
	(18)	1.0-4.0	-	-	194
	(45)	1.56-3.15	-	-	193
	(11)	-	-	2.0	197
nalidixic acid	(3)	>64->1600	>64->1600	>64->1600	75
norfloxacin	(30)	4.0-50.0	4.0-50.0	8.0-50.0	75
	(45)	12.5-25.0	-	-	193
	(29)	-	-	16.0	194
ofloxacin	(23)	0.5-8.0	0.5-1.0	0.5-4.0	75
	(18)	1.0-4.0	-	-	194
	(27)	-	-	<1.0	195
	(45)	0.39-0.78	-	-	193
pefloxacin	(18)	2.0-8.0	-	-	196
rosoxacin	(76)	>1.0-80.0	>1.0-10.0	5.0-80.0	75
sparfloxacin	(12)	-	0.25	0.5	198
	(50)	0.03-0.06	-	-	199
	(9)	-	-	0.063	73
	(6)	0.62-0.125	-	-	198
temafloxacin	(50)	0.125-0.25	-	-	199
	(49)	0.03-0.5	0.125	0.25	202
	(18)	0.5-1.0	-	-	196

shows no Gram-negative intracellular diplococci. The term "bacteriological cure" implies that after treatment, the causing microbes can no longer be cultured. The term "clinical cure" is understood by some investigators as resolution of complaints and by others as resolution of both complaints and abnormalities in the urine sediment.

Ofloxacin

Eleven patients with *C. trachomatis* positive urethritis and 19 patients with *C. trachomatis* negative urethritis were treated with 200 mg ofloxacin twice daily for 7 days [210]. The follow up was done one day and 14 days after therapy. All cultures were negative. The results showed that 20 of the 30 (67%) patients had no symptoms or complaints of urethritis after treatment. Mild side-effects not lasting more than 3 days were observed in 13% of the patients. At this dose,

Table 8. In vitro activity of quinolones against *Ureaplasma urealyticum*.

Quinolone (no. of isolates)		MIC (µg/ml)			references
		range	50%	90%	
rosoxacin	(58)	0.5->62.0	4.0	8.0	75
	(51)	2.0-4.0	4.0	4.0	206
norfloxacin	(49)	4.0-64.0	16.0	32.0	75
	(31)	4.0-16.0	8.0	16.0	206
	(19)	4.0-64.0	32.0	64.0	208
ciprofloxacin	(68)	0.5->64.0	2.0	8.0	75
	(56)	2.0-64.0	8.0	16.0	206
	(20)	2.0-16.0	4.0	8.0	208
enoxacin	(49)	4.0-64.0	32.0	64.0	75
	(32)	4.0-16.0	8.0	16.0	206
ofloxacin	(49)	1.0-16.0	4.0	8.0	75
	(58)	0.5-4.0	2.0	2.0	206
difloxacin	(29)	0.5-2.0	1.0	2.0	206
	(28)	2.0->256.0	8.0	16.0	209
pefloxacin	(40)	1.0-4.0	2.0	4.0	206
fleroxacin	(50)	2.0-8.0	4.0	4.0	206
lomefloxacin	(32)	0.5-8.0	4.0	4.0	206
	(104)	4.0-16.0	8.0	8.0	207
cinoxacin	(49)	-	>128.0	>128.0	206
nalidixic acid	(60)	-	64.0	256.0	206
temafloxacin	(28)	2.0->256.0	4.0	32.0	209
sparfloxacin	(34)	-	0.5	1.0	198
	(50)	-	-	0.5	200

ofloxacin is highly effective in eradicating *C. trachomatis*. In patients with NGU, the therapeutic effectiveness of ofloxacin is similar to that of tetracycline. In an investigation of 17 patients with chlamydia positive urethritis, who were treated with 200 mg ofloxacin twice daily for 7 days, it was observed that after treatment, chlamydia could not be cultured in any case [211]. Patients were examined 7 and 14 days after treatment. Side-effects were minimal. Whether these patients were also clinically cured was not explicitly reported in this study.

A randomized comparison study in patients with *C. trachomatis* positive and *C. trachomatis* negative urethritis who were treated either with 300 mg ofloxacin twice daily or with 100 mg doxycycline, twice daily, showed that there was no difference in the cure rates obtained with these two drugs. Patients were evaluated 21 to 28 days after therapy. Minimal side-effects were observed [212].

In a randomized study 17 men with NGU were treated with 300 mg ofloxacin twice daily for 7 days or doxycycline 100 mg twice daily for 7 days. Patients were examined 2 and 3 weeks after therapy. Nine of the men had positive cultures for *C. trachomatis* and all had negative cultures after therapy. Four patients with

Table 9. Multidose quinolone regimens in the treatment of acute non-gonococcal urethritis.

regimen (one week unless given otherwise)	culture before therapy (%)	bacteriological cure (%)	clinical cure (%)	references
ofloxacin 200 mg BID				
Ct neg	19/30 (63)	-	11/19 (58)	210
Ct pos	11/30 (37)	11/11 (100)	9/11 (82)	
ofloxacin 200 mg BID				
Ct pos	17/17 (100)	17/17 (100)	-	211
ofloxacin 300 mg BID				
Ct pos	11/29 (38)	10/11 (91)		212
Uu pos	4/29 (4)	3/4 (75)	(98)	
Ct pos/Uu pos	5/29 (17)	5/5 (100)		
Ct neg/Uu neg	9/29 (31)	-	9/9 (100)	
ofloxacin 300 mg BID				
Ct pos	5/9 (55)	5/5 (100)	9/9 (100)	213
Uu pos	1/3 (33)	1/3 (33)		
fleroxacin 400/800 mg				
Ct pos	19/19 (100)	19/19 (100)		
fleroxacin 400 mg				
Ct pos	9/16 (56)	6/9 (67)		215
Uu pos	7/16 (44)	5/7 (71)	10/16 (62)	
fleroxacin 600 mg				
Ct pos	5/18 (28)	4/5 (80)		215
Uu pos	10/18 (72)	9/13 (69)	11/18 (61)	
fleroxacin 800 mg				
Ct pos	7/15 (47)	3/7 (43)		215
Uu pos	8/15 (53)	6/8 (75)	14/15 (93)	
fleroxacin 600 mg				
Ct pos	27/27 (100)	25/27 (92)	-	216
fleroxacin 600 mg				
Ct pos	26/26 (100)	25/26 (100)	25/26 (96)	217
ciprofloxacin 750 mg BID				
Ct neg/Uu pos	12/71 (17)	9/12 (75)	-	218
Ct pos/Uu pos	14/71 (20)	4/14 (29)	-	
Ct pos/Uu neg	22/71 (31)	10/22 (46)	-	
Ct neg/Uu neg	23/71 (32)	-	14/23 (61)	
ciprofloxacin 500 mg BID				
Ct pos	14/37 (38)	3/14 (21)	3/14 (21)	219
Uu pos	9/37 (24)	4/9 (44)	-	
Ct neg/Uu neg	14/37 (38)	-	4/14 (28)	
ciprofloxacin 1.5 g				
Ct pos	94/94 (100)	88/94 (94)	84/94 (89)	220
ciprofloxacin 750 mg BID				
Ct pos	21/38 (55)	10/21 (48)	20/38 (53)	221
Ct neg	17/38 (45)	-		
ciprofloxacin 1 g BID				
Ct pos	16/41 (39)	10/16 (62)	21/41 (51)	221
Ct neg	25/41 (61)	-		
rosoxacin 150 mg BID x 10 days				
Ct neg/Uu pos	11/31 (35)	3/15 (20)	2/15 (13)	222
Ct pos/Uu pos	4/31 (13)	1/13 (8)	0/13 (0)	
Ct pos/Uu neg	9/31 (29)	-	-	
Ct neg/Uu neg	7/31 (23)	-	4/7 (57)	
norfloxacin 400 mg BID x 10 days				
Ct pos	25/78 (32)	4/25 (16)	6/25 (24)	223
Ct neg/Uu neg	27/78 (35)	17/27 (63)	15/27 (56)	
Ct neg/Uu neg	26/78 (33)	-	16/26 (62)	
temafloxacin 400 mg BID				
Ct pos	-	25/26 (96)		
Uu pos	-	49/85 (58)	72/77 (94)	224

Ct = *Chlamydia trachomatis*; Uu = *Ureaplasma Urealyticum*; pos = positive culture; neg = negative culture.

a positive culture for *U. urealyticum* were treated with doxycyline and were negative after therapy and out of three patients with a positive culture for *U. urealyticum* treated with ofloxacin two had positive cultures after therapy. Side effects were seen in 14 (44%) of the total of 32 patients treated. This study demonstrated that ofloxacin and doxycycline were equally effective in the treatment of NGU [213].

Fleroxacin

In an open study, 11 patients were treated with 400 mg fleroxacin twice daily for 7 days, 6 patients were treated with 400 mg fleroxacin once daily for 7 days and 5 patients were treated with 800 mg fleroxacin once daily for 7 days [214]. Patients were examined 3 weeks after therapy. Three patients were not evaluable. *C. trachomatis* could not be demonstrated in any of the 19 patients after therapy. All patients were without complaints and no abnormalities in the urine sediments were observed.

Side-effects were observed in 7 patients.

Although the number of patients in this study was small, excellent results were obtained.

In a dose-finding study with 400 mg, 600 mg and 800 mg fleroxacin, it was observed that acceptable clinical effects were obtained only with 800 mg fleroxacin [215]. Because of the large number of side-effects, frequently serious, observed in 15% of the patients who were treated with 400 mg and in 81% of the patients who were treated with 800 mg fleroxacin, this study was discontinued.

In two other randomized studies with 600 mg fleroxacin good results were obtained [216,217]. The drug was well tolerated.

Ciprofloxacin

In a double-blind randomized study, patients with NGU were treated either with 750 mg ciprofloxacin twice daily for 7 days or with 100 mg doxycycline, twice daily for 7 days [218]. Patients were examined 3 to 6 weeks after treatment. From a total of 225 patients who were treated, 56 (25%) patients were not evaluable. Thirty-seven of the 71 (52%) patients who were treated with ciprofloxacin were cured and 45 of the 74 (61%) patients who were treated with doxycycline were cured. In patients with *C. trachomatis* infections only, ciprofloxacin was significantly less effective than doxycycline (46% versus 75% respectively). In patients with *U. urealyticum* infection only ciprofloxacin is more effective than doxycycline (69% versus 45% resp.). In patients from whom cultures were negative, ciprofloxacin treatment was as effective as doxycycline treatment (61% versus 64% respectively). Side-effects were mild and were observed in 19 of the 83 (23%) patients who were treated with doxycycline and in 14 of the 87 (16%) patients who were treated with ciprofloxacin. In a study of patients with NGU, who were treated with 500 mg ciprofloxacin twice daily for 7 days, the following results were obtained [219]. Patients were examined 1, 2, 3, 6 and 12 days after treatment. *C. trachomatis* was cultured from 14 patients at the start of the therapy. After therapy, *C. trachomatis* was cultured once again from 11 patients. In these

11 patients, urethritis was also still present. Ten of the 14 chlamydia negative patients had persistent urethritis or had a relapse.

In a study with 1.5 g ciprofloxacin or doxycycline 100 mg daily for seven days in 200 patients with *C. trachomatis* infections (157 males and 43 females), neither treatment was effective enough in the treatment of uncomplicated urogenital infections caused by *C. trachomatis* [220]. Out of the 9 patients from whom *U. urealyticum* cultures were positive prior to treatment, from only 4 patients the cultures were negative after 2 weeks or more. This study showed that ciprofloxacin at the dose used was ineffective in treating NGU.

In a randomized, double blind study 178 men suffering from NGU were treated with 750 mg and 1000 mg ciprofloxacin twice daily for 7 days or 100 mg doxycycline twice daily for 7 days [221]. Patients were examined 2 and 4 weeks after therapy. All the 10 patients with a positive *C. trachomatis* culture treated with doxycycline were bacteriologically cured. In 10 (48%) of the 21 patients with a positive *C. trachomatis* culture, treated with 750 mg ciprofloxacin and 10 (62%) of the 16 patients with a positive *C. trachomatis* culture, treated with 1000 mg ciprofloxacin, chlamydial infection was eradicated.

Urethritis had resolved in 20 (53%) of the 38 men in the 750 mg ciprofloxacin group, in 21 (51%) of 41 men in the 1000 mg ciprofloxacin group and in 20 (56%) of the 36 men in the doxycycline group.

Side effects (gastrointestinal) were reported by 10 (19%) of the 52 patients treated with doxycycline, by 20 (35%) of the 57 patients treated with 750 mg ciprofloxacin and by 20 (38%) of the 53 patients treated with 1000 mg ciprofloxacin. The conclusion of this study was that ciprofloxacin in dosages as high as 2 g daily is inadequate for treatment of *C. trachomatis* infections.

Rosoxacin

The effectiveness of 150 mg Rosoxacin twice daily for 10 days was compared with that of tetracycline in treating patients with NGU [222]. Patients were examined 10 and 17 days after start of therapy. After treatment with rosoxacin, chlamydia was cultured once again in 12 of the 13 patients, who had chlamydia positive cultures at the beginning of the therapy. All 10 patients with *C. trachomatis* infections, treated with tetracycline, were bacteriologically cured. Six of the 31 patients, treated with rosoxacin, had no complaints or any abnormalities in the urine sediment indicative of urethritis. Eighteen of the 31 patients, treated with tetracycline, were clinically cured. After therapy, *U. urealyticum* was isolated from 12 of the 15 (80%) patients, treated with rosoxacin and from 3 of the 18 (17%) patients, treated with tetracycline. Rosoxacin was ineffective in treating patients with NGU.

Norfloxacin

Twenty-five patients with NGU and positive chlamydia cultures were treated with 400 mg norfloxacin twice daily for 7 days [223]. Patients were examined 7, 14, 28 and 42 days after treatment. At 7 days after therapy, 21 of the 25 patients still had positive chlamydia cultures. At later examinations, two more patients had

chlamydia positive cultures once again. Norfloxacin was ineffective in treating patients with NGU.

Temafloxacin

In a randomized study 189 patients with NGU or non-gonococcal cervicitis were treated with 400 mg temafloxacin twice daily for 7 days, or 100 mg doxycycline twice daily for 7 days [224]. Patients were examined twice during the 28 days post-therapy. A bacteriological cure rate for *C. trachomatis* was observed in 25 of the 26 chlamydia positive patients in both groups. The clinical cure was 93.5% in the temafloxacin group and 86% in the doxycycline group. Both drugs were generally well tolerated.

Conclusion

Quinolones are unsuitable for treating NGU, if NGU is considered as a whole, without any distinction of the causative agents isolated. Ofloxacin can be used successfully if only chlamydia-positive patients are considered. Quinolones should be used with reservations for treating patients with NGU since, in The Netherlands, there are only a few centers, which possess the necessary expertise in routinely culturing *C. trachomatis*. An alternative policy would be to treat patients, with complaints of urethritis, only after the results of tissue cultures are known and only then contemplate treating chlamydia-positive patients with ofloxacin.

The Macrolides

Macrolides are still important agents for treating infectious diseases 40 years after their introduction. The recent development of new macrolides has rekindled interest in this group of antibiotics. These new macrolides have better activity against Gram-negative and Gram-positive bacteria. They achieve high tissue and intracellular peak levels in contrast to aminoglycosides and β-lactam antibiotics. In addition, macrolides also have non-specific immuno-stimulatory properties, such as a more efficient migration and phagocytosis by polymorphonucleated leukocytes. Macrolides inhibit the RNA dependent protein synthesis by stimulating the dissociation of ribosomal peptidyl tRNA. Macrolides can be both bacteriostatic and bactericidal. Their activity profiles includes, among others, *N. gonorrhoeae* and *Chlamydiae* species. The development of resistance to macrolides is seldom plasmid mediated. A review of the in vitro activity of different macrolides against *C. trachomatis* is given in Table 10. In vitro effectiveness of azithromycin and roxithromycin against *C. trachomatis* is comparable to those of erythromycin and tetracycline. In vitro effectiveness of clarithromycin against *C. trachomatis* was lower as those of erythromycin and tetracycline.

A review of the in vitro activity of different new macrolides against *U. urealyticum* is given in Table 11. In vitro, roxithromycin was as effective as josamycin against *U. urealyticum*, but more effective than erythromycin. Five

Table 10. In vitro activity of new macrolides against *Chlamydia trachomatis*.

