Original Article

Gonorrhea: Data on antibiotic resistance and accompanying infections at the University Hospital Dresden over a 10-year time period

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Introduction

Gonorrhea is a disease with worldwide distribution, high morbidity and far-reaching socioeconomic consequences. It is caused by Neisseria gonorrhoeae, a gram-negative diplococcus identified by Albert Neisser in 1879. In infected men N. gonorrhoeae often elicits acute urethral signs and symptoms, which is why therapy is usually initiated rapidly. An infection in women more often takes an asymptomatic course and is associated with a greater risk of ascending infections of the lesser pelvis and consecutive infertility.

Also rectal and pharyngeal infections are frequently not recognized and treated due to the lack of symptoms. The worldwide incidence of gonorrhea is estimated at 62 million cases annually [1].

Since the German Protection against Infection Act (Infektionsschutzgesetz, IfSG) came into effect in 2001 there is no longer compulsory reporting of gonorrhea in Germany. Reliable epidemiological data are therefore not available; only a few reports on the regional level exist on the status of resistance. Therefore, a high number of unreported cases must be assumed; about 25,000–40,000 new infections yearly are estimated [2].

An exception exists in Saxony, where on the basis of the Saxonian IfSG reporting ordinance gonococcal infections remain notifiable. Partially published data demonstrate an increase in reported gonorrhea cases until the year 2007, with the highest incidence being found in the Dresden county [2]. Data on antibiotic resistance of N. gonorrhoeae in this county have not yet been published.

Summary

Background: Over the last years, growing resistance of gonorrhea to quinolones has emerged worldwide. Currently, cases with resistance to oral and parenteral third-generation cephalosporins are increasingly reported. Because gonorrhea is not a notifiable infection in Germany, data on epidemiology and antimicrobial surveillance of gonorrhea are scarce. We present the results of N. gonorrhoea testing at the University Hospital Dresden over the course of ten years.

Patients and Methods: The results of cultural and nucleic acid amplification testing of gonorrhea and accompanying infections of 1,850 smears between 2001 and 2010 were analyzed retrospectively.

Results: Among 181 patients (mean age 30.4 years) 159 had positive PCR analyses for gonorrhea and 50 positive cultures of Neisseria gonorrhoea. The rate of ciprofloxacin resistance was high (in 46 % of all isolates), primarily in men. Resistance to cefotaxime and ceftriaxone could not be detected so far. In 40 % of the cases at least one accompanying urogenital infection occurred, predominantly with Chlamydia trachomatis.

Conclusions: Similar to other regions of Germany, a high percentage of resistance to quinolones was found in gonorrhea, but not to cephalosporins. Additionally, accompanying infections were frequent and warrant screening. In the light of recent cases of resistance to cephalosporins currently emerging in Europe, cultural diagnosis of gonorrhea should definitely be intensified by all means. The results should be integrated into a surveillance system.
The detection of gonococcal DNA using nucleic acid amplification techniques (NAAT) is today considered the diagnostic standard in routine diagnostics. The sensitivity of these tests is generally higher than the comparatively time-consuming culture [3, 4], so that these have in the meantime been pushed into the background. Antibiotic resistance can, nevertheless, only be determined by culture. This is of particular significance due to the in addition to the worldwide quinolone resistance, cases of resistance to oral and parenteral third-generation cephalosporins as the last substance class for untargeted, empirical therapy of gonorrhea have been observed.

Materials and methods

The results of 1,850 samples for gonococcus diagnostics submitted over 10 years (2001–2010) at the University Hospital Dresden were analyzed. The smears with positive PCR results were taken from the urethra, cervix/vagina, pharynx, anus and intraoperatively from the adnexa uteri. The smears with positive culture were taken from the urethra, cervix/vagina, rectum or from a Bartholin abscess (Table 1). For molecular detection with PCR, transport was using either the appropriate transport medium (Cobas® Amplicor transport medium, Roche Diagnostics) or 2-sucrose-phosphate (2-SP) cell culture medium. For cultural detection conventional Amies transport medium was predominantly employed. In individual cases a selective transport medium (modified Thayer-Martin [MTM] agar of the BD company) was used to isolate gonococci from mixed flora.

