Antimicrobial susceptibility patterns of gonococcal isolates in Pretoria, South Africa, over a 20-year period (1984-2004)

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This paper reviews the susceptibility profiles of Neisseria gonorrhoeae *over a 20-year period in the Pretoria region. Endourethral specimens were collected from adult men with symptoms of urethritis attending primary health care clinics and private medical practitioners. These swabs were plated on enriched media for isolation of* N. gonorrhoeae. *Antimicrobial susceptibility of the organisms was performed using the disc diffusion and agar dilution methods. Plasmid analyses were performed on beta-lactamase-producing isolates. Penicillase-producing* N. gonorrhoeae *strains increased from 4% to 16%, whilst chromosomally mediated penicillin-resistant strains increased dramatically from 0% to 16% from 1984 to 2004. There was an equal distribution of the 3.2 MDa African and 4.4 MDa Asian plasmids. High-level tetracycline-resistant strains (36%) were detected for the first time in 2004. Ciprofloxacin resistance emerged at 7% in the same year. Gonococcal isolates remained susceptible to cefoxitin, ceftriaxone, cefpodoxime, and spectinomycin. However, the minimum inhibitory concentration values for spectinomycin were very close to the breakpoint. We have shown a continuing increase in resistance to penicillin (plasmid and chromosomal), the emergence of high-level tetracycline resistance and an emergence of resistance to ciprofloxacin. Susceptibility testing is essential for successful therapeutic outcomes and needs to be performed in an ongoing basis.*

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Introduction

Gonococcal urethritis is a common clinical presentation in men presenting to primary health care clinics and private medical general practitioners in Southern Africa. In public health care clinics and in many private medical practices, treatment for sexually transmitted infections is reliant on syndromic management.[1] For successful syndromic management, constant monitoring of the antimicrobial susceptibility of the causative organisms is required.

Penicillin was the antibiotic of choice for the treatment of gonorrhoea up to the early 1980s in South Africa. Thereafter, the emergence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) and chromosomally mediated resistance to penicillin (CMRNG), as well as tetracycline resistance, prompted the use of alternative treatment regimens for urethritis.[2]

The mechanism of resistance of *N. gonorrhoeae* to penicillin and tetracycline resulted in the profiling of the plasmids that revealed the presence of the 4.4 MDa ('Asian') plasmids and the 3.2 MDa ('African') plasmids, as well as high-level tetracycline-resistant plasmid carrying the 'American type' TetM gene.[3-5]

In view of the ongoing resistance of the gonococcal isolates to penicillin and tetracycline, the national Department of Health of South Africa advocated the use of ciprofloxacin (500 mg stat dose) as first-line therapy for uncomplicated gonococcal urethritis as part of syndromic management in 1996.[1] Subsequent antimicrobial surveillance of gonococcal isolates from various centres in South Africa indicated that the isolates were susceptible to ciprofloxacin.[6-7]

More recently, the first ciprofloxacin-resistant *N. gonorrhoeae* isolates were detected in KwaZulu-Natal.[8] Subsequent to the KwaZulu-Natal publication, there were reports of quinolone resistance from other centres in South Africa.[9]

We describe the antimicrobial susceptibilities and plasmid profiles of gonococcal isolates isolated in Pretoria, South Africa, over a 20-year period (1984-2004).

Materials and methods

Patients

Aetiological surveys (published and unpublished) for acute urethritis were conducted by the Department of Microbiological Pathology at the then Medical University of Southern Africa in Pretoria in 1984,[10] 1991,[11] 1992 and 2004.[12] The patients were men attending the primary health care clinics and private medical practitioners in the city of Pretoria.

Specimen collection and isolation of *Neisseria gonorrhoeae*

Endourethral swabs were taken from men with complaints of burning on micturition and/or having urethral discharge. The swabs were inoculated onto modified New York City media at the bedside or placed into Amies' charcoal transport media and transported to the laboratory, where they were subsequently plated out onto the Thayer Martin medium and, in the more recent studies, onto New York City medium. Plates were incubated at 35ºC in a humidified atmosphere of 5-10% CO_2 for 48 hours. Isolates were identified as *N. gonorrhoeae* by Gram-staining, oxidase reaction and by their ability to produce acid from glucose, but not from maltose or sucrose.

Antimicrobial susceptibility testing

All gonococcal isolates were tested for penicillinase production using the chromogenic cephalosporin, nitrocephin (Oxoid). Antibiotic susceptibility testing of the gonococcal isolates was carried out using the Kirby Bauer disc diffusion method on GC agar base (Oxoid, UK) supplemented with 1% IsoVitaleX. The diameter of the inhibition zone was measured in millimetres and interpreted according to National Committee for Clinical Laboratory Standards (CLSI) criteria.[13]

The minimum inhibitory concentration (MIC) of ciprofloxacin for *N. gonorrhoeae* strains was determined by the Etest® (AB Biodisk) on GC medium enriched with 1% IsoVitaleX. For both the methods, an inoculum density of 0.5 MacFarland's standard was used. The standard agar dilution method was used to determine the MICs of penicillin, cefoxitin, ceftriaxone, cefpodoxime, tetracycline, ciprofloxacin, ofloxacin and spectinomycin. One microlitre volumes containing 10^4 cfu were inoculated onto media containing appropriate dilutions of the antibiotic under test in GC agar base (Oxoid, UK) supplemented with 1% IsoVitaleX.