Macrolide (no. of isolates)		MIC (μg/ml)			references
		range	50%	90%	
azithromycin	(4)	0.26-1.02	-	-	225
	(10)	0.064-0.25	-	-	226
	(6)	-	0.075	0.111	227
clarithromycin	(?)	<0.002-0.008	0.004	0.008	228
roxithromycin	(10)	0.03-0.125	-	-	230
	(3)	-	-	0.8	231

Table 11. In vitro activity of new macrolides against *Ureaplasma urealyticum*.

Macrolide (no. of isolates)		MIC (μg/ml)			references
		range	50%	90%	
roxithromycin	(100)	0.1-2.0	-	0.5	232
clarithromycin	(28)	0.004-256.0	0.5	256.0	209
	(104)	0.05-0.5	0.2	0.2	207
A-63075	(28)	0.5-256.0	16.0	256.0	209
azithromycin	(30)	0.125-0.5	0.25	0.5	247
	(4)	0.26-1.02	-	-	248
	(65)	1.0-4.0	2.0	4.0	207

erythromycin-resistant *U. urealyticum* strains were also resistant to clarithromycin and A-63075 [209]. The remaining *U. urealyticum* strains were susceptible to clarithromycin. A-63075 showed higher MICs against *U. urealyticum*.

Clinical trials are necessary to determine the efficacy of these macrolides in patients with NGU. A review of the clinical trials conducted with these new macrolides is given in Table 12.

Satisfactory results were obtained in patients with NGU who were treated with 400 mg erythromycin acistrate (EA) thrice daily for 10 days [233]. Patients were examined 4 to 10 days after treatment. There was a 100% bacteriological cure in 16 patients with chlamydia positive cultures. Side-effects were observed in 50% of the patients and 2 patients had to discontinue the therapy due to side-effects. Same results as those obtained with EA were also obtained with 500 mg erythromycin stearate (ES) thrice daily for 10 days [233]. Side-effects were observed in 52% of the patients.

Roxithromycin

No difference in the effectiveness was observed in a study in which 300 mg roxithromycin once daily for 7 days was compared with 500 mg erythromycin twice daily for 7 days [234]. Patients were examined 1 and 21 days after therapy. Bacteriological cures were observed in 55 of the 75 (73%) patients with

Table 12. New macrolides in the treatment of acute non-gonococcal urethritis.

regimen	culture before therapy (%)	bacteriological cure (%)	clinical cure (%)	references
erythromycin acistrate 400 mg TID x 10 days				
Ct pos.	16/48 (33)	16/16 (100)	11/16 (69)	233
Ct pos.	32/48 (67)	-	25/32 (78)	
erythromycin stearate 500 mg TID x 10 days				
Ct pos.	21/50 (42)	20/21 (95)	12/21 (57)	233
Ct neg.	29/50 (58)	-	25/29 (86)	
roxithromycin 300 mg x 7 days				
Ct pos.	75/75 (100)	55/75 (73)	63/75 (84)	234
roxithromycin 150 mg BID x 10 days				
Ct pos.	94/143 (66)	93/94 (99)	-	235
Uu pos.	40/143 (28)	40/40 (100)	-	
roxithromycin 150 mg BID x 10 days				
Ct pos.	75/94	-	89/94 (93)	235
Uu pos.	33/94	-		
roxithromycin 450 mg x 10 days				
Ct pos.	68/96	-	92/96 (96)	235
Uu pos.	41/96	-		
roxithromycin 300 mg x 10 days				
Ct pos.	29/48 (60)	28/29 (96)		236
Uu pos.	4/48 (8)	4/4 (100)	45/48 (94)	
Ct neg/Uu pos.	15/48 (32)	-		
roxithromycin 300 mg x 7 days				
Ct neg.	43/43 (100)	-	38/43 (88)	237
roxithromycin 150 mg BID x 10 days				
Ct pos.	51/60 (85)	43/47 (91)	43/47 (91)	238
Ct neg.	9/60 (15)	-		
azithromycim 500 mg + 250 mg x 2 days				
Ct pos.	25/30 (83)	22/25 (88)	-	239
Uu pos.	5/30 (17)	5/5 (100)	-	
azithromycin 500 mg + 250 mg x 2 days				
Ct pos.	18/18 (100)	15/15 (100)	14/15 (93)	240
azithromycin 1 g				
Ct pos.	17/17 (100)	17/17 (100)	17/17 (100)	240
azithromycin 1 g				
Ct pos.	44/58 (76)	43/44 (98)	-	239
Uu pos.	14/58 (24)	11/14 (78)	-	
azithromycin 1 g				
Ct pos.	42/96 (44)	42/42 (100)	96/96 (100)	241
Ct neg.	54/96 (56)	-	-	

Ct = *Chlamydia trachomatis*; Uu = *Ureaplasma urealyticum*; pos = positive culture; neg = negative culture.

chlamydia positive cultures, who were treated with roxithromycin and in 50 of the 71 (70%) similar patients treated with erythromycin.
Side-effects were observed in 15% of the patients in both groups, but there was no termination of therapy due to side-effects. Lassus et al [235] treated a total of 637 patients with 150 mg roxithromycin twice daily. Clinical cure was observed in 91% of the patients. Bacteriological cure was observed in 90% of the patients. A higher dose did not improve the cure rates. Side-effects were observed in 3.5% of the patients.
No differences in the effectiveness were observed in a comparative study with 300 mg roxithromycin once daily for 10 days and 200 mg doxycycline once daily for 10 days [236]. The overall cure rates were 94% for roxithromycin and 89% for doxycycline. In the group of patients with chlamydial infections, treatment with roxithromycin produced bacteriological cure in 96% of the patients, whereas, bacteriological cure was observed in 95% of patients with chlamydial infections, who were treated with doxycycline. Side-effects were observed in 6.5% of the patients, who were treated with roxithromycin and in 7% of the patients, who were treated with doxycycline.
In a randomized study, 87 men with a *C. trachomatis* negative NGU were treated with 300 mg roxithromycin once daily for seven days or 500 mg erythromycin twice a day for seven days [237]. In the roxithromycin group the clinical efficacy rate was 88% on day 8 and between 78% and 84% on day 21. Side effects were mainly gastrointestinal and occurred in 15% of the patients treated with roxithromycin. There was no difference with the erythromycin group.
In a study with roxithromycin 150 mg twice daily good results were obtained. No side effects were observed [238].

Azithromycin

Twenty-two of the 25 (88%) patients with *C. trachomatis* positive urethritis and 5 of the 5 patients with *U. urealyticum* positive urethritis, who were treated with 500 mg azithromycin on the first day and 250 mg on the second and third day were cured [239]. Fourty-three of the 44 (98%) *C. trachomatis* positive patients and 11 of the 14 (78%) *U. urealyticum* positive patients who were treated with a single oral dose of 1.0 g azithromycin were cured [239]. The drug was well tolerated. Lassus observed that 32 of the 32 *C. trachomatis* positive patients, who were treated with either of the dose schedules were bacteriologically and clinically cured [240].
In a study with 1.0 g azithromycin in a single oral dose all patients were cured. Side-effects were mild [241].

Conclusion

Of the new macrolides, roxithromycin is as effective as erythromycin. Azithromycin is very effective in a single oral dose in treating *C. trachomatis* urethritis. Futher studies with azithromycin in treating patients with chlamydia negative

Table 13. Pharmacokinetics of the various drugs, used in the treatment of non-gonococcal urethritis.

drugs	dose (mg)	mean peak serum level (µg/ml)	T 1/2 (hours)	MIC 90 (µg/ml)		efficiency (%)	references
				Ct	Uu		
Quinolones:							
ofloxacin	400	5.64	7.4	0.5-4	2-8	58-100	75, 139, 193, 194, 206, 210-213
fleroxacin	400/800	5.0	10.0	2-8	4	61-100	75, 140, 194, 205, 206, 215-217
ciprofloxacin	500/750	2.3	3.9	1-5	8-16	21-94	75, 137, 193, 197, 206, 208, 218-221
rosoxacin	250	6.4	5.0	5-80	4-8	0-57	75, 109, 206, 222
norfloxacin	400	2.4	4.0	8-50	16-64	24-62	75, 136, 193, 194, 206, 208, 223
temafloxacin	400	5.3	7.6	0.25	32	94-96	202, 204
Macrolides:							
erythromycin acistrate	400	2.23	3.0	2	2	69-100	233, 243, 244
erythromycin stearate	500	0.93	3.0	2	2	57-95	233, 244, 245
roxithromycin	300	12.0	8.0	0.5	1	73-99	232, 234, 235, 236-238, 242
azithromycin	500/1000	0.37	7.0	0.111	0.5	93-100	227, 239, 240, 241, 246, 247
clarithromycin	400	2.1	4.7	0.008	256	-	209, 228, 246

Ct = *Chlamydia trachomatis*; Uu = *Ureaplasma Urealyticum*

urethritis and patients with chronic recurrent NGU are necessary to establish its efficacy. No clinical studies with clarithromycin in treating patients with NGU are done till now. The MIC values of clarithromycin for *C. trachomatis* are promising.

In Table 13 a summary of the pharmacokinetics and therapeutic effectiveness is shown of the various quinolones and macrolides, used in the treatment of NGU.

1.4. OBJECTIVES OF THE STUDY

The main aim of the studies, reported in this thesis was:

- to investigate the clinical efficacy and benefit of new drugs.
  Drugs demonstrating satisfactory in vitro effectiveness against microorganisms that cause gonorrhea and those which play a role in the development of non-gonococcal urethritis were evaluated.
  Since coinfections with *N. gonorrhoeae* and *C. trachomatis* occur rather frequently, it is essential to search for antibiotics that are effective in eradicating both these microbes.
- to look for the so called 'one minute treatment'.
  The fact that patients with sexually transmitted diseases are inclined to default treatment and often fail to return for examination after completing treatment, a short and simple treatment schedule is necessary.

- to monitor the clinical effectiveness of the currently used and newly developed antibiotics.
- to monitor the increasing number of multiple drug resistant *N. gonorrhoeae* and *C. trachomatis* strains.
- to obtain detailed insight into the origin of microbes causing sexually transmitted urethritis and coinfections.
- to up-to-date systematic review of in vitro and in vivo studies of gonococcal and non-gonococcal infections.
  This provides an excellent opportunity to assess the difficulties faced in their effective treatment.
- to gain insight into the therapeutic effectiveness of new antibiotics from different classes and with different modes of administration.

The investigations described in this thesis can be divided into two parts. In the first part, the clinical efficacies of enoxacin (a new quinolone), cefodizime (a third generation parenteral cephalosporin) and ceftetrame (a new oral cephalosporin) were assessed in patients with gonococcal urethritis. In the second part, the therapeutic effectiveness of ciprofloxacin (a new quinolone) and roxithromycin (a new macrolide) were assessed in patients with NGU.

Susceptibilities of *N. gonorrhoeae* and *C. trachomatis* to different antibiotics were also assessed.

N. gonorrhoeae and *C. trachomatis* strains that were isolated from patients before and after treatment were also tested for possible resistance formation to the particular antibiotic used for treatment. These strains were also typed wherever possible. Side-effects and laboratory abnormalities caused by these trial drugs were also strictly monitored and critically evaluated.

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CHAPTER 2

Comparative double-blind study of 200- and 400-mg enoxacin given orally in the treatment of acute uncomplicated urethral gonorrhea in males

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SUMMARY

In a double-blind randomized study, 155 male patients with uncomplicated urethral gonorrhea were given 200 mg (one capsule with 200 mg and one capsule with placebo; n = 77) or 400 mg (two capsules with 200 mg; n = 78) of enoxacin orally. The cure rates in the 200- and 400-mg treatment groups were 90 and 92%, respectively. The enoxacin MIC for the isolated *Neisseria gonorrhoeae* strains ranged from 0.015 to 0.12 μg/ml. Postgonocccal urethritis was diagnosed in 29 (42%) patients in the 200-mg treatment group and 19 (26%) patients in the 400-mg treatment group. Side effects (nausea, headache, and vomiting) occurred in 2 (3%) of the 77 patients in the 200-mg treatment group and in 3 (4%) of the 78 patients in the 400-mg treatment group.

INTRODUCTION

The increasing resistance of *Neisseria gonorrhoeae* to penicillins and tetracycline calls for new therapeutic agents. The 4-quinolone group has yielded some derivatives that are active against *N. gonorrhoeae* and can be given orally. Cure rates of up to 100% have been reported in previous studies with ciprofloxacin in the treatment of uncomplicated urogenital gonorrhea in males (12) and with enoxacin in the treatment of uncomplicated urogential gonorrhea in females (4,13). In vitro studies have shown that enoxacin, one of the new quinolone agents, is effective against *N. gonorrhoeae* with MICs of 0.03 to 0.25 μg/ml (8). Our double-blind randomized study involved clinical evaluation of the treatment of uncomplicated urethral gonorrhea in males with a single oral dose of either 200 or 400 mg of enoxacin.

MATERIALS AND METHODS

Patient population

All patients were men attending the outpatient clinic for sexually transmitted diseases at the University Hospital Rotterdam-Dijkzigt. Patients who were under 18, had disseminated gonococcal infections, had solitary rectal or pharyngeal gonococcal infections, had syphilis, liver, or kidney diseases, had allergies to quinolones, and were recently treated with enoxacin or on theophylline medication were excluded. This was a double-blind study.

A randomization list ensured that patients received a single oral dose of either 400 mg of enoxacin in two 200-mg capsules or 200 mg of enoxacin in one 200-mg capsule and one placebo capsule. The capsules were taken with water under the supervision of a nurse. The patients were advised to abstain from sexual contacts throughout the follow-up period.

All subjects gave oral informed consent before they were enrolled, and the study was approved by the committee for the protection of human subjects at the University Hospital Rotterdam-Dijkzigt.

Venereological study

A standard history was taken, and all patients underwent the following tests before and after therapy. Gram stains were made of the discharge, and samples for *Chlamydia trachomatis* and *N. gonorrhoeae* cultures were taken from the urethra. *N. gonorrhoeae* culture samples were taken from the pharynx (and from the rectum in homosexual men), and the sediment of 10 to 15 ml of the first-voided urine was collected. Blood was drawn for Venereal Disease Research Laboratory (VDRL), fluorescent treponemal antibody (FTA-ABS), and THPA tests (only before therapy) and general hematology, liver and kidney function determinations. Therapy was started if Gramnegative diplococci were found in the Gram stain or if *N. gonorrhoeae* was isolated from the culture.

The effect of therapy was evaluated 7 to 14 days after patients began to receive medication. Postgonococcal urethritis was diagnosed if, at follow up, more than 10 leukocytes per field were seen in the sediment of the first voided urine at a magnification of 250 x and the Gram stain contained no intracellular Gram-negative diplococci. At follow-up, all patients were asked about side effects and whether dysuria or discharge was still present.

***N. gonorrhoeae* cultures**

For *N. gonorrhoeae* cultures, samples were taken with a carbon-impregnated cotton swab and, after tranport in Stuart medium, transferred within 6 h to a selective medium which consisted of GC agar base (Oxoid Ltd., Londen, England) supplemented with 2% hemoglobin (Oxoid Ltd.) and 1% IsoVitaleX (BBL Microbiology Systems, Cockeysville, Md).

MICs of enoxacin, tetracycline, penicillin, ampicillin and cefuroxime for the *N. gonorrhoeae* strains were determined by an agar dilution technique on gonococcal agar base (11) with twofold serial antibiotic dilutions between 4 and 0.004 µg/ml and a bacterial inoculum of 10^4 CFU/spot. All *N. gonorrhoeae* strains were tested for beta-lactamase production using the chromogenic cephalosporin (nitrocephin) test (5).

Susceptibility to spectinomycin was tested by the disk diffusion method. Auxotyping of the *N. gonorrhoeae* strains was performed at the National Institute of Public Health and Environmental Hygiene (1). Pairs showing similarity before and after therapy were sent to the Centers for Disease Control for lectin agglutination (9) and serotyping (3) tests as described previously.

C. trachomatis cultures

For *C. trachomatis* cultures, samples were taken by inserting white-cotton-tipped metal swabs deep into the urethra, rotating them, and suspending them in buffer containing 0.2 M sucrose and 0.02 M phosphate with 10% fetal calf serum, 25 mg of gentamicin per ml, and 25 U of nystatin per ml. Samples were frozen to −70°C within 6 h of being obtained. Chlamydiae were cultured on HeLa 229 monolayers washed with DEAE-dextran in 96-well microtiter plates. Staining was done with fluorescent monoclonal antibodies (Syva Inc.), after 48 h of incubation (10). Subpassage was not performed.