Molecular biological diagnostics

Since 2001 detection of gonococci has been performed using a commercial multiplex-PCR system (Cobas Amplicor® Chlamydia trachomatis/ Neisseria gonorrhoeae [CT/NG] test, Roche Diagnostics). The manufacturer states a sensitivity of this method of about 5 copies of the target sequence per reaction. For extinction values (E660 value) slightly above the cut-off or for sample materials not licensed by the manufacturer (e.g. pharyngeal smears), where due to colonization by atpathogenic Neisseria false-positive results have to be expected, a 16S rDNA sequencing as a “confirmatory reaction” was performed for verification. In 2010 PCR diagnostics were switched to a real-time PCR technique LightCycler (LightMix® Neisseria gonorrhoeae test, TIB MOLBIOL, Berlin, Germany). The specific binding of the hybridization probes on the PCR amplification product was registered as a measurable fluorescence signal using the FRET (fluorescence-resonance energy transfer) principle. The lower detection threshold of the test is stated by the manufacturer to be about 10 copies per reaction.

Cultural detection of gonococci and sensitivity testing

For cultural detection the colonies were subcultured after testing the oxidase reaction on boiled blood or blood agar for 18 to 24 hours at 5 % CO2, and 37 °C. Identification and differentiation of the gonococci were performed employing specific metabolic reactions in conventional manual (Api NH, bioMérieux) or automated test systems (NH card VITEK 2, bioMérieux). From each isolate identified as N. gonorrhoeae sensitivity testing was performed using agar diffusion on blood agar. Incubation was at 37 °C and 5 % CO2. Routinely, the following antibiotics were tested: penicillin G, cefotaxime, ceftriaxone, ciprofloxacin, doxycycline and cotrimoxazole. In the event of unusual resistances or implausible sensitivity results an additional ellipsometer test (E-test) was conducted for the antibiotic in question: Classification of the isolate as “sensitive”, “intermediate” and “resistant” was performed according to the limit specifications of the CLSI (Clinical and Laboratory Standards Institute). Every sensitivity test was accompanied by appropriate ATCC (American Type Culture Collection) strains as quality control.

Cultural diagnostics of ureaplasmas and mycoplasmas

Culture of Ureaplasma urealyticum or Mycoplasma hominis was performed using ureaplasma bouillon and ureaplasma agar under anaerobic conditions. Testing of the charges of ureaplasma agar or bouillon was done with the help of the ATCC strain Ureaplasma urealyticum serovar 6 (ATCC 27818). The first clues after culture were colony morphology or simple biochemical reactions (e.g. urease activity). Further identification was performed using PCR and sequencing. Sensitivity testing was performed using the detection of antibiotic inhibition of metabolic activity (arginine or urea
cleavage) with serial dilution tests in a fluid medium. Using the appropriate limit concentration of the antibiotics strains were classified as sensitive, intermediate and resistant.

Results

Frequency of infections with *Neisseria gonorrhoeae* at the University Hospital Dresden (UKD)

In 1,850 submitted samples in a total of 181 patients gonorrhea was diagnosed with PCR in 159 cases and in 50 with positive gonococcus culture (Figure 1). Over the course of time since 2001 an increase in the number of detected infections with up to a maximum of 36 infections in the year 2007 was seen.

The submitters of the positive samples were the Department of Dermatology in 75 %, the Department of Gynecology and Urology in 11 % and 9 %, respectively, in a few cases the Department of Pediatrics and Adolescent Medicine and the Department of Internal Medicine as well as external clinics (Figure 2).

Gender and age distribution of infection with *Neisseria gonorrhoeae*

Of affected patients 81 % were men (Figure 3). The mean age of the patients was 30.4 years overall, while one-third of affected women were under the age of 20 years, with this age group being represented twice as often as in men. The mean age of infected men was 32.5 years, of affected women 20.7 years. Above the age group 20–24 years men already predominated distinctly, in the age group over 40 years men were found almost exclusively. In gonorrhea detected by culture about one-third of the material came from women (16 infections detected by culture in women vs. 34 in men), which is clearly over the female share of total patients diagnosed with gonorrhea (19 %).

Resistance profile

In sensitivity testing a total of 46 % of the gonococcus isolates were resistant or intermediately sensitive to ciprofloxacin
It is notable in this regard, that the first strain resistant to ciprofloxacin was already detected in 2001 and in 2010 already 66% of the tested strains were resistant or had intermediated sensitivity (4 of 6). Of the patients with a quinolone-resistant gonococcus strain 78% were men (18 men vs. 5 women). Among women in 32% (5 of 16) and among men in 53% (18 of 34) ciprofloxacin-resistant gonococci could be identified. The mean age of men with ciprofloxacin-resistant gonococci was 33 years, in women 26 years. Taking the entire resistance profile in the past 10 years, it is seen that 14% of the gonococcus isolates are resistant to or not adequately sensitive to penicillin-G; the same holds true for 26% of the isolates with respect to tetracyclines. To date no resistance to cefotaxime and ceftriaxone has been observed in our isolates.