Statistical analysis

Results were statistically analyzed by the two-tailed Fisher exact test.

RESULTS

Of the 243 patients treated, 88 (36%) could not be evaluated, 45 in the 200-mg and 43 in the 400-mg treatment group. Reasons included failure to report for follow-up (47 patients), follow-up later than 2 weeks (17 patients), sexual contact during the study (11 patients), capsules vomited up immediately after ingestion (1 patient), negative *N. gonorrhoeae* culture at start of study (3 patients), and material not obtained for *N. gonorrhoeae* culture at follow-up (9 patients).

Treatment with 200 mg of enoxacin

In the 200-mg treatment group, 77 patients were available for evaluation; 69 (90%) patients were culture negative for *N. gonorrhoeae* at follow-up. Of the 77 patients with positive initial *N. gonorrhoeae* cultures, the strains were isolated from the urethra in all 77; none were isolated from the rectum or the pharynx. The auxotyping patterns of the *N. gonorrhoeae* strains obtained from six treatment failures were identical (Table 1). In two failures, one or both strains were no longer available for auxotyping. The typing patterns of pre- and posttreatment strains obtained from two nonassessable patients (one for sexual contant during the study and one for follow-up later than 2 weeks) showed similarity in auxo-and serotyping. One pair showed a similar and one pair a different lectin agglutination pattern (Table 1). Five (6%) of the 77 *N. gonorrhoeae* strains isolated produced penicillinase; the cure rate for this group of patients was 100%. Postgonococcal urethritis (PGU) was diagnosed in 29 (42%) of the 69 patients negative for *N. gonorrhoeae* at follow-up. *C. trachomatis* was isolated from the urethra in 8 (28%) of the 29 patients; only 3 of them still had complaints. Samples from 20 patients (69%) were *C. trachomatis* negative, and only 5 of these patients still had complaints. In one symptomatic case,

no *C. trachomatis* culture was obtained. In four cases, *C. trachomatis* was isolated in the absence of urinary sediment changes. One (1%) patient complained of nausea and one (1%) of headache and nausea.

Treatment with 400 mg of enoxacin

In the 400-mg treatment group, 78 patients were available for evaluation; 72 (92%) patients were culture negative for *N. gonorrhoeae* at follow-up. Of the 78 patients with positive *N. gonorrhoeae* cultures, the strains were isolated from the urethra in 78, the rectum in 2, and the pharynx in 0.

The auxotyping patterns of the *N. gonorrhoeae* strains from the two treatment failures were identical (Table 1). In four failures, one or both strains were no longer available for auxotyping. The typing patterns of pre- and posttreatment strains obtained from two nonassessable patients (one for follow-up later than 2 weeks and one for capsule vomited immediately after ingestion) showed similarity in auxo- and serotyping. One pair showed a similar and one pair a different lectin agglutination pattern (Table 1). Two strains isolated from two patients after therapy had enoxacin MICs of 1 and 2 μg/ml. The pretreatment strain of the first patient was no longer available for MIC determination. The pretreatment strain of the latter patient showed a MIC of 0.03 μg/ml (Table 1).

Table 1. MIC's of enoxacin for *N. gonorrhoeae* strains isolated before and after therapy with enoxacin and their typing patterns.

Patient group and enoxacin dose (mg)	Pretreatment strains				Posttreatment strains			
	MIC (μg/ml)	Auxotype[a]	Serovar	Lectin group	MIC (μg/ml)	Auxotype[a]	Serovar	Lectin group
Assessable								
200	0.03	NR	IB-3	6	0.03	NR	IB-3	6
	0.06	Pro-	IB-5	7	0.06	Pro-	IB-5	7
	0.03	Pro-	IB-4	7	0.03	Pro-	IB-4	7
	0.06	Pro-	IB-4	7	0.06	Pro-	IB-4	7
	0.06	NR Phe	IB-6	7	0.06	NR Phe	IB-6	7
	0.03	Pro-	IB-4	6	0.06	Pro-	IB-4	6
400	0.03	Amac-	IB-3	6	0.03	Amac-	IB-3	6
	0.03	Amac-	IB-2	7	2	Amac-	IB-2	7
Nonassessable								
200	0.03	NR Phe	IB-6	7	0.03	NR Phe	IB-6	7
	0.06	Amac-	NT[b]	12	0.06	Amac-	NT	6
400	0.03	NR	IB-6	6	0.06	NR	IB-6	6
	0.03	NR Phe	IB-6	12	0.03	NR Phe	IB-6	7

[a]NR Phe, not requiring and inhibited by Phenylalanine; Pro-, proline requiring; Amac-, amino acid mixture requiring (1); NR, no requirement.
[b]NT, Not typable.

None of the strains produced penicillinase. Two patients also had a positive rectal gonococcal culture. The follow-up cultures were negative for both patients.

PGU was diagnosed in 19 (26%) of the 72 patients negative for *N. gonorrhoeae* at follow-up. *C. trachomatis* was isolated from the urethra in 9 (47%) of the 19 patients; only 2 of these patients still had complaints, whereas 10 (53%) samples from asymtomatic patients were *C. trachomatis* negative. In five cases, *C. trachomatis* was isolated in the absence of urinary sediment changes. Two (3%) patients complained of nausea, and one (1%) vomited a few minutes after ingestion of the medication.

C. trachomatis cultures

Before therapy, *C. trachomatis* was isolated from 17 (11%) of the 155 patients and after therapy from 26 (17%).

Abnormal laboratory findings

The following abnormal laboratory findings were obtained for the 200-mg treatment group: enlarged platelets in two (3%) patients, a slightly increased serum glutamic oxalacetic transaminase value in two (3%), slight anisocytosis in two (3%), and a few macrocytes in one (1%). The only abnormal finding for the 400-mg treatment group was a slightly increased serum glutamic pyruvic transaminase value in one (1%) patient.

Antimicrobial susceptibility and typing patterns

Eleven (5%) of the 239 *N. gonorrhoeae* strains isolated produced penicillinase. The MICs of enoxacin ranged from 0.004 to 0.12 μg/ml. The enoxacin MIC for 90% of isolates was 0.06 μg/ml (Table 2). One *N. gonorrhoeae* strain showed an increase in the MIC from 0.03 to 2 μg/ml after treatment with 400 mg of enoxacin.

Table 2. In vitro susceptibility of *N. gonorrhoeae* strains isolated before treatment with enoxacin.

	MIC (μg/ml)[a]		
Antibiotic	Range	50%	90%
Enoxacin[b]	0.015 - 0.12	0.03	0.06
Penicillin[c]	0.015 - 4	0.25	1
Ampicillin	0.03 - 4	0.25	0.5
Cefuroxime[b]	0.008 - 2	0.03	0.12
Tetracycline[b]	0.06 - 4	0.25	0.5

[a]50% and 90%, MIC for 50 and 90% of isolates, respectively.
[b]Tested against all strains, including six penicillinase-producing strains (n = 134).
[c]Tested against non-penicillinase-producing strains only (n = 128).

The lectin agglutination patterns and serotyping of each of the eight pre- and post-treatment pairs of isolates obtained from assessable patients were identical (Table 1).

DISCUSSION

A study of 12 healthy control volunteers (16) demonstrated peak plasma levels of 1.2 μg/ml after a single dose of 200 mg and 2.1 μg/ml after 400 mg of enoxacin. The half-life of enoxacin is ca. 5 h. In view of the good results of our previous pilot study with enoxacin (4), the above-mentioned data, and low MICs of enoxacin for *N. gonorrhoeae*, we decided to investigate this quinolone in a large group of patients suffering from uncomplicated gonorrhea.

The cure rate found in this study, 90% in the 200-mg treatment group, and 92% in the 400-mg treatment group, was lower than that in our previous study of enoxacin with indentical doses in the treatment of uncomplicated female urogenital gonorrhea (13). The cure rates in the latter study were 98.7% in the 200-mg treatment group and 100% in the 400-mg treatment group. Although the MICs of enoxacin for penicillinase-producing and non-penicillinase-producing *N. gonorrhoeae* strains were low (0.3 to 0.12 μg/ml) and agreed with those determined in previous studies (2,4,8) the cure rates in both groups of treated males were lower than expected from other studies with quinolones (4,12,13).

Pre- and posttreatment isolates were available from six treatment failures in the 200-mg group and two failures in the 400-mg group. Auxotyping, serotyping, and lectin agglutination patterns were the same for each pair of *N. gonorrhoeae* strains obtained before and after therapy. This suggests either failure of therapy or reinfection with the same strain. In six therapy failures auxotyping was impossible, and no conclusions about whether the strains were the same before and after therapy could be drawn.

The fact that in two nonassessable patients the pre- and posttreatment pairs of strains showed the same auxo- and serotyping and lectin agglutination patterns underscored the importance of additional typing methods.

The MICs of enoxacin for one *N. gonorrhoeae* strain rose from 0.03 μg/ml before therapy to 2 μg/ml after therapy with 400 mg of enoxacin. Van Klingeren et al. (14) demonstrated that the sensitivity in vitro of *N. gonorrhoeae* strains to quinolones may show a 10-fold to 100-fold decrease compared with the parent strain at a frequency of 1 in 10^8 to 10^9. These findings indicate that resistance to quinolones can develop in vivo.

Enoxacin in a single oral dose is not effective against *C. trachomatis*; this is in accordance with the high MICs of enoxacin against *C. trachomatis* (MIC for 90% of isolates, 16 μg/ml) and data reported for other antibiotics given in a single dose (6,7,15). This probably explains the PGU in the 42% of the 200-mg treatment group and 26% of the 400-mg treatment group. Like Oriel et al. (7), we found a larger number of positive *C. trachomatis* cultures after therapy than before.

The low percentage of positive *C. trachomatis* cultures (28 to 47%) for patients suffering from PGU is probably due to the short follow-up period. It is possible that some patients may have persistent urethral leukocytosis due to gonorrhea alone. Also, enoxacin may cause temporary suppression of *C. trachomatis*. Of the PGU patients, 31% in the 200-mg treatment group and 10% in the 400-mg treatment group still had complaints. These low percentages are probably also due to the short follow-up period. The peak incidence of symptomatic PGU occurs 2 to 3 weeks after treatment.

The differences in cure of gonococcal infections between the 200-mg treatment group and the 400-mg treatment group were not statistically significant ($P > 0.05$). The differences in PGU rate were not statistically significant either ($P > 0.05$). The side effects observed (headache and nausea) were mild and transient. Abnormal laboratory findings occurred more often in the 200-mg treatment group, than in the 400-mg treatment group demonstrating that there was no dose-dependent relation with the medication given.

Our conclusion is that enoxacin is an effective drug in the treatment of uncomplicated male urethral gonorrhea, although the cure rate in this study was lower than that in previous studies of female patients and previous studies with other quinolones (13).

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CHAPTER 3

Randomized comparative study of 0.5 and 1 g of cefodizime (HR 221) versus 1 g of cefotaxime for acute uncomplicated urogenital gonorrhea

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SUMMARY

Uncomplicated urogenital and concomitant oropharyngeal gonorrhea in 424 male and female patients was treated in a randomized comparative study with 0.5 g of cefodizime (89 men and 54 women), 1 g of cefodizime (87 men and 52 women), or 1 g of cefotaxime (86 men and 56 women). The cure rates were 100% for men and women in the group given 0.5 g of cefodizime, 100% for men and women in the group given 1 g of cefodizime, and 99% for men and 100% for women in the group given 1 g of cefotaxime. The MICs of cefodizime and cefotaxime for the isolate of *Neisseria gonorrhoeae* ranged from 0.004 to 0.06 μg/ml. *Chlamydia trachomatis* was isolated before treatment in 15% and after treatment in 13% of all patients. Side effects, such as nausea, diarrhea, abdominal pain, genital candidiasis, and pain at the site of injection, developed in 4% of the patients given cefodizime. Side effects, such as vertigo, genital candidiasis, fatigability, and diarrhea, developed in 4% of the patients treated with cefotaxime. In both groups of patients, the side effects were mild and transient. Cefodizime and cefotaxime are safe and effective agents in the treatment of uncomplicated urogenital gonorrhea.

INTRODUCTION

Previous studies (12–14) performed in our department during the past 2 years have shown that some 5% of *Neisseria gonorrhoeae* infections are caused by penicillinase-producing strains. The use of β-lactamase-stable chemotherapeutics may aid in the reduction of treatment failures.

Cefodizime is a new β-lactamase-stable chemotherapeutic (8) for parenteral use. It has the same structure as cefotaxime, with a 1-mercapto-1.3-thiazole chain on the third position of the dihydrothiazine ring.

In vitro studies have shown that cefodizime is effective against strains of *N. gonorrhoeae*, with MICs ranging from < 0.004 to 0.016 μg/ml (4). A study of eight healthy volunteers (3) revealed that peak levels in serum of about 59 μg/ml were obtained after a single intra-muscular dose of 1 g of cefodizime. The half-life was about 3.8 h.

This report presents a clinical evaluation of the treatment of uncomplicated urogenital gonorrhea in men and women with 0.5 or 1 g of cedofizime in a single intramuscular dose compared with a single intramuscular dose of 1 g of cefotaxime.

Although the aim of this study was to evaluate the antibacterial effect of cefodizime against *N. gonorrhoeae*, we also decided to evaluate concomitant *Chlamydia trachomatis* infections and the urinary sediment, because of the epidemiological interest.

MATERIALS AND METHODS

All the patients attended the venereal diseases outpatient clinic of University Hospital Rotterdam-Dijkzigt. Patients excluded from this study included patients younger than 18; those suffering from disseminated gonococcal infections, solitary pharyngeal gonorrhea, and hepatic or renal diseases; patients with cephalosporin and/or penicillin allergy; those recently treated with antibiotics, probenecid, and/or immunosuppressive agents; pregnant or lactating women; and patients with gastrointestinal pathology. Metronidazole or tinidazole medication was accepted as concomitant antibiotic treatment during the study. All patients gave oral consent before study.

Pre- and posttreatment cultures for *N. gonorrhoeae* and *C. trachomatis* were done, and tests were performed as follows. For men, Gram stain of discharge, urethral *C. trachomatis* culture, and urethral and tonsillar *N. gonorrhoeae* cultures were done. Rectal cultures were obtained from homosexual men. The urinary sediment was tested for the presence of leukocytes in the first 10 to 15 ml. For women, urethral and cervical Gram stain, urethral, cervical, rectal, and tonsillar *N. gonorrhoeae* cultures; and cervical *C. trachomatis* cultures were done.

Treatment was given on the basis of a Gram stain, positive *N. gonorrhoeae* culture, or enzyme-linked immunosorbent assay (1) positive for *N. gonorrhoeae.* Women who had negative Gram stains but were exposed to *N. gonorrhoeae* through sexual contact were also treated.

By using a randomization list, patients were given a single intramuscular injection of 0.5 g of cefodizime, 1 g of cefodizime, or 1 g of cefotaxime.

The effect of therapy was evaluated 2 to 7 days after the patients began to receive medication. At follow-up visits, explicit questions were asked about side effects, dysuria, discharge, and abstinence from sexual contact. The trial protocol was approved by the medical ethics committee of the University Hospital Rotterdam-Dijkzigt.

Before and after treatment, blood was drawn for Venereal Disease Research Laboratory, fluorescent treponemal antibody-abs, and TPHA tests (only before therapy); general hematology; and liver and kidney function determinations.

N. gonorrhoeae cultures

Specimens for *N. gonorrhoeae* culture were taken from the urethra, cervix, tonsil, and rectum as described above with a carbon-impregnated swab stick; transported in Stuart medium; and transferred within 6 h to a selective medium consisting of GC agar base (Oxoid Ltd.) supplemented with 2% hemoglobin (Oxoid) and 1% IsoVitaleX (BBL Microbiology Systems).

Phenotypic characterization

The MICs of cefodizime, cefotaxime, ceftriaxone, tetracycline, and penicillin for the gonococcal strains were determined by using a standard agar dilution method (11) with twofold dilutions between 4 and 0.004 mg/liter. All gonococcal strains were tested for β-lactamase production by using the chromogenic cephalosporin (Nitrocephin) test (6).

Susceptibility to spectinomycin was tested by the disk diffusion method.

Auxotyping of the gonococcal strains was done by the National Institute of Public Health and Environmental Hygiene (2). Pre- and posttreatment gonococcal strains with the same auxotypes were sent to the Centers for Disease Control, Atlanta, Ga., for examination by lectin agglutination (7) and serologic typing (5) as decribed previously.

C. trachomatis cultures

Specimens for *C. trachomatis* culture were taken by using a metal swab stick with a white cotton swab inserted deep into the urethra or cervix and rotated. The specimens were suspended in 0.02 M phosphate buffer containing 0.20 M sucrose with 10% fetal calf serum, 25 ug of gentamicin per ml, and 25 U of nystatin per liter. The samples were frozen at −70°C. The culture was made on HeLa 229 monolayers prerinsed with DEAE-dextran in 96-well microdilution plates. Fluorescent staining was accomplished with a monoclonal antibody reagent (Syva Inc.) after 48 h (9) without subpassage.

Statistical analysis

Statistical analysis was performed by using Fisher's exact test (two tailed).

RESULTS

Treatment with 0.5 g of cefodizime

Of 108 male patients treated with 0.5 g of cefodizime, 19 (18%) proved to be nonassessable (Table 1). Results of *N. gonorrhoeae* cultures of the assessable patients according to the site of isolation are shown in Table 2. In all 89 patients (100%), the gonococcal cultures became negative after antibiotic treatment. One patient developed candidiasis of the glans penis. Three patients (3%) showed transient slightly increased serum glutamic oxalacetic and pyruvic transaminase activities.

Of the 67 female patients treated with 0.5 g of cefodizime, 13 (19%) were found to be nonassessable (Table 1).

Table 1. Assessability of patients treated with various antibiotics.

Characteristic	No. (%) of patients					
	0.5 g of cefodizime		1 g of cefodizime		1 g of cefotaxime	
	Male (n = 108)	Female (n = 67)	Male (n = 103)	Female (n = 66)	Male (n = 108)	Female (n = 72)
Assessable	89(82)	54(81)	87(84)	52(79)	86(80)	56(78)
Nonassessable	19(18)	13(19)	16(16)	14(21)	22(20)	16(22)
No follow-up	17(89)	5(38)	14(88)	8(57)	16(73)	4(25)
No laboratory confirmation of gonoccal infection	2(11)	5(38)	2(12)	5(36)	5(23)	12(75)
Sexual contact and positive *N. gonorrhoeae* culture	0	2(16)	0	1(7)	1(4)	0
No *N. gonorrhoeae* culture at follow-up	0	1(8)	0	0	0	0

Table 2. Assessable patients with positive culture of *N. gonorrhoeae* by culture site.

Site	No (%) of patients					
	0.5 g of cefodizime		1 g of cefodizime		1 g of cefotaxime	
	Male (n = 89)	Female (n = 52)	Male (n = 87)	Female (n = 52)	Male (n = 86)	Female (n = 56)
Urethra	89(100)	41(79)	87(100)	12(23)	86(100)	43(77)
Throat	0	3(6)	0	0	0	2(4)
Rectum	0	20(38)	2(2)	17(33)	0	17(30)
Cervix	11(12)	48(92)	6(7)	44(85)	9(10)	49(88)
PPNG[a]		1(2)		1(2)		4(7)

[a]Penicillinase-producing *N. gonorrhoeae* strains

For 2 of the 54 assessable patients, no *N. gonorrhoeae* cultures had been made; the diagnosis in these patients was made on the basis of a positive enzyme-linked immunosorbent assay (Gonozyme) (1) and a positive Gram stain. Both patients had negative *N. gonorrhoeae* cultures after therapy. Results of *N. gonorrhoeae* cultures of the assessable patients according to the site of isolation are shown in Table 2. The 52 female patients (100%) with positive gonococcal cultures became negative. The estimated cure rate in this group of patients was 100%.

No laboratory (hematological and liver and kidney function) changes were found. One patient complained of nausea, and one reported nausea, diarrhea, and abdominal pain.

Treatment with 1 g of cefodizime

Of the 103 male patients treated with 1 g of cefodizime, 16 (16%) were found to be nonassessable (Table 1). Results of *N. gonorrhoeae* cultures of the assessable patients are shown in Table 2. In all 87 patients (100%), the gonococcal cultures had become negative after treatment. One patient (1%) complained of nausea, two (2%) complained of diarrhea, and one (1%) complained of pain at the injection site. No laboratory (hematological and liver and kidney function) changes were found.

Of the 66 female patients given 1 g of cefodizime, 14 (21%) proved to be nonassessable (Table 1). Results of *N. gonorrhoeae* cultures of the assessable patients are shown in Table 2. In all 52 female patients (100%), the gonococcal cultures had become negative after treatment. One patient (2%) complained of abdominal pain, one (2%) complained of diarrhea, one (2%) complained of nausea; one patient (2%) developed genital candidiasis, and one (2%) showed a slightly increased lactate dehydrogenase value.

Treatment with 1 g of cefotaxime

Of 108 male patients treated with 1 g of cefotaxime, 22 (20%) proved to be nonassessable (Table 1). Results of *N. gonorrhoeae* cultures of the assessable patients are shown in Table 2. In 85 male patients (99%), the gonococcal culture had become negative. One patient again had a positive urethral *N. gonorrhoeae* culture at follow-up. He denied having had any sexual contact. Auxotypings (proline requiring) of the gonococcal strain before and after treatment were identical, as were the lectin agglutination patterns (7) and the serovar (IA-4). One patient (1%) complained of vertigo, and one (1%) developed candidiasis of the glans penis. No abnormal laboratory (hematological and liver and kidney function) findings were obtained.

Of 72 female patients treated with 1 g of cefotaxime, 16 (22%) were non-assessable (Table 1). Results of *N. gonorrhoeae* cultures of the assessable patients are shown in Table 2. In all 56 female patients (100%), the gonococcal cultures had become negative. One patient (2%) complained of fatigability, one (2%) complained of diarrhea, and one (2%) developed vaginal candidiasis. No abnormal laboratory findings were obtained.

C. trachomatis cultures

C. trachomatis was isolated before treatment from 63 (15%) of the total of 418 patients and after treatment from 51 (13%) of the total of 403 patients (Table 3); 29 patients had persistent *C. trachomatis* infections at follow-up, and 18 patients had positive *C. trachomatis* cultures at follow-up with a negative culture at the first visit.

Table 3. Results of *C. trachomatis* cultures of assessable patients before and after treatment.

Drug and dose (g)	No patients with isolates/total			
	Male		Female	
	Before	After	Before	After
Cefodizime				
0.5	7/88[a]	7/89	14/54	13/52[b]
1.0	10/85[b]	9/89	12/50[b]	8/50[b]
Cefotaxime				
1.0	7/85[a]	9/86	13/56	5/54[b]

[a] No *C. trachomatis* culture was performed for one patient.
[b] No *C. trachomatis* cultures were performed for two patients.

Table 4. In vitro susceptibility of *N. gonorrhoeae* strains (n = 424)[a] isolated before treatment with cefodizime or cefotaxime.

Antibiotic	Organisms[b]	MIC (μg/ml)[c]	
		50%	90%
Cefodizime	Non-PPNG	< 0.004	0.008
	PPNG	< 0.004	0.016
	All strains	< 0.004	0.008
Cefotaxime	Non-PPNG	0.004	0.008
	PPNG	< 0.004	0.016
	All strains	< 0.004	0.008
Ceftriaxone	Non-PPNG	< 0.004	< 0.004
	PPNG	< 0.004	0.008
	All strains	< 0.004	< 0.004
Tetracycline	Non-PPNG	0.5	2
	PPNG	1	4
	All strains	0.5	2
Penicillin	Non-PPNG	0.12	0.5
	PPNG	> 4	> 4
	All strains	0.12	1

[a] Including penicillinase-producing strains (n = 32).
[b] PPNG. Penicillinase-producing *N. gonorrhoeae*.
[c] 50% and 90%. MIC for 50 and 90% of strains tested, respectively.

Antimicrobial susceptibility and typing patterns

Of the 424 *N. gonorrhoeae* strains, 32 (8%) produced penicillinase. The MICs of cefodizime ranged from < 0.004 to 0.06 μg/ml. The MIC of cefodizime for 90% of isolates tested was 0.008 μg/ml (Table 4). The MICs of cefotaxime ranged from < 0.004 to 0.06 μg/ml. The MIC of cefotaxime for 90% of isolates tested was 0.008 μg/ml (Table 4). The MICs of ceftriaxone ranged from 0.004 to 0.03 μg/ml.

The MICs of tetracycline ranged from < 0.016 to 4 μg/ml, and the MICs of penicillin ranged from < 0.016 to 4 μg/ml.

All *N. gonorrhoeae* strains were susceptible to spectinomycin. From three patients, *N. gonorrhoeae* was again isolated at the follow-up visit.

Auxo- and serotypes and the lectin agglutination patterns of each of the strains isolated before and after treatment in the two nonassessable patients, who had sexual contact during the study, were likewise identical. Differences in MICs were not observed.

DISCUSSION

So far there have been no clinical trials with cefodizime in the treatment of patients with acute uncomplicated gonorrhea. In view of the long half-life, high peak levels in serum, and the low MIC of cefodizime for *N. gonorrhoeae*, this agent is eminently suitable for the treatment of uncomplicated gonorrhea. The cure rate in the male and female patients treated with 0.5 and 1 g of cefodizime was 100%. That in the male patients given 1 g of cefotaxime was 99%, compared with 100% in the female patients.

The low percentage of positive pharyngeal gonococcal cultures (1%) precludes any conclusion about the effect of cefodizime on pharyngeal gonorrhea.

The high percentage of nonassessable female patients, for whom no laboratory confirmation of gonococcal infection (Table 1) was found, was due to the fact that in our clinic a woman with a proved sexual contact with a man suffering from gonorrhea is treated simultaneously with her partner. The number of patients with side effects (nausea, diarrhea, abdominal pain, genital candidiasis, pain at the injection site, fatigability, and vertigo, always mild and transient) was smaller in the group treated with 0.5 g of cefodizime (2%) than in the group given 1 g of cefodizime (6%) and in the group treated with 1 g of cefotaxime (4%), although the differences were not statistically significant.

No abnormal laboratory findings were obtained for the patients treated with 1 g of cefotaxime. Transient slightly increased serum glutamic oxalacetic and pyruvic transaminase activities were found in three patients given 0.5 g of cefodizime, and one patient given 1 g of cefodizime showed a transient slightly increased lactate dehydrogenase value. A relation to the medication received cannot completely be excluded.

The MICs of cefodizime against penicillinase-producing and non-penicillinase-producing *N. gonorrhoeae* ranged from < 0.004 to 0.06 μg/ml and corresponded with the values found in previous in vitro studies (4,8).

The succes rates and toxicity profiles of cefodizime in the treatment of uncomplicated gonorrhea (penicillinase-producing and non-penicillinase-producing *N. gonorrhoeae*) are similar to those of other broad-spectrum cephalosporins, such as ceftriaxone, and quinolones, such as ciprofloxacin and spectinomycin.

The pre- and posttherapeutic strains from one assessable patient and those from two nonassessable patients were identical in terms of auxo- and serotyping and lectin agglutination patterns; although the two patients were nonassessable because of sexual contact during the study, therapy failure cannot be excluded. In the case of the assessable patient, too, the possibility of reinfection with the same strain cannot be excluded.

The brief follow-up period of 2 to 7 days precludes any conclusion about the occurrence of postgonococcal urethritis in the three treatment groups. The peak of symptomatic postgonococcal urethritis comes 2 to 3 weeks after treatment. The low percentages of positive *C. trachomatis* cultures (10 to 11%) should likewise be ascribed to the brief follow-up period.

An agent which could cure both gonorrhea and *C. trachomatis* infections is becoming more and more desirable. Our conclusion is that cefodizime in doses of 0.5 and 1.0 g and cefotaxime in a dose of 1.0 g are effective, safe agents in the treatment of uncomplicated urogenital gonorrhea.

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CHAPTER 4

A preliminary study of ceftetrame in acute uncomplicated gonorrhoea in males

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SUMMARY

Ten male patients suffering from uncomplicated urethral gonorrhoea were treated orally with 1200 mg Ceftetrame (Ro 19-5248). Nine of the 10 patients were cured. The sensitivity of the isolated *Neisseria gonorrhoeae* strains to Ro 19-5247, the active metabolite of Ceftetrame, ranged from < 0.002 to 0.12 µg/ml. *Chlamydia trachomatis* was isolated before and after treatment in one patient. No side effects were reported. Ceftetrame is an effective drug in the treatment of uncomplicated urogenital gonorrhoea in males.

List of abbreviations

MIC = Minimum Inhibitory Concentration.
VDRL = Venereal Disease Research Laboratory-test.
FTA-abs = Fluorescent Treponemal Antibody Absorption.
TPHA = Treponema Pallidum Haemagglutination Assay.
TKA = Trichosanthes Kinlowii.
GSI = G. simplicifolia I.
SBA = Glycine max.
STA = Solanum Tuberosum.
WGA = Wheat Germ Agglutinin.
N.R. = Non-requiring.

INTRODUCTION

In a previous study with cefuroxime axetil ester, an oral cephalosporin in the treatment of urethral and rectal gonorrhoea, a cure rate of 99.5% was observed. Side effects were noted in 38% of the patients[1]. Since then, a new generation of oral cephalosporins has been developped. We studied Ceftetrame (Ro 19-5248), an oral cephalosporin of the ester type. After absorption from the intestinal tract Ceftetrame is hydrolysed by esterase to an active metabolite (Ro 19-5247) which shows a very promising activity against *Neisseria gonorrhoeae*, including penicillinase-producing strains. The MIC for the strains previously studied ranged from < 0.004-0.25 µg/ml[2]. A study of nine healthy volunteers revealed that peak serum levels of about 7.25 mg/ml were obtained after a single oral dose of 1200 mg Ceftetrame. The half life-time was about 1.5 h[3]. This pilot study was initiated for evaluating the efficacy of 1200 mg Ceftetrame in the treatment of uncomplicated acute gonococcal urethritis in male patients.

MATERIALS AND METHODS

All patients were men attending the out-patient clinic for sexually transmitted diseases at the University Hospital Rotterdam-Dijkzigt. Excluded were patients under 18, patients with complicated gonococcal infection, patients with only rectal or pharyngeal gonococcal infection, patients with syphilis, liver or kidney diseases, or cardiovascular decompensation, patients with a hypersensitivity to cephalosporin or penicillin and patients treated with other antimicrobials within the preceding 2 weeks.

A treatment was given on the basis of a Gram stain or positive culture for *Neisseria gonorrhoeae*.

13 Patients were treated with a single oral dose of 1200 mg Ceftetrame (12 capsules of 100 mg). The capsules were taken with water under the supervision of a nurse. The patients were advised to abstain from sexual contacts during the study period. All subjects gave oral informed consent before they were enrolled, and the study was approved by the committee for the protection of human subjects at the University Hospital Rotterdam-Dijkzigt.

Of the 13 patients treated, 3 could not be evaluated. Reasons were: 2 patients failed to report for follow-up and in one patient the culture for *N. gonorrhoeae* at start of the study turned out to be negative. A standard history was taken and all patients underwent the following tests before and 7-8 days after therapy: Gram stain of discharge, urethral *Chlamydia trachomatis* culture, *N. gonorrhoeae* cultures from urethra and tonsillae. Rectal cultures were obtained from homosexual men. The sediment of 10 to 15 ml of the first urine voided at least 4 hours after the previous voiding was collected. Blood was drawn for VDRL, FTA-ABS, and TPHA test (only before therapy), and general haematology, liver and kidney function determinations.

Nongonococcal urethritis was diagnosed if at follow-up, more than 10 leucocytes per field were seen in the sediment at a magnification of 250 x, and the Gram stain contained no intracellular Gram-negative diplococci. *N. gonorrhoeae* cultures were taken from the urethra, tonsil, and rectum as described above, using a carbon impregnated swab stick. They were transported in Stuart medium and inoculated within six hours, onto a selective medium consisting of GC agar base (Oxoid Ltd, London, England) and supplemented with 2% hemoglobin (Oxoid Ltd) 1% Isovitalex (BBL Microbiology Systems, Cockeysville, USA). The MIC values of the *N. gonorrhoeae* strains for the active metabolite and cefotaxime were determined by the standard agar dilution method with serial twofold dilutions between 4 and 0.004 μg/ml. All *N. gonorrhoeae* strains were tested for β-lactamase production using the chromogenic cephalosporin (Nitrocefin^r) test[4]. Auxotyping of the *N. gonorrhoeae* strains was performed at the National Institute of Public Health and Environmental Hygiene[5]. The pair of strains showing similarity before and after therapy were sent to the Centers for Disease Control, Atlanta, Georgia, USA, for lectin agglutination[6] and serotyping[7]. For *C. trachomatis* cultures, samples were taken by inserting white cotton-tipped metal swabs deep into the urethra, rotating and suspended in 0.02 M

phosphate buffer containing 0.2 M sucrose en 10% fetal calf serum, 25 µg/ml gentamicin and 25 U/ml nystatin. Samples were placed at −70°C within 6 hours.