Co-infections

In 152 patients additional testing for chlamydiae and in 126 patients complete diagnostics of urogenital co-infections
with *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Chlamydia trachomatis* were performed. In addition to the gonococcal infection in a total of 40 % of the patients at least one urogenital co-infection could be detected here.

In 17 % of the men and 41 % of the women a chlamydial infection was detected in addition to the infection with gonococci (n = 152) (Figure 6). In 4 % of the men and 17 % of the women the additional cultural identification of
Mycoplasma hominis and in 24 % of the men (29 of 120) and 83 % of the women (5 of 6) detection of Ureaplasma urealyticum succeeded. In 9 % of all men infected with N. gonorrhoeae (50 % of the women) two additional co-infections were present (mixed infections with chlamydiae and/or mycoplasmas and/or ureaplasmas).

**Discussion**

*Neisseria gonorrhoeae* possesses numerous mechanisms to develop resistance, which on a worldwide basis significantly limits empirical antibiotic therapy of gonorrhea. In the meantime there are growing indications that the cephalosporins as the last available substance class for empirical therapy are also affected. Due to the rapid, technically uncomplicated and more sensitive detection of a gonococcal infection using NAATs, cultural detection has been pushed into the background. Even though it is possible using genetic markers to find clues as to sensitivity to particular antibiotics, specificity and sensitivity do not equal those of cultural sensitivity testing [5].

**Resistance situation in Germany**

The West European sentinel data from 12 countries in the European Surveillance of Sexually Transmitted Infections (ESSTI) with almost 1,000 gonococci strains in the year 2004 demonstrated the following resistance rates: ciprofloxacin 30.9 % (low-level resistance), penicillin 21.3 %, tetracyclines 49.8 %, azithromycin 8.2 % and ceftriaxone 0.3 % [6]. The continuation of data collection in the years 2006 to 2008 showed across Europe an increase of resistances to ciprofloxacin (42–52 %) and an increase of the minimal inhibitory concentration (MIC) for ceftriaxone, although clinical resistance had still not been observed. In this study 84 gonococcal strains from Germany were included; 59.5 % of these were resistant to ciprofloxacin, 2.4 % to azithromycin [7].

Otherwise only individual publications exist for Germany with resistance data from various time periods that impressively document the development of resistance to the respective standard therapy.

In an early study from the years 1988–1992 in Frankfurt/Main in 150 gonococcal isolates complete resistance to penicillin was seen in 21.3 % as well as to doxycycline in 2.0 % [8]. In Berlin in the year 2000 18.8 % of the isolates were resistant to penicillin and 12.9 % to tetracyclines; only in one of the 85 cultures ciprofloxacin resistance was found [9]. In a study from Northern Germany in 2001 already 34 % of the gonococcal isolates (n = 268) possessed resistance to ciprofloxacin [10]. In the years 2004/2005 in 65 gonococcal isolates from the Stuttgart-Heidelberg area resistance to quinolones was seen in 47.4 % and in 7.7 % elevated MIC of azithromycin [11]. A study from the Rhein-Main
region in the years 2005–2008 reports a resistance rate to ciprofloxacin of about 55 % (in 2008: 64 %) with an unusually high share of azithromycin resistances (17 %). Resistances to cefixime were not observed [12].

Data regarding epidemiology and resistance of gonorrhoea from Saxony have until now been published only partially. In a laboratory in the Leipzig area in 33 % of 61 gonococcal strains resistance to ciprofloxacin was observed. Further published data from East Saxony (Görlitz/Bautzen) demonstrated in presumably pre-treated patients ciprofloxacin resistance in a total of 62 %, but no resistances to cefuroxime and cefitinbuten [2].

In the present study from the Dresden University Hospital a total of 38 % of the gonococcal isolates were resistant (19/50) and 8 % intermediate sensitive (4/50) to ciprofloxacin. The first quinolone-resistant strain was already detected in 2001; in 2010 16.5 % of tested strains displayed resistance (1/6) and 50 % intermediate sensitivity (3/6), so that in this year 66 % of the cultures were not or only to a limited extent sensitive to ciprofloxacin. Of the patients with ciprofloxacin-resistant gonococci 78 % were men (18 men, 5 women) with a mean age of 33 years.

To date no resistance to third-generation cephalosporins has been observed in our isolates. Overall the isolates reported here display a resistance spectrum similar to the published cases from the Leipzig area. In summary, in Saxony, too, resistance to quinolones in N. gonorrhoeae has been observed already since 2001, with resistance rates lying between 30 % and 60 % in the various regions. This corresponds to other regions in Germany.