Chlamydia was cultured on HeLa 229 monolayers rewashed with DEAE-dextran in 96-well microtiter plates. Staining was done with Microtrak fluorescent monoclonal antibodies (Syva Corp.) after 48 hours incubation[8]. Subpassage was not performed.

RESULTS

Ten patients were available for evaluation. In 9 patients cultures were negative for *N. gonorrhoeae* at follow-up. *C. trachomatis* was isolated before and after treatment in one patient. In 3 patients more than 10 leucocytes were found at follow-up, although *C. trachomatis* cultures were negative. No side effects were reported. One patient showed a slightly increased SGPT value and 3 patients a slightly increased lymphocyte count. None of the 11 *N. gonorrhoeae* strains produced penicillinase. The MIC values for the active metabolite ranged from < 0.002 to 0.12 µg/ml. The MIC90 for the active metabolite was 0.03 µg/ml. Auxotyping of the pre-and post-treatment gonococcal strain was identical (N.R.) as was the lectin agglutination pattern (TKA, GS1, SBA. STA, WGA) and the serovar (I-B1). The pre- as well as the posttreatment strain showed the same MIC value.

DISCUSSION

There have been no previous published studies on the oral treatment of gonococcal urethritis with Ceftetrame. In this study, 9 of 10 patients treated orally with Ceftetrame had negative gonococcal cultures at follow-up. The pre- and posttreatment strain from one assessable patient was identical in terms of auxo-and serovar typing and lectin agglutination pattern which suggests treatment failure or reinfection with the same strain. The results of this pilot study suggest that Ceftetrame is an effective drug in the treatment of uncomplicated urogenital gonorrhoea.

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CHAPTER 5

Resistance of *Neisseria Gonorrhoeae* to enoxacin

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Enoxacin shows favourable minimum inhibitory concentration (MIC) values, ranging from 0.03 to 0.12 mg/l against *Neisseria gonorrhoeae*. Of 78 male patients, treated in our Dermato-venereologic out-patient department for gonococcal urethritis with 400 mg enoxacin in one oral dose, 92% were cured. Of the failures, one patient reported back with a relapse of his infection. As renewed sexual intercourse was denied reinfection seemed probable. Determination of the MIC values by means of the agar dilution technique, using a gonococcal agar base with an inoculum of 10^4 CFU per spot showed a substantial increase in MIC (2.0 mg/l) of the post-treatment strain in comparision with the pre-treatment strain (0.03 mg/l). These results were confirmed at the National Institute of Public Health and Environmental Hygiene. Both strains were susceptible to penicillin (MIC 0.06 mg/l) and of similar auxotype. Mutants of gonococci showing a ten to hundred-fold decreased susceptibility to quinolones as compared to the parent strains can be isolated in vitro with a frequency of 10^8–10^9 (Van Klingeren, Dessens-Kroon & Verheuvel, 1985).

These findings indicate, that resistance has to be considered when therapy fails.

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CHAPTER 6

Decreased sensitivity of *Neisseria gonorrhoeae* to quinolone compounds

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Recently the use of quinolone compounds was reviewed in this journal (1). The compounds are reported to show favourable activity in vitro against *Neisseria gonorrhoeae* (2). In 1985, however, three penicillin-sensitive (MIC < 0.03, 0.06 and 0.6 μg/ml) *Neisseria gonorrhoeae* strains with decreased sensitivity to several quinolone compounds were isolated at our dermato-venereological out-patient department from as many patients suffering from uncomplicated urogenital gonococcal infection. Occurrence of this decreased sensitivity was relatively rare, as several hundred *Neisseria gonorrhoeae* strains were tested with various quinolone compounds.

MIC values of ciprofloxacin, enoxacin, ofloxacin and RO 23-6240 (AM 833) were determined for these strains by means of the agar dilution technique, using a gonococcal agar base with a bacterial inoculum of 10^4 CFU per spot. A decrease in sensitivity to these compounds was noted, MIC values being between 0.12 and 2.0 μg/ml. The MIC values of ciprofloxacin, enoxacin, ofloxacin and RO 23-6240 for the three strains were 0.12, 1.0-2.0, 0.50 and 0.50-1.0 μg/ml respectively. These results were reproducible. Of the compounds tested ciprofloxacin was the most active. The auxotype patterns of the three strains were not identical. Typical MIC 90 values of ciprofloxacin, enoxacin, ofloxacin (1) and RO 23-6240 (2) for *Neisseria gonorrhoeae* are 0.01, 0.25, 0.05 and 0.06 μg/ml respectively.

As with other bacterial species the existence of crossresistance to quinolones (1) in *Neisseria gonorrhoeae* must be considered when choosing a compound for therapy.

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CHAPTER 7

In vitro activities of seven quinolone derivatives against *Neisseria gonorrhoeae*

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SUMMARY

The in vitro activities of ciprofloxacin, enoxacin, fleroxacin, lomefloxacin, norfloxacin, ofloxacin and pefloxacin against 10 penicillinase-producing *N. gonorrhoeae* strains (PPNG's) 10 non PPNG's, 10 non-penicillinase-producing penicillin-resistant chromosomally mediated *N. gonorrhoeae* strains (CMRNG's), 3 *N. gonorrhoeae* strains with a decreased sensitivity against quinolones compounds and one tetracycline resistant penicillinase-producing *N. gonorrhoeae* (TRNG/PPNG) strain were evaluated in this study. The non PPNG, PPNG, CMRNG and TRNG/ PPNG strains showed good to excellent sensitivity to the quinolones tested. The *N. gonorrhoeae* strains with a decreased sensitivity against quinolones showed higher MIC (Minimum Inhibitory concentration) values.

INTRODUCTION

The search for new agents to treat gonococcal infections is still going on. Till now all of the quinolones tested in clinical trials have proved effective as single-dose therapy of gonorrhoeae, including the infections caused by penicillinase-producing isolates of *Neisseria gonorrhoeae* (PPNGs) [1,4]. Reports of resistance to enoxacin from The Netherlands and to norfloxacin from Thailand necessitates both the development of new therapeutic regimens as well as ongoing assessment of the effectiveness of old and new regimens. [5] There are indications for the existence of a cross-reacting diminished sensitivity pattern to quinolones in *Neisseria gonorrhoeae*. [6] In this paper the activity of seven quinolone derivatives against different penicillinase-negative and positive *N. gonorrhoeae* isolates is reported.

MATERIALS AND METHODS

A total of 34 *N. gonorrhoeae* strains isolated from patients, attending the venereologic out-patient department of the University Hospital of Rotterdam (The Netherlands) were tested. Ten were penicillinase-producing (or beta-lactamase) (PPNG's) as determined by the chromogenic cephalosporin test. [7]

Ten were non-penicillinase producing penicillin sensitive strains (non-PPNG's) (MIC 0.25 U/ml) and ten were non-penicillinase-producing penicillin-resistant chromosomally mediated *N. gonorrhoeae* strains (CMRNGs) with a MIC of penicillin of 1 μg/ml. Three strains had a decreased sensitivity against quinolones and one strain was a tetracycline-resistant penicillinase-producing *N. gonorrhoeae* (TRNG/PPNG) isolate with a MIC of tetracycline of 32 mg/l [8].

The following antimicrobial agents were tested: ciprofloxacin, enoxacin, fleroxacin, lomefloxacin, norfloxacin, ofloxacin, pefloxacin. MICs were determined by using a standard agar dilution method with GC agar base (Oxoid Ltd., London,

England) supplemented with 2% hemoglobin (Oxoid Ltd.) and 1% IsoVitalex (BBL Microbiology Systems, Cockeysville, Md.). Twofold dilutions of antibiotic concentrations, ranging from 8-0.004 mg/l, were used. The bacterial inoculum was 10^4 CFU per spot. The plates were incubated for 18 h at 37°C in an aerobic CO_2 atmosphere. Four bacterial strains with known MIC values were used as control strains.

RESULTS AND DISCUSSION

The ranges of MIC values and MIC values, required to inhibit up to 50% and 90% (MIC 50, MIC 90) of the quinolones against ten non-PPNG, ten PPNG and ten CMRNG strains are summarized in Table 1.

Table 1. In vitro activity of 10 non-penicillinase-producing penicillin sensitive (non-PPNG), 10 penicillinase-producing (PPNG) and 10 non-penicillinase-producing penicillin-resistant chromosomally mediated resistant (CMRNG) *N. gonorrhoeae* strains.

		MIC (mg/l)		
Compound		Range	50%	90%
ciprofloxacin	non-PPNG	0.004-0.008	0.004	0.008
	PPNG	0.004-0.015	0.008	0.008
	CMRNG	0.004-0.008	0.004	0.004
	total	0.004-0.15	0.004	0.008
enoxacin	non-PPNG	0.015-0.06	0.03	0.06
	PPNG	0.06 -0.12	0.06	0.06
	CMRNG	0.04 -0.12	0.06	0.12
	total	0.004-0.12	0.06	0.12
fleroxacin	non-PPNG	0.001-0.03	0.03	0.03
	PPNG	0.03	0.03	0.03
	CMRNG	0.001-0.06	0.06	0.06
	total	0.001-0.06	0.03	0.06
lomefloxacin	non-PPNG	0.015-0.06	0.015	0.015
	PPNG	0.015-0.06	0.015	0.015
	CMRNG	0.015-0.12	0.06	0.06
	total	0.015-0.12	0.015	0.06
norfloxacin	non-PPNG	0.015-0.03	0.015	0.03
	PPNG	0.015-0.03	0.015	0.03
	total	0.015-0.25	0.03	0.06
ofloxacin	non-PPNG	0.008-0.03	0.008	0.03
	PPNG	0.008-0.03	0.0015	0.015
	CMRNG	0.015-0.06	0.03	0.06
	total	0.008-0.06	0.015	0.03
pefloxacin	non-PPNG	0.008-0.03	0.015	0.015
	PPNG	0.015-0.06	0.03	0.03
	CMRNG	0.015-0.06	0.06	0.06
	total	0.008-0.06	0.03	0.06

Table 2. In vitro activity of three *N. gonorrhoeae* strains with decreased sensitivity against quinolones (A, B, C), and one tetracycline-resistant strain (D).

	MIC (mg/l)			
Compound	A	B	C	D
ciprofloxacin	0.12	0.12	0.12	0.004
enoxacin	2	1	2	0.03
fleroxacin	1	0.5	1	0.03
lomefloxacin	0.5	0.5	1	0.015
norfloxacin	0.5	1	2	0.015
ofloxacin	0.5	0.5	1	0.015
pefloxacin	1	0.5	2	0.15

The values of the three *N. gonorrhoeae* strains with a decreased sensitivity to quinolones (strain A, B, C) and the one TRNG/PPNG strain (D) are listed in Table 2.

All three groups of *N. gonorrhoeae* strains (non-PPNG, PPNG, CMRNG) showed good to excellent sensitivity to the quinolone derivatives tested. No appreciable differences between the groups are noted. These findings are in agreement with previous reports. [9-12]. Of all quinolones tested ciprofloxacin MIC values are lowest. The TRNG/PPNG strain shows the same sensitivity pattern regarding the quinolones as other gonococci from either group. As expected the phenomenon of decreased sensitivity of strains to quinolone compounds resulted in higher MIC values than usually expected. Sofar evaluation of the clinical relevance has not been evaluated for fleroxacin, lomefloxacin and pefloxacin is in progress.

Although these new drugs are promising candidates as alternative drugs for the single-dose treatment of gonorrhoeae, world wide use in the future remains to be carefully monitored.

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CHAPTER 8

Evaluation of roxithromycin in the treatment of non-gonococcal urethritis in males

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SUMMARY

One-hundred and fifty-two male patients suffering from non-gonococcal urethritis were treated with an oral dose of 300 mg roxithromycin daily for seven days. *Chlamydia trachomatis* was isolated from the urethra in 53 patients (35%), and *Ureaplasma urealyticum* in 42 patients (28%). After treatment, 49 (92%) of the 53 patients with positive *Chlamydia trachomatis* cultures and 34 (81%) of the 42 patients with positive *Ureaplasma urealyticum* cultures had negative cultures at follow-up. A clinical cure was observed in 137 patients (90%). Ten patients (7%) showed side effects consisting of nausea, sensation of distended abdomen, headache and fatigue. Seventy-eight male patients suffering from non-gonococcal urethritis were treated with an oral dosage of 2 x 150 mg roxithromycin daily for seven days. *Chlamydia trachomatis* was isolated from the urethra in 22 patients (28%), and *Ureaplasma urealyticum* in 30 patients (38%). After treatment, all of the 22 patients with formerly positive *Chlamydia trachomatis* cultures and 23 (77%) of the 30 patients with formerly positive *Ureaplasma urealyticum* cultures were negative at follow-up. A clinical cure was observed in 70 patients (90%). Three patients (4%) showed side effects consisting of nausea and headache. It is concluded that roxithromycin is a good alternative to tetracycline and erythromycin in the treatment of non-gonococcal urethritis in males.

INTRODUCTION

Tetracycline and erythromycin are still drugs of choice in the treatment of male non-gonococcal urethritis or uncomplicated *Chlamydia trachomatis* infections (1). Scheibel (2) demonstrated that a seven-day course of erythromycin is as effective as a seven-day course of tetracycline in treatment of *Chlamydia trachomatis* urethritis in males. Tetracyclines may result in phototoxicity in patients, while a frequent side effect of erythromycin is gastric symptoms. However, erythromycin is safe for children and pregnant women. In view of the high prevalence of *Chlamydia trachomatis* infections, an increase of such infections in these patients might be expected (3). Therefore it seemed useful to evaluate the efficacy of medication with an alternative macrolide antibiotic, roxithromycin (RU 28965).

This agent has the same structure as erythromycin but has a 2-methoxy-ethoxy-methoxy-imino group on the 9-position. In vitro studies (4) have shown that roxithromycin is effective against *Chlamydia trachomatis* and *Ureaplasma urealyticum*, with MIC values of 0.03-0.125 mcg/ml and 1.0-4 mcg/ml respectively. A study in 70 healthy control volunteers (5) demonstrated peak plasma levels of 2.5 mcg/ml after a single oral dose of 50 mg roxithromycin and 16.3 mcg/ml after 400 mg. Investigations indicated that roxithromycin was safe, well tolerated and well absorbed after oral administration. The half-life of roxithromycin is about 8 h.

The effectiveness of roxithromycin in treatment of non-gonococcal urethritis in males was investigated in two consecutive open trials. Patients in the first trail received 300 mg daily while those in the second trail were given 2 x 150 mg daily. Fractional doses might ensure a more constant plasma concentration, more effective therapy and fewer side effects.

MATERIALS AND METHODS

Patients Population

A total of 296 male patients with non-gonococcal urethritis who attended the out-patient clinic for sexually transmitted diseases of the University Hospital Rotterdam were enrolled for the study. One group of 186 patients received an oral dosage of 300 mg roxithromycin daily for seven days. The other group of 110 patients received an oral dosage of 150 mg roxithromycin twice daily for seven days. Excluded from the study were patients younger than 18 years, patients who had used antibiotics within seven days prior to the start of therapy, patients taking theophylline, anti-epileptics, ergot alkaloids, antacids or H_2-blockers, patients hypersensitive to macrolides and patients with a history of renal disease. All subjects gave oral informed consent before they were enrolled, and the study was approved by the Committee for the Protection of Human Subjects at the University Hospital Rotterdam-Dijkzigt.

Microbiogical Investigations

All patients underwent the following investigations before and one day after completion of therapy: Gram stain of urethral discharge, and culture of urethra samples for *Ureaplasma urealyticum*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*; blood samples were subjected to treponemal antibody test (only before therapy), general haematological investigations and chemical analysis. Then the sediment of the first 10-15 ml of urine was collected when the patient had not voided for 4 h or longer. Non-gonococcal urethritis was diagnosed if more than ten leucocytes were observed in the sediment at magnification 250 times and if the Gram stain revealed no intracellular Gram-negative diplococci. Clinical cure was defined as the absence of all symptoms after therapy. A negative culture after therapy was considered evidence of bacteriological cure. At the control visit (day 8) all patients were asked whether they had experienced side-effects.

Swabs for culture of *Ureaplasma urealyticum* were taken from the urethra with a wooden swab-stick and suspended in transport medium consisting of 22.5 g trypticase soy broth (Oxoid, UK), 200 ml inactivated horse serum, 30 ml yeast extract according to Hers (6), 50 ml 20% urea, 12.5 ml 0.2% phenol red. 50,000 U penicillin G and 750 ml distilled water. Swab-stick and suspensions were frozen at

−70°C within 6 h. The culture was performed according to the technique of Shephard (7,8). Swabs for cultures of *Chlamydia trachomatis* were taken with a white cotton-tipped metal swab-stick, which was inserted deep into the urethra, rotated, withdrawn and suspended in 0.2 m sucrose and 0.02 M phosphate buffer with 10% fetal calf serum, 25 mcg/ml gentamicin and 25 U/ml nystatin. The samples were frozen at −70°C within 6 h. The culture was performed on HeLa 229 monolayers pre-washed with DEAE-dextran in 96-well titre plates. Staining was carried out with fluorescent monoclonal antibodies (Syva, USA) after 48 h of incubation (9). Subpassage was not performed. Swabs for culture of *Neisseria gonorrhoeae* were taken from the urethra with a carbon-impregnated swabstick which, after transport in Stuart medium, was inoculated within 6 h on a selective medium comprising GC agar base (Oxoid) supplemented with 2% haemoglobin (Oxoid) and 1% Isovitalex (BBL, USA).

Statistical Analysis

The results were analysed using the two-tailed Fisher's exact test.

RESULTS

Of the 186 patients treated with 300 mg roxithromycin daily (Table 1), 34 (18%) could not be evaluated due to failure to report for follow-up in 19 (56%) cases, sexual contact in three (9%), no chlamydia culture at follow-up in six (17%), positive *Neisseria gonorrhoeae* culture in one (3%), improper drug intake in one (3%) and no *Neisseria gonorrhoeae* culture at the start of the study in four (12%). The remaining 152 patients available for evaluation included 53 (35%) in whom *Chlamydia trachomatis* and 42 (28%) in whom *Ureaplasma urealyticum* was isolated from the urethra. After treatment 49 (92%) of the 53 patients with formely positive *Chlamydia trachomatis* cultures and 34 (81%) of the 42 patients with

Table 1. Results of therapy in relation of results of culture for *Chlamydia trachomatis* and *Ureaplasma urealyticum* in 230 male patients suffering from non-gonococcal urethritis before and after oral treatment with 1 x 300 mg or 2 x 150 mg roxithromycin daily for seven days.

Culture results	1 x 300 mg (n=152)			2 x 150 mg (n=78)		
Chlamydia trachomatis/ Ureaplasma urealyticum	Before treatment	After treatment	Resolution of pyuria	Before treatment	After treatment	Resolution of pyuria
Positive/positive	15	0	13	5	0	2
Negative/positive	27	3	21	25	4	13
Positive/negative	38	7[a]	33	17	0	12
Negative/negative	72	142	56	31	74	22

[a]Three positive only at follow-up.

formely positive *Ureaplasma urealyticum* cultures had negative cultures. Clinical cure was observed in 137 (90%) of the 152 patients. In three cases only the follow-up *Chlamydia trachomatis* culture was positive. *Chlamydia trachomatis* was isolated in six and *Ureaplasma urealyticum* in three clinically cured patients with a negative urinary sediment. Both a negative urinary sediment and clinical cure were noted in 123 (81%) of the 152 patients. Side effects included nausea in five (3%) patients, sensation of distended abdomen in one (1%), nausea and headache in one (1%) and fatigue in three (2%). The only abnormal laboratory finding was a slight increase in SGOT and SGPT values in one patient.

Of the 110 patients treated with 2 x 150 mg roxithromycin daily (Table 1), 32 (29%) could not be evaluated due to failure to report for follow-up in 25 (78%) cases, sexual contact in two (6%), no chlamydia culture at follow-up in three (19%), improper drug intake in one (3%) and no *Neisseria gonorrhoeae* culture at the start of the study in one (3%).

The 78 patients available for evaluation included 22 (28%) in whom *Chlamydia trachomatis* and 30 (38%) in whom *Ureaplasma urealyticum* was isolated from the urethra. After treatment all of the 22 patients with formerly positive *Chlamydia trachomatis* cultures and 23 (77%) of the 30 patients with formerly positive *Ureaplasma urealyticum* cultures had negative cultures. Clinical cure was observed in 70 (90%) of the 78 patients. *Ureaplasma urealyticum* was isolated in one clinically cured patient with a negative urinary sediment. Both a negative urinary sediment and a clinical cure was noted in 49 (63%) of the 78 patients. Side effects included nausea in two patients (3%) and headache in one patient (1%). One patient had a slightly increased SGOT value and three patients a slightly increased total bilirubin concentration.

At statistical analysis both within and between groups, only the difference between clinically cured patients with a negative urinary sediment was statistically in favour of a single dose of 300 mg ($p < 0.01$).

DISCUSSION

The clinical cure rates obtained with both dosage regimens (90%) are within the range reported for tetracyclines of 70-90% (10-13). Unlike Bowie (13) we were not able to demonstrate a more favourable therapeutic result in patients with cultures positive for *Chlamydia trachomatis* and/or *Ureaplasma urealyticum* compared to those with negative cultures. Due to the latency or temporary suppression of the organism seen in *Chlamydia trachomatis* infections, the cure rate obtained immediately after therapy might be higher than would have been the case with a follow-up period of two weeks. However, Bowie (13) demonstrated that none of 24 men who had become *Chlamydia trachomatis* negative after a seven day course of 1 g tetracycline daily were positive again after a follow-up period of more than two weeks. Arya (12) demonstrated that relapses occured after 3 to 4 weeks in 11 of 62

patients and after 5 weeks in 64 of 90 patients who admitted sexual contact during this period.

The fact that the difference between the clinically cured patients with a negative urinary sediment was statistically significant in favour of the 300 mg dose may reflect better tissue penetration when a higher single dose is administered, with a consequently more pronounced anti-bacterial effect.

The mild side effects reported by 5% of the patients were never a reason for discontinuation of treatment. Roxithromycin would thus seem to be effective in the treatment of non-gonococcal urethritis in males, and suitable for use as an alternative to tetracycline and erythromycin.

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CHAPTER 9

Clinical efficacy of ciprofloxacin versus doxycycline in the treatment of non-gonococcal urethritis in males

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SUMMARY

In a randomized study the clinical efficacy of ciprofloxacin was compared with that of doxycycline administered in two different dosage schemes to male patients suffering from non-gonococcal urethritis. Fourteen days after completion of therapy (day 21) pyuria was absent in 30 of 100 patients in the ciprofloxacin group; *Chlamydia trachomatis* was isolated from five and *Ureaplasma urealyticum* from eight patients. In the 100 mg doxycycline group (n=60) pyuria was absent in 36 patients (60%) and *Ureaplasma urealyticum* was isolated from six patients on day 21. In the 200 mg doxycycline group (n=45) pyuria was absent in 18 patients (40%) and *Ureaplasma urealyticum* was isolated from two patients on day 21. Side effects were mild and transient in all groups. It is concluded that ciprofloxacin given in a dosage of 1 g for seven days is not effective in the treatment of non-gonococcal urethritis.

INTRODUCTION

Epidemiological research into the prevalence of genital pathogens in male patients attending a venereological out-patient clinic revealed that *Chlamydia trachomatis* was isolated in 30% and *Ureaplasma urealyticum* in 30%. It was also found that up to 40% of the men suffering from gonorrhoea were also infected with *Chlamydia trachomatis* (1). These percentages indicate that *Chlamydia trachomatis* and perhaps also *Ureaplasma urealyticum* play an important role in the aetiology of sexually transmitted diseases.

Ciprofloxacin, one of the new quinolones, is effective against *Neisseria gonorrhoeae* both in vitro and in vivo (2). The MIC values of ciprofloxacin for both *Chlamydia trachomatis* and *Ureaplasma urealyticum* range from 0.5 to 2 μg/ml (3). An agent which could cure both gonorrhoeae and chlamydial infections is becoming more and more desirable. Moreover, an addition to the current drugs used in the treatment of non-gonococcal urethritis (tetracyclines or macrolides (4)) would be welcome. Ciprofloxacin is a possible candidate (5). In a randomized study the clinical efficacy of ciprofloxacin versus doxycycline administered in two different dosage schemes was tested in male patients suffering from non-gonococcal urethritis.

MATERIALS AND METHODS

All patients were men attending the outpatient clinic for sexually transmitted diseases at the University Hospital Rotterdam-Dijkzigt. Patients younger than 18 were excluded, as were patients with a history of allergy to tetracycline or carboxyquinolone derivatives, those with severe liver and kidney disease, those with underlying conditions making it unlikely that treatment with the test drugs and

follow-up could be completed, and those likely to require treatment with a concomitant antimicrobial agent with a range of activity similar to that of the test drugs.

Three hundred and eight patients were enrolled in the study; 155 patients were treated with ciprofloxacin and 153 with doxycyline. In the first part of the study a randomisation list ensured that patients received either 1 g ciprofloxacin in a single oral dose daily for seven days or 200 mg doxycycline on day 1, followed by 100 mg daily for six days. In the second part of the study a randomisation list ensured that patients received either 1 g ciprofloxacin daily for seven days or 2 x 100 mg doxycycline daily for seven days. Patients were advised to abstain from sexual contacts or to use condoms throughout the follow-up period of three weeks. All subjects gave oral informed consent before enrollment. The study was approved by the committee for the protection of human subjects at the University Hospital Rotterdam-Dijkzigt.

A standard history was taken and all patients submitted to the following tests before and one day after completion of therapy (day 8): Gram stain of urethral discharge and culture of urethral samples for *Ureaplasma urealyticum* (6-9), *Chlamydia trachomatis* (6,10) and *Neisseria gonorrhoeae* (6), test of blood samples for treponemal antibodies (only before therapy), general haematological tests and blood chemical analysis. The sediment of the first 10-15 ml urine was collected when the patient had not voided for 4 h or longer.

Non-gonococcal urethritis was diagnosed if more than ten leucocytes were observed in the sediment at a magnification of 250 times and if the Gram stain revealed no intracellular Gram-negative diplococci. At the control visit urinary sediment was examined by microscopy for the presence of casts and crystals. Clinical cure was defined as the disappearance of complaints following therapy. Bacteriological cure was defined as a negative culture following therapy. At the first assessment (day 8) all patients were asked whether they had experienced any side-effects. The patients whose pyuria was cured were asked to report back for a follow-up two weeks after completion of therapy. At this third visit (day 21) the previously performed tests were repeated except for the blood analysis. At both control visits all patients were asked whether dysuria and/or discharge were still present. The results were analysed statistically by applying Fisher's one-tailed exact test.

RESULTS AND DISCUSSION

Table 1 summarizes the reasons why patients could not be included in this study. The results of *Chlamydia trachomatis* and *Ureaplasma urealyticum* cultures, and the incidence of resolution of pyuria and clinical cure in the assessable patients are summarized in Table 2 and 3. Of the total of 155 male patients treated with 1 g ciprofloxacin, 55 (35%) were not assessable (Table 1); 100 patients were bacteriologically assessable and 101 patients were assessable with respect to side effects. Of the nine patients who were *Ureaplasma urealyticum* positive on day 21 (Table 3)

Table 1. Non-assessable patients treated with ciprofloxacin and doxycycline.

Reason for non-assessment	Ciprofloxacin	Doxycycline
Sexual contact	8	1
Failure to return for assessment	22	22
Failure to return for follow-up	9	16
Failure to take medication	6	0
Gonorrhoea	3	2
No gonococcal culture at start of study	1	0
No control *C. trachomatis* culture	5	7
Recent syphilis	1	0
Total	55	48

one was positive only on that day, one had first become positive one day after completion of therapy, five had relapses, and two had been positive throughout. Of the eight patients who were positive for *Chlamydia trachomatis* on day 21, three were positive only on that day and five had relapses. Ten patients had a positive culture for *Chlamydia trachomatis* or *Ureaplasma urealyticum* without sediment changes on day 21.

Of the total of 153 patients treated with doxycycline, 48 were not assessable (Table 1). In the 100 mg doxycycline group, 60 patients were bacteriologically assessable and 63 patients were assessable with respect to safety. Of the eight patients who were *Ureaplasma urealyticum* positive on day 21 (Table 3) two were positive only on that day. One had first become positive one day after completion of therapy, three had relapses and two had been positive throughout. One patient was positive for *Chlamydia trachomatis* on day 21. Three patients had a positive culture for *Chlamydia trachomatis* or *Ureaplasma urealyticum* without sediment changes on day 21.

In the 200 mg doxycycline group 45 patients were bacteriologically assessable and 47 were assessable with respect to side-effects. Of the three patients who were positive for *Ureaplasma urealyticum* on day 21 (Table 3), one was positive only on that day, one had a relapse and one had been positive throughout. One patient had a positive culture without pyuria on day 21.

One of the 101 patients (1%) treated with ciprofloxacin had abdominal pain. Abnormal laboratory findings included slightly increased alkaline phosphatase, SGOT and SGPT values in one case, and slight anisocytosis in one case. Side effects and abnormal laboratory findings were not observed in the 100 mg doxycycline group.

One of the 47 patients treated with 200 mg doxycycline showed a photoallergic reaction, one complained of abdominal pain, one of vertigo and one of general malaise. Abnormal laboratory findings included slightly increased total bilirubin, SGOT and SGPT values in one case, a slightly total bilirubin value in one case, and slightly increased total bilirubin and SGPT values in one case.

Table 2. Results of antimicrobial therapy in males with non-gonococcal urethritis after 8 days.

Oral therapy	No. of patients	Culture before therapy		Bacteriological cure		Resolution of pyuria	Clinical cure
		C. tracho-matis	U. urealy-ticum	C. tracho-matis	U. urealy-ticum		
Ciprofloxacin 1 g x 7 days	100	32/100	32/100	29/32 (+1)[a]	28/32 (+1)[a]	44/100	74/100
Doxycycline 200 mg x 1 day 100 mg x 6 days	60	15/60	20/60	14/15 (+1)[a]	15/20 (+1)[a]	44/60	50/60
Doxycycline 2 x 100 mg x 7 days	45	13/45	20/45	13/13	18/20	23/45	36/45

[a] Number of cultures not previously positive.

Table 3. Results of antimicrobial therapy in males with non-gonococcal urethritis after 21 days.

Oral therapy	No. of patients	Culture before therapy		Bacteriological cure		Resolution of pyuria	Clinical cure
		C. tracho-matis	U. urealy-ticum	C. tracho-matis	U. urealy-ticum		
Ciprofloxacin 1 g x 7 days	100	32/100	32/100	24/32	23/32	30/100	35/100
Doxycycline 200 mg x 1 day 100 mg x 6 days	60	15/60	20/60	14/15	12/20	36/60	38/60
Doxycycline 2 x 100 mg x 7 days	45	13/45	20/45	13/13	17/20	18/45	20/45

Statistical analysis disclosed significant differences only between the group given ciprofloxacin and the group given 200 mg doxycycline on day 1 followed by 100 mg daily for six days, in terms of resolved pyuria one day (44% versus 73%) and 14 days (30% versus 60%) after completion of therapy ($p < 0.01$). A difference in cure rate between *Chlamydia trachomatis* and/or *Ureaplasma urealyticum* positive and negative non-gonococcal urethritis was not observed.

Nor was a difference seen between the bacteriological and clinical cure rates in the three treatment groups.

In view of the bacteriological and clinical findings after three weeks, it is evident that neither ciprofloxacin nor doxycycline in dosages administered in this study are sufficient to cure non-gonococcal urethritis.

An interesting finding is the decreasing rates of clinical cure and resolution of pyuria observed in non-gonococcal urethritis patients treated with doxycycline. The factors responsible are not known.

A possible reason for the low efficacy of ciprofloxacin in the treatment of non-gonococcal urethritis is underdosage. Another possible reason is insufficient duration

of treatment. It has been reported (11) that a dosage of 500 mg ciprofloxacin given twice daily for ten days was effective in the treatment of non-gonococcal urethritis. A clinical study (12) comparing the efficacy of 750 mg ciprofloxacin twice daily versus 100 mg doxycycline twice daily for seven days revealed no differences in the clinical and bacteriological cure or relapse rates in non-gonococcal urethritis patients. An earlier study (13) using a dosage of 500 mg ciprofloxacin twice daily for seven days revealed a cure rate of 22% in patients suffering from *Chlamydia trachomatis* positive urethritis.