Current data on the worldwide development of resistance of N. gonorrhoeae to cephalosporins

Oral and parenteral cephalosporins of the third generation such as cefixime and ceftriaxone have for several years been recommended in international guidelines for empirical therapy of gonorrhoea [14, 15]. In recent years starting in Japan and also in the USA and Europe a trend to rising MIC of cephalosporins has been observed in vitro [7, 16, 17]. In Great Britain the spread of a gonococcal clone with reduced sensitivity to cefixime was reported in 2011 [18]. In the meantime cases of therapy failure with cefixime have been reported first in Asia [19, 20] and currently in Europe [21–23].

At the beginning of the year 2011 the discovery of a new multi-resistant gonococcal mutant H041 that is highly resistant to all cephalosporins including ceftriaxone drew great attention. This mutant was isolated from pharyngeal gonorrhoea of a female sex worker [24]. Shortly thereafter therapy failure of ceftriaxone in a pharyngeal gonorrhoea acquired in Asia was observed in Sweden; an elevated MIC to ceftriaxone could be found [25].

To date no case of therapy resistance to cefixime or ceftriaxone has been published in Germany; recently, however, cefixime resistance was reported in Austria in urethral gonorrhoea acquired in Munich [23].

Co-infections

In our patient collective 67 % of the women and 38 % of the men had a urogenital co-infection. Of the men 17 % and of the women 41 % tested positive for chlamydiae. In the STD sentinel survey of the Robert Koch Institute a high rate of co-infections was found: 17.2 % of all female patients and 11.8 % of the male patients with verified gonorrhoea were also positive for chlamydiae [26]. In international studies the prevalence varies greatly depending on the collective studied.

In a population-based American study within the context of the National Health and Nutrition Examination Survey (NHANES) in about 6,600 participants the presence of gonococcal infection of 0.24 % was found, of which 46 % had a co-infection with chlamydiae, underscoring the necessity of appropriate testing and co-treatment of chlamydial infection [27].

Less is known about genital mycoplasmas, with the pathogenic relevance being discussed controversially. Older studies found a co-infection with Ureaplasma urealyticum in 27–38 % in gonorrhoea [quoted in 28]. A recent Japanese study on 390 men with culturally confirmed gonorrhoea showed the following co-infection rates before therapy: Chlamydia trachomatis 21.8 %, Mycoplasma genitalium 4.1 %, Mycoplasma hominis 2.1 % and Ureaplasma urealyticum 10.6 %. A significant association could be shown here of Mycoplasma genitalium and Ureaplasma urealyticum biovar 2 with a post-gonococcal urethritis appearing after therapy, while biovar 1 (U. parvum) was interpreted more likely as colonization [28].

International guidelines on the therapy of gonorrhoea

Since the increase of resistance to quinolones, in Germany cefixime 400 mg orally or ceftriaxone i.m. are recommended as single-dose therapy [29]. Spectinomycin, a relative of the aminoglycosides, is also effective, but not sufficient for pharyngeal gonorrhoea.

In the STD guideline of the CDC from 2010 for therapy of uncomplicated gonorrhoea, independent of the presence of an additional chlamydial infection, dual therapy with ceftriaxone 250 mg i.m. in combination with azithromycin 1 g or alternatively doxycycline is recommended [14], also with the intent of preventing the further development of resistance to cephalosporins [16].

The current British guideline for 2011 recommends in dual therapy with azithromycin 1 g an even higher dose of
ceftixaxone with 500 mg i.m. [30]. Cefixime 400 mg orally, also in combination with azithromycin 1 g, is only recommended if the patient declines parenteral therapy [30].

Due to the worldwide increasing resistance to azithromycin in gonorrhea, this substance is no longer suitable for empirical monotherapy [6, 20].

Conclusions

In view of the worldwide increasing antibiotic resistance spectrum of N. gonorrhoeae performing adequate diagnostics with sensitivity testing is urgently needed for effective therapy. High resistance rates to quinolones have been confirmed on a regional basis including Saxony. Due to recent reports of treatment failure and increased MIC of cefixime and ceftriaxone, the substance class of third-generation cephalosporins as the last available possibility for untargeted therapy is in danger. Therefore, in updated international guidelines combination therapy with other antibiotics with an in part significantly increased dose of ceftriaxone is recommended.

The spread of cephalosporin-resistant gonococcal strains in Germany is likely only a question of time, as the infections are not rarely acquired in foreign countries, as shown in a recent study from Norway [17]. Moreover, due to the lack of a reporting ordinance in Germany, a considerable number of unreported cases can be presumed, so that data on antibiotic resistance of N. gonorrhoeae even at the regional level is an important information source. Due to the frequency of co-infections, appropriate diagnostics for chlamydial and urogenital mycoplasma infections should additionally be performed in gonorrhea.

References


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