Although the bacteriological cure rates in our three treatment groups were within the range reported in the literature of 70-90% (14-16), in terms of resolution of pyuria and clinical cure the rates were lower in all three treatment groups. A study (14) using the same doxycycline regimens as those used in our study showed no difference between the two groups in the clinical and bacteriological cure rate in male patients suffering from non-gonococcal urethritis. Evaluation took place one day after completion of treatment. Although not statistically significant, the results in terms of resolution of pyuria are better in our study in the 100 mg doxycycline group than the 200 mg doxycycline group. Futher studies are necessary to confirm these findings. Side-effects and laboratory changes were minimal in number and of a transient nature in the three treatment groups in our study.

In conclusion, ciprofloxacin administered in a dosage of 1 g for seven days is not sufficiently effective in the treatment of non-gonococcal urethritis in male patients.

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CHAPTER 10

Antimicrobial susceptibility and serotyping of *Chlamydia trachomatis* strains isolated before and after treatment with ciprofloxacin and doxycycline

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In evaluating new therapies for treatment of sexually transmitted diseases it is necessary to examine carefully patients in whom treatment fails in order to rule out factors as poor compliance in taking medication or continued sexual contact during the study period. Alternatively, development of antimicrobial resistance in the microorganism involved may account for treatment failure. In this study we investigated cases of treatment failure occurring in an earlier therapy study of patients with non-gonococcal urethritis who were treated with ciprofloxacin or doxycycline (1). Randomly selected *Chlamydia trachomatis* strains isolated before therapy and all available pairs of strains isolated before and after therapy with either drug were serotyped, and the in vitro activity of these two drugs and erythromycin against the strains was determined.

Five *Chlamydia trachomatis* strains isolated only before therapy with ciprofloxacin or doxycycline were serotyped and the MICs and MBCs of ciprofloxacin, erythromycin and doxycycline for these strains determined. Likewise, serotype, MIC and MBC were determined for four pairs of strains isolated before and after therapy with ciprofloxacin (1 g daily in a single oral dose for seven days) and one strain isolated before and after therapy with doxycycline (200 mg on day 1, followed by 100 mg daily for six days).

The MIC of each drug was determined using the method described by Segreti et al (2). DEAE-dextran treated McCoy cells were inoculated with 10^3-10^4 inclusion-forming units of each chlamydial isolate per well, centrifuged for 60 min, and incubated at 37°C in a 5% CO_2 atmosphere for 2 days. The inoculum was then replaced with culture media (0.1 ml per well) containing serial twofold dilutions of each antimicrobiol agent and 0.1 µg/ml cycloheximide.

The drugs were diluted from 16 to 0.015 µg/ml. After 48h of incubation two wells per dilution were fixed and stained with a fluorescine-conjugated monoclonal antibody to *Chlamydia trachomatis* (3). The MIC was defined as the lowest concentration at which 90% inhibition of inclusion body formation was observed. The remaining wells were incubated for another 48h in culture media without antibiotics. The MBC was defined as the lowest concentration of antimicrobial agent yielding no inclusions after passage (2).

Staphylococcus aureus ATCC and 25923 and 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 with known MIC values for the test drugs were used as control strains.

The culture medium containing suspended *Chlamydia trachomatis* and host cell debris was centrifuged at 11.000 x g for 15 minutes. The resulting pellet was suspended in phosphate buffered saline (of pH 7.6 containing 0.02% formalin). Suspensions of prototype antigens or clinical isolates to be typed were applied to nitrocellulose membranes and dried. The dot enzyme immunoassay for serotyping was performed as described perviously (4).

The MICs of doxycycline before treatment were 0.06-0.25 µg/ml (Table 1).

Unfortunately the only posttreatment strain in the doxycycline group died in storage and could not be tested further. The MICs of ciprofloxacin before and after

Table 1. Serotypes of and MICs of doxycycline, ciprofloxacin and erythromycin for five *Chlamydia trachomatis* strains isolated only before antibiotic treatment or before and after treatment.

Therapeutic Agent	Serotype		MIC (μg/ml)					
			Ciprofloxacin		Doxycycline		Erythroycin	
	Before	After	Before	After	Before	After	Before	After
Ciprofloxacin	D		2		0.25		0.5	
	I		2		0.12		0.25	
	E		2		0.25		0.5	
	H		2		0.12		0.25	
	F		2		0.06		0.25	
	K	K	2	2	0.25	0.25	0.5	0.5
	I	I	1	1	0.12	0.12	0.25	0.25
	F	F	2	2	0.25	0.25	0.12	0.12
	D	D	2	2	0.25	0.25	0.12	0.12
Doxycycline	D		2		0.12		0.25	
	E		2		0.06		0.25	
	E		2		0.12		0.25	
	D		2		0.12		0.25	
	J		J		0.12		0.25	
	G	[a]	1	[a]	0.12	[a]	0.5	[a]

[a]Strain not viable for testing.

therapy were 1–2 μg/ml (Table 1). No elevation of MICs was observed in the strains isolated after treatment. The MICs of erythromycin for all strains were 0.12–0.5 μg/ml (Table 1). All of the MIC and MBC values determined in this study were identical or within one dilution step.

All strains isolated before and after therapy were of the same serotype (Table 1).

The MIC values of doxycycline, ciprofloxacin and erythromycin were within the ranges previously reported (5).

The fact that the pre-treatment and post-treatment strains were the same does not rule out a new infection, but makes it less probable, as condom use was advised during the treatment and follow-up period. Thus we conclude that these cases should be regarded as genuine instances of treatment failure of ciprofloxacin without development of resistance.

Recently, Jones et al. (6) isolated *Chlamydia trachomatis* strains resistant to tetracycline from five patients. These isolates were also resistant to doxycycline and erythromycin. The clinical significance of this resistance is not known.

To our knowlegde, there are no reports of resistance to ciprofloxacin in *Chlamydia trachomatis.*

Although successful treatment of *Chlamydia trachomatis* infections with ciprofloxacin depends not only on the MIC/MBC values but also on other pharmacokinetic parameters, clinical studies have demonstrated that ciprofloxacin in increasing dosages did not display increased efficacy (1,7,8).

Thus the findings in this investigation support our previous conclusion that *C. trachomatis* infections in these patients were not cured with ciprofloxacin therapy (1).

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CHAPTER 11

General discussion

In view of the AIDS catastrophe, one may question whether research into the causes and the treatment of sexually transmitted urethritis is still meaningful. In my personal opinion, it is far from it. All sexually transmitted diseases including HIV infections are closely related to each other as far as their history, biology, behavioural science, economics and therapeutics are concerned [1]. Worldwide, Gonorrhea is the most common veneral disease. In the Western World, non-gonococcal urethritis (NGU) is the most common bacterial Sexually Transmitted Disease (STD).

Historically, it appears that the incidence of gonococcal infections occurs in waves and serves as a scale for measuring the changes in sexual behaviour [2]. The downward trend in gonococcal infections of the past years has recently reversed into an upward trend [3]. Since, vaccination against gonococcal infections is unavailable at present and will not be available in the near future, treatment with antibiotics remains essential [4].

The fact that *N. gonorrhoeae* are able to survive for prolonged periods in an untreated patient and frequently infect the same individiual again makes this micro-organism a "survivor" [5]. At present, it appears that inspite of the worldwide use of protocoled treatment strategies, it has not yet been possible to check the problem of gonorrhea. The number of resistant and multiple resistant gonococcal strains has increased steadily [3,6,7]. In the game of chess between the new antibiotics and the development of resistance, *N. gonorrhoeae* appear to have the upper hand.

Resistance against quinolones (a new group of drugs for treating gonorrhea), first reported by us for enoxacin, is being increasingly reported in the international literature [8]. Resistance against ciprofloxacin has been reported in different studies [9,10]. Development of resistance against norfloxacin has also been reported [11]. The second group of drugs that occupy a prominent place for treating gonorrhea are the cephalosporins. The reduced susceptibility of gonococci for this group of antibiotics in vitro is of great concern [12]. In vivo resistance to cephalosporins has not yet been reported.

The knowledge that at present there is no new group of antibiotics which is able to eliminate the newly arising highly resistant *N. gonorrhoeae* strains appears to present a rather gloomy picture for the treatment of gonorrhea in the future.

Another noteworthy point is the frequent occurrence of mixed infections of *N. gonorrhoeae* with *C. trachomatis* (about 15%).

To date, infection caused by *C. trachomatis* is the most common bacterial STD in the Western World.

It has been estimated that between 35 and 60% of the NGU cases are caused by *C. trachomatis*. At present, this figure is somewhere between 15 and 20%.

The possible polymicrobial aetiology of NGU often pose therapeutic problems. Even when the therapy of NGU is strictly according to the protocol, treatment-failures still occur in a not to be neglected group of patients [13].

At present, the therapy of NGU is mainly directed at eliminating *C. trachomatis* infections.

Taking into account that *C. trachomatis* is a frequent causative agent of NGU, the complications that arise and the checkable follow-up after treatment suggest that this approach for dealing with NGU is both useful and essential. In fact, the problem is clearly more complicated. The persistent and recurrent NGU presents a dilemma which cannot be easily resolved since the exact causative agent(s) remains elusive [14].

Recently, resistance of *C. trachomatis* strains against erythromycin, tetracycline and other antibiotics has been reported [15]. Although the clinical implication of this is not yet clear, it should be taken into account, there will be problems in treating infections caused by these strains in the future. Evaluation of new drugs for treating NGU are progressing. The new quinolones which are effective against *C. trachomatis* in vitro appear not to offer any distinct clinical advantage and/or are observed to be less effective as compared with other normally used therapies. Another group of drugs, the new macrolides are as effective as other normally used therapies. Azithromycin has the advantage that the period of treatment can be shortened by 1 to 3 days. To date, this drug has mostly been clinically evaluated in patients with *C. trachomatis* positive urethritis. Whether the same results would be obtained in NGU as a whole remains to be investigated.

The coincidence of gonococcal and non-gonococcal urethritis, the nature of the patient population in relation to therapy compliance and check-ups, the size of the problem, the resulting complications and the development of resistance by the micro-organisms justify the view to return to the old thinking that sexually transmitted urethritis is a single disease. The solution would be a single dose of one drug or a cocktail of drugs administered to a patient with urethritis. However, that is not to say that culturing of the different causative agents, the determining of antibiograms, the research into vaccins and the development of new drugs should be discontinued.

SUMMARY

Chapter 1

In this chapter, a historical survey of sexually transmitted urethritis is presented. The different causative agents, the clinical presentations and the complications are all discussed. A review of different therapies and the problems encountered through the years is presented. The development of drug-resistance by the causative microbes is discussed in detail. The epidemiology of gonorrhea, *C. trachomatis* and NGU is described. A literature survey of new drugs for treating gonorrhea, the quinolones and the third generation and oral cephalosporins is presented and discussed with respect to their in vitro effectiveness against *N. gonorrhoeae*, their clinical efficacy and their side-effects.

At the same time, a literature survey on new drugs for treating NGU and *C. trachomatis* infections is also presented. The quinolones and new macrolides are

discussed in relation to their in vitro effectiveness against *C. trachomatis* and *U. urealyticum*, their clinical efficacy and their side-effects.

Chapter 2

In a double-blind randomized study, 155 male patients with uncomplicated urethral gonorrhea were given 200 mg (n = 77) or 400 mg (n = 78) of enoxacin orally. The cure rates in the 200- and 400-mg treatment groups were 90 and 92%, respectively. The enoxacin MIC for the isolated *N. gonorrhoeae* strains ranged from 0.015 to 0.12 μg/ml. Postgonocccal urethritis was diagnosed in 29 (42%) patients in the 200-mg treatment group and 19 (26%) patients in the 400-mg treatment group. Side effects (nausea, headache, and vomiting) occurred in 2 (3%) of the 77 patients in the 200-mg treatment group and in 3 (4%) of the 78 patients in the 400-mg treatment group.

Chapter 3

Uncomplicated urogenital and concomitant oropharyngeal gonorrhea in 424 male and female patients was treated in a randomized comparative study with 0.5 g of cefodizime (89 men and 54 women), 1 g of cefodizime (87 men and 52 women), or 1 g of cefotaxime (86 men and 56 women). The cure rates were 100% for men and women in the group given 0.5 g of cefodizime, 100% for men and women in the group given 1 g of cefodizime, and 99% for men and 100% for women in the group given 1 g of cefotaxime. The MICs of cefodizime and cefotaxime for the isolate of *N. gonorrhoeae* ranged from 0.004 to 0.06 μg/ml. *C. trachomatis* was isolated before treatment in 15% and after treatment in 13% of all patients. Side effects, such as nausea, diarrhea, abdominal pain, genital candidiasis, and pain at the site of injection, developed in 4% of the patients given cefodizime. Side effects, such as vertigo, genital candidiasis, fatigability, and diarrhea, developed in 4% of the patients treated with cefotaxime. In both groups of patients, the side effects were mild and transient. Cefodizime and cefotaxime are safe and effective agents in the treatment of uncomplicated urogenital gonorrhea.

Chapter 4

Ten male patients suffering from uncomplicated urethral gonorrhoea were treated orally with 1200 mg Ceftetrame. Nine of the 10 patients were cured. The sensitivity of the isolated *N. gonorrhoeae* strains to Ro 19-5247, the active metabolite of Ceftetrame, ranged from < 0.002 to 0.12 μg/ml.

C. trachomatis was isolated before and after treatment in one patient. No side effects were reported. Ceftetrame is an effective drug in the treatment of uncomplicated urogenital gonorrhoea in males.

Chapters 5 and 6

The development of resistance of *N. gonorrhoeae* against the new quinolones and the crossresistance of *N. gonorrhoeae* to various quinolone derivatives are discussed here.

Chapter 7

The in vitro activities of ciprofloxacin, enoxacin, fleroxacin, lomefloxacin, norfloxacin, ofloxacin and pefloxacin against 10 penicillinase-producing *N. gonorrhoeae* strains (PPNG's) 10 non PPNG's, 10 non-penicillinase-producing penicillin-resistant chromosomally mediated *N. gonorrhoeae* strains (CMRNG's), 3 *N. gonorrhoeae* strains with a decreased sensitivity against quinolones compounds and one tetracycline resistant penicillinase-producing *N. gonorrhoeae* (TRNG/PPNG) strain were evaluated in this study. The non PPNG, PPNG, CMRNG and TRNG/ PPNG strains showed good to excellent sensitivity to the quinolones tested. The *N. gonorrhoeae* strains with a decreased sensitivity against quinolones showed higher MIC values.

Chapter 8

One-hundred and fifty-two male patients suffering from non-gonococcal urethritis were treated with an oral dose of 300 mg roxithromycin daily for seven days. *C. trachomatis* was isolated from the urethra in 53 patients (35%), and *U. urealyticum* in 42 patients (28%). After treatment, 49 (92%) of the 53 patients with positive *C. trachomatis* cultures and 34 (81%) of the 42 patients with positive *U. urealyticum* cultures had negative cultures at follow-up. A clinical cure was observed in 137 patients (90%). Ten patients (7%) showed side effects consisting of nausea, sensation of distended abdomen, headache and fatigue. Seventy-eight male patients suffering from non-gonococcal urethritis were treated with an oral dosage of 2 x 150 mg roxithromycin daily for seven days.

C. trachomatis was isolated from the urethra in 22 patients (28%), and *U. urealyticum* in 30 patients (38%). After treatment, all of the 22 patients with formerly positive *C. trachomatis* cultures and 23 (77%) of the 30 patients with formerly positive *U. urealyticum* cultures were negative at follow-up. A clinical cure was observed in 70 patients (90%). Three patients (4%) showed side effects consisting of nausea and headache. It is concluded that roxithromycin is a good alternative to tetracycline and erythromycin in the treatment of non-gonococcal urethritis in males.

Chapter 9

In a randomized study the clinical efficacy of ciprofloxacin was compared with that of doxycycline administered in two different dosage schemes to male patients suffering from non-gonococcal urethritis. Fourteen days after completion of therapy (day 21) pyuria was absent in 30 of 100 patients in the ciprofloxacin group; *C. trachomatis* was isolated from five and *U. urealyticum* from eight patients. In the 100 mg doxycycline group (n = 60) pyuria was absent in 36 patients (60%) and *U. urealyticum* was isolated from six patients on day 21. In the 200 mg doxycycline group (n = 45) pyuria was absent in 18 patients (40%) and *U. urealyticum* was isolated from two patients on day 21. Side effects were mild and transient in all groups. It is concluded that ciprofloxacin given in a dosage of 1 g for seven days is not effective in the treatment of non-gonococcal urethritis.

Chapter 10

In this chapter, the susceptibility of *C. trachomatis* strains isolated in the study described in chapter 9 for ciprofloxacin, doxycycline and erythromycin are determined. At the same time five strains which were isolated before, and the strains that were isolated before and after therapy were sero-typed. The MICs for these three antibiotics corroborated well with those reported in the literature.

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SAMENVATTING

Men kan zich afvragen of, in het licht van de AIDS catastrofe, onderzoek naar de oorzaak en behandeling van sexueel overdraagbare urethritiden nog wel zinvol is. Niets is mijns inziens minder waar. Alle sexueel overdraagbare aandoeningen, inclusief HIV infecties, zijn historisch, biologisch, gedragswetenschappelijk, economisch en therapeutisch nauw met elkaar verbonden [1]. Wereldwijd is gonorroe de meest voorkomende geslachtsziekte. In de westerse wereld is NGU de meest voorkomende bacteriële SOA.

Historisch gezien blijkt de incidentie van gonorroïsche infecties een golfbeweging te beschrijven en een graadmeter te zijn voor veranderingen in sexueel gedrag [2]. De neergaande lijn van het aantal gonorroïsche infecties van de afgelopen jaren is recentelijk omgebogen naar een opwaartse [3]. Omdat vaccinatie tegen *N. gonorrhoeae* infecties nog niet op korte termijn te verwachten valt, blijft behandeling met antibiotica noodzakelijk [4].

Het feit dat de gonococ in staat is gedurende langere tijd in de onbehandelde patiënt te overleven en frequent eenzelfde persoon kan besmetten, maakt dit microorganisme tot een "overlever" [5]. Op dit moment is het, ondanks wereldwijde protocollaire behandelingsstrategieën, niet mogelijk gebleken het gonorroe probleem in te dammen. Het aantal resistente en multipel resistente gonococcen stammen neemt overhand toe [3,6,7]. In het schaakspel van nieuw antibioticum en resistentievorming lijkt de gonococ in het voordeel te zijn.

Resistentie tegen quinolonen, een nieuwe geneesmiddelengroep bij de behandeling van gonorroe, door ons voor het eerst beschreven voor enoxacin, wordt steeds vaker gemeld in de internationale literatuur [8]. Resistentie tegen ciprofloxacin werd verschillende malen beschreven [9,10]. Ook voor norfloxacin is resistentie vorming beschreven [11].

De tweede groep geneesmiddelen die een voorname plaats innemen bij de behandeling van gonorroe zijn de cephalosporinen. De verminderde in vitro gevoeligheid van de gonococ voor deze groep antibiotica baart grote zorgen [12]. In vivo resistentie is echter nog niet gemeld.

Het gegeven dat er op dit moment geen nieuwe groep antibiotica is, die in staat is de opkomende hoog resistente gonococcen stammen te elimineren stemt somber wat betreft de behandeling van gonorroe in de toekomst.

Een ander aandachtspunt is het veelvuldig voorkomen van menginfecties van *N. gonorrhoeae* met *C. trachomatis* (ongeveer 15 procent).

Infecties veroorzaakt door *C. trachomatis* zijn heden de meest voorkomende bacteriële SOA in de Westerse Wereld.

Het aandeel van *C. trachomatis*, als verwekker van NGU werd geschat tussen de 35 en 60 procent en ligt op dit moment tussen de 15 en 20 procent.

De mogelijk polymicrobiële aetiologie van NGU veroorzaakt therapeutische problemen. Ook al is de behandeling van NGU protocolair vastgelegd toch faalt deze behandeling bij een niet te verwaarlozen groep [13].

De therapie van NGU wordt op dit moment toegespitst op *C. trachomatis* infecties.

Met het oog op het veelvuldig voorkomen van *C. trachomatis* als verwekker van NGU, de bijkomende complicaties en de controleerbare follow-up na behandeling, is deze benadering van het NGU probleem nuttig en wezenlijk. Echter het probleem is duidelijk complexer. Het voorkomen van persisterende en recidiverende NGU is een niet weg te cijferen dilemma [14], omdat de verwekker(s) onbekend zijn.

Recentelijk werd er melding gemaakt van resistentie van *C. trachomatis* stammen tegen erythromycine, tetracycline en andere antibiotica [15]. Alhoewel de klinische relevantie hiervan nog niet duidelijk is, moet in de toekomst met behandelingsproblemen bij infecties met dit soort stammen rekening worden gehouden.

De evaluatie van nieuwe geneesmiddelen bij de behandeling van NGU is in volle gang. De nieuwe quinolonen met een goede in vitro activiteit tegen *C. trachomatis* blijken in de praktijk bij klinische studies geen duidelijke voordelen te bieden en/of slechtere resultaten te geven in vergelijking met de gebruikelijke therapieën. Een andere groep geneesmiddelen, de nieuwe macroliden, voldoen even goed als de gangbare therapieën. Azithromycin heeft als voordeel, dat de behandelingsduur verkort kan worden van 1 tot 3 dagen. Tot nu toe vond klinische evaluatie hoofdzakelijk plaats bij *C. trachomatis* positieve urethritis. Of dezelfde resultaten zouden zijn behaald bij NGU als geheel blijft de vraag.

Het samengaan van gonorroïsche en niet-gonorroïsche urethritis, met betrekking tot de aard van de patiënten-populatie, therapie-trouw, controle-bezoek, en de omvang van het probleem, met de er uit voort vloeiende complicaties en resistentievorming van de betrokken micro-organismen, rechtvaardigen het denkbeeld terug te grijpen op de oude gedachte dat sexueel overdraagbare urethritis één ziektebeeld is. De oplossing zou zijn, een eenmalige gift van één geneesmiddel of een cocktail van geneesmiddelen. Dit wil niet zeggen dat het kweken van de verschillende verwekkers, het bepalen van het antibiogram, het onderzoek naar vaccins en het ontwikkelen van nieuwe middelen geen doorgang moet vinden.

Hoofdstuk 1

Geeft een historisch overzicht van de sexueel overdraagbare urethritiden.

De verschillende verwekkers worden besproken, de klinische beelden en de complicaties. Er wordt een overzicht gegeven van de verschillende therapieën door de jaren heen en de problemen die zich daarbij voordeden. De resistentie vorming van de veroorzakende micro-organismen wordt uitgebreid besproken. Er wordt ingegaan op de epidemiologie van gonorroe, *C. trachomatis* en NGU.

In een literatuurstudie worden de nieuwe geneesmiddelen bij de behandeling van gonorroe, de quinolonen en de derde generatie- en orale cephalosporinen bediscussieerd wat betreft in vitro activiteit tegen *N. gonorrhoeae*, effectiviteit en bijwerkingen. Tevens werd een literatuurstudie verricht naar de nieuwe geneesmiddelen bij de behandeling van NGU en *C. trachomatis* infecties.

De quinolonen en nieuwe macroliden worden besproken wat betreft in vitro aktiviteit tegen *C. trachomatis* en *U. urealyticum*, effectiviteit en bijwerkingen.

Hoofdstuk 2

In een dubbel-blind gerandomiseerd onderzoek werden 155 mannen lijdend aan een ongecompliceerde gonorroische urethritis behandeld met 200 mg (n=77) of 400 mg (n=78) enoxacin, een nieuw quinoloon, in een eenmalige orale gift.

De genezingspercentages in de met 200- en 400-mg behandelde groep patiënten waren respectievelijk 90% en 92%. De MIC voor enoxacin van de in deze studie geïsoleerde *N. gonorrhoeae* stammen varieerde van 0.015 tot 0.12 µg/ml. De diagnose postgonorroische urethritis werd gesteld bij 29 (42%) patiënten behandeld met 200 mg enoxacin en bij 19 (26%) van de met 400 mg enoxacin behandelde patiënten.

Bijwerkingen (misselijkheid, hoofdpijn en braken) traden op bij 2 (3%) van de 77 patiënten in de met 200 mg behandelde groep en bij 3 (4%) van de 78 patiënten in de met 400 mg behandelde groep.

Hoofdstuk 3

In een gerandomiseerd vergelijkend onderzoek met 0.5 g cefodizime, 1 g cefodizime of 1 g cefotaxime werden in totaal 424 mannelijke en vrouwelijke patiënten met een urogenitale en bijkomende oropharyngeale gonorroe behandeld. Met 0.5 g cefodizime (eenmalige intramusculaire gift) werden 89 mannen en 54 vrouwen, met 1 g cefodizime 87 mannen en 52 vrouwen en met 1 g cefotaxime 86 mannen en 56 vrouwen behandeld.

De genezingspercentages waren 100% voor zowel de mannelijke als de vrouwelijke patiënten behandeld met 0.5 g en 1 g cefodizime.

Het genezingspercentage in de met 1 g cefotaxime behandelde groep patiënten was 99% bij de mannen en 100% bij de vrouwen. De MICs voor cefodizime en cefotaxime van de geïsoleerde *N. gonorrhoeae* stammen varieerde van 0.004 tot 0.06 µg/ml. *C. trachomatis* werd bij 15% van de patiënten geïsoleerd voor behandeling en bij 13% na behandeling. Bijwerkingen bestaande uit misselijkheid, diarree, buikpijn, genitale candidiasis en pijn op de injectie plaats werden gezien bij 4% van de patiënten behandeld met cefodizime.

Bijwerkingen bestaande uit duizeligheid, genitale candidiasis, vermoeidheid en diarree traden op bij 4% van de patiënten behandeld met cefotaxime. Alle bijwerkingen waren mild en van voorbijgaande aard. Cefodizime en cefotaxime zijn veilige en effectieve geneesmiddelen voor de behandeling van ongecompliceerde gonorroe.

Hoofdstuk 4

In een pilot-studie werden 10 mannelijke patiënten met een ongecompliceerde urethrale gonorroe behandeld met 1200 mg ceftetrame in een eenmalige orale gift. Negen van de tien patiënten waren na behandeling genezen. De gevoeligheid van de geïsoleerde *N. gonorrhoeae* stammen voor Ro 19-5247, de actieve metaboliet van ceftetrame, varieerde van < 0.002 tot 0.12 µg/ml.

C. trachomatis werd bij 1 patiënt zowel voor als na behandeling geïsoleerd. Bijwerkingen traden niet op. Ceftetrame kan een effectief geneesmiddel zijn bij de behandeling van ongecompliceerde urogenitale gonorroe.

Hoofdstuk 5 en 6

Geven een beschouwing over resistentievorming van *N. gonorrhoeae* voor de nieuwe quinolonen en het bestaan van een kruisresistentie tussen de verschillende quinoloon derivaten.

Hoofdstuk 7

In deze studie worden de in vitro gevoeligheden bepaald van ciprofloxacin, enoxacin, fleroxacin, lomefloxacin, norfloxacin, ofloxacin en pefloxacin voor 10 PPNG's, 10 non PPNG's, 10 CMRNG's, 3 *N. gonorrhoeae* stammen met een verminderde gevoeligheid voor quinoloon derivaten en een TRNG/PPNG stam.

De non PPNG, PPNG, CMRNG en TRNG/PPNG stammen hadden een uitstekende gevoeligheid voor de geteste quinolonen. De *N. gonorrhoeae* stammen met een verminderde gevoeligheid voor quinolonen lieten hogere MIC waarden zien.

Hoofdstuk 8

In deze studie werden 152 mannelijke patiënten met een NGU behandeld met 300 mg roxithromycin per dag gedurende 7 dagen. *C. trachomatis* werd bij 53 (35%) patiënten en *U. urealyticum* bij 42 (28%) patiënten uit de urethra geïsoleerd. Na behandeling waren bij 49 (92%) van de 53 patiënten met een positieve *C. trachomatis* kweek en bij 34 (81%) van de 42 patiënten met een positieve *U. urealyticum* kweek de kweken negatief geworden. Klinische genezing werd bij gezien bij 137 (90%) patiënten.

Bijwerkingen, bestaande uit misselijkheid, opgeblazen gevoel in de buik, hoofdpijn en vermoeidheid werden waargenomen bij 10 (7%) patiënten.

In het tweede deel van deze studie werden 78 mannelijke patiënten behandeld met 2 x 150 mg roxithromycin per dag gedurende 7 dagen.

C. trachomatis werd bij 22 (28%) patiënten en *U. urealyticum* bij 30 (38%) patiënten uit de urethra geïsoleerd. Na behandeling waren alle *C. trachomatis* kweken negatief geworden. Bij 23 (77%) van de 30 patiënten met een positieve *U.*

urealyticum kweek waren de kweken negatief geworden na behandeling. Klinische genezing werd gezien bij 70 (90%) patiënten.

Bijwerkingen, bestaande uit misselijkheid en hoofdpijn traden op bij 3 (4%) patiënten. De conclusie van deze studie was dat roxithromycin een goed alternatief is voor tetracycline bij de behandeling van NGU.

Hoofdstuk 9

In een gerandomiseerde studie werd de klinische effectiviteit van ciprofloxacin vergeleken met doxycycline bij patiënten lijdend aan NGU. Veertien dagen na behandeling werd geen pyuria meer waargenomen bij 30 van de 100 met ciprofloxacin behandelde patiënten; *C. trachomatis* werd geïsoleerd bij 5 en *U. urealyticum* bij 8 patiënten. In de met 100 mg doxycycline behandelde groep patiënten (n=60) werd 14 dagen na behandeling bij 36 (60%) patiënten geen pyuria meer waargenomen; *U. urealyticum* werd bij 6 patiënten geïsoleerd. In de met 200 mg doxycycline behandelde groep patiënten (n=45) werd 14 dagen na behandeling bij 18 (40%) patiënten geen pyuria meer waargenomen; *U. urealyticum* werd bij 2 patiënten geïsoleerd. Bijwerkingen waren mild en van voorbijgaande aard.

Ciprofloxacin in een dosering van 1 g per dag gedurende 7 dagen is niet geschikt voor de behandeling van NGU.

Hoofdstuk 10

Deze studie beschrijft de gevoeligheid van de in hoofdstuk 9 geïsoleerde *C. trachomatis* stammen voor ciprofloxacin, doxycycline en erythromycine. Tevens vond serotypering plaats van verschillende stammen die voor behandeling en de stammen die voor en na behandeling werden geïsoleerd. De MICs van bovengenoemde antibiotica waren in overeenstemming met die welke in de literatuur worden gemeld.

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 8 december 1954 te Dordrecht geboren.
Hij behaalde in 1975 het Atheneum-B diploma aan het Gemeentelijk Lyceum te Dordrecht.
Aan de Medische Faculteit te Rotterdam werd in 1976 de studie geneeskunde aangevangen.
In 1983 werd het arts examen behaald. Alvorens met de specialisatie tot dermato-venereoloog op de afdeling Dermatologie en Venereologie van het Academisch Ziekenhuis Dijkzicht Rotterdam (Prof. Dr. E. Stolz en Prof. Dr. Th. van Joost) in 1986 aan te vangen heeft hij als AGNIO gewerkt bij Dr. J.D.R. Peereboom-Wynia huidarts in Scheveningen en op bovengenoemde afdeling in Rotterdam.
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ABBREVIATIONS

AIDS	- acquired immunodeficiency syndrome
B. ureolyticus	- *Bacteroides ureolyticus*
C. trachomatis	- *Chlamydia trachomatis*
CMRNG	- chromosomally mediated resistant *N. gonorrhoeae*
DEAE	- diethylaminoethanol
DGI	- disseminated gonococcal infection
E. coli	- *Escherichia coli*
HIV	- Human Immunodeficiency Virus
HLA	- Human Leucocyt Antigen
MBC	- Minimum Bactericidal Concentration
MIC	- Minimum Inhibitory Concentration
MTR-GENE	- Envelope phenotype
M. genitalium	- *Mycoplasma genitalium*
N. gonorrhoeae	- *Neisseria gonorrhoeae*
NGU	- non-gonococcal urethritis
NON-PPNG	- non penicillinase-producing *N. gonorrhoeae*
PID	- pelvic inflammatory disease
PPNG	- penicillinase-producing *N. gonorrhoeae*
SOA	- sexueel overdraagbare aandoening
STD	- sexually transmitted disease
TEM-1 type b-lactamase	- type of extra-chromosomal DNA
TetM	- streptococcal tetracycline resistance determinant
T. pallidum	- *Treponema pallidum*
TRNG	- Plasmid mediated tetracycline resistant *N. gonorrhoeae*
WHO	- World Health Organization
CDC	- Centers for Disease Control