The World Health Organization reference strains (WHO E) of *N. gonorrhoeae,* as well as the ATCC 49226, were used as controls. The susceptibility of penicillin, tetracycline and ciprofloxacin were defined as follows: penicillin-susceptible strains (MIC ≤0.06 mg/l); penicillin intermediate resistance strains (MIC 0.125-1 mg/l); penicillin-resistant strains (CMRNG) (MIC ≥2 mg/l); tetracycline-susceptible strains (MIC ≤0.25 mg/l); tetracycline intermediate resistant strains (MIC 0.5-1 µg/ml); chromosomal-mediated tetracycline-resistant *N. gonorrhoeae* strains (CMRTNG) (MIC 2-8 mg/l); high-level plasmid-mediated tetracycline-resistant *N. gonorrhoeae* (TRNG) strains (MIC ≥16 mg/l); ciprofloxacin-susceptible strains (MIC ≤0.06 mg/l); ciprofloxacin intermediate resistance strains (MIC 0.125-0.5 mg/l); ciprofloxacin-resistant strains (MIC ≥1 mg/l).

Tetracycline-resistant *Neisseria gonorrhoeae* (TRNG)

TRNG strains were screened by growth on GC agar medium containing 10 mg/l tetracycline for 24 h. Growths of *N. gonorrhoeae* on this medium indicated high-level resistance and were designated as TRNG; this was confirmed with polymerase chain reaction (PCR). [4]

Plasmid analysis

Plasmids were extracted from PPNG strains using the rapid alkaline lysis method of Birnboim and Doly[14] and separated by electrophoresis in 1% agarose gel. Plasmids were visualised by ultraviolet light transillumination of ethidium bromide-stained gels.

Results

The prevalence of PPNG and CMRNG strains in the four surveys undertaken is shown in Table 1. PPNG strains had increased from 4% in 1984 to 16% in 2004, whilst the CMRNG (≥2 mg/l) strains have also increased dramatically, from 0% in 1984 to 16% in 2004. However, in 1991, the MICs were not determined.

The susceptibility profiles of the most recent gonococcal isolates (2004) in the city of Pretoria are shown in Table 2. Nineteen of the 119 non-PPNG isolates (16%) were considered totally resistant (CMRNG ≥2 mg/l)

Table 1: PPNG and CMRNG strains in the city of Pretoria over 20 years

Year	No. strains tested	Method used	PPNG[1] (%)	CMRNG[2] (%)
1984	172	Agar dilution	7 (4%)	0
1991	155	Agar dilution	17 (11%)	ND[3]
1992	47	Agar dilution	6 (13%)	5/41 (12%)
2004	141	Agar dilution Etest® for ciprofloxacin	22 (16%)	19/119 (16%)

[1]PPNG: Penicillinase-producing *N. gonorrhoeae.*
[2]CMRNG: Chromosomally mediated resistant *N. gonorrhoeae.* [3]ND: Not done

Table 2: Susceptibility profile of gonococci isolates in 2004 (n=141) (Courtesy of De Jongh et al, 2007)

Antimicrobial agent	No. of isolates	%
Penicillin		
[1]PPNG	22/141	16
Non-PPNG (fully susceptible, MIC ≤ 0.06 mg/l)	13/119	11
Decreased susceptibility (intermediate resistance, MIC 0.125-1 mg/l)	87/119	73
Chromosomally mediated resistance [2](CMRNG) (MIC ≥2 mg/l)	19/119	16
Tetracycline		
Susceptible (MIC ≤1 mg/l)	46	33
Intermediate resistance (MIC 0.5-1 mg/l)	19	13
[3]CMTRNG (MIC 2-8 mg/l)	25	18
[4]TRNG (MIC ≥16 mg/l)	51	36
Ciprofloxacin		
Susceptible (MIC ≤0.03 mg/l)	131	93
Decreased susceptibility (MIC 0.06-0.5 mg/l)	0	0
Resistant (MIC ≥1 mg/l)	10	7

[1]PPNG: Penicillinase-producing *N. gonorrhoeae.* [2]CMRNG: Chromosomally mediated resistant *N. gonorrhoeae.* [3]CMTRNG: Chromosomally mediated tetracycline-resistant *N. gonorrhoeae.*
[4]TRNG: Plasmid-mediated tetracycline-resistant *N. gonorrhoeae*

to penicillin. Decreased susceptibility (MIC 0.125-1 mg/l) was detected in 73% of the isolates. Resistance to tetracycline (MIC ≥2 mg/l) has increased from 7% in 1992 to 54% in 2004. Only 46 (33%) isolates remained susceptible to tetracycline, while 13% showed intermediate resistance to tetracycline. Low level resistance was detected in 18% of the isolates. There was a dramatic increase of high-level tetracycline resistance (TRNG; MIC ≥ 16 mg/l) strains from 0% in 1992 to 36% in 2004.

All high-level tetracycline-resistant gonococcal isolates (MIC ≥16 mg/l) were identified as TRNG in the 2004 study by growth on the gonococcal sensitivity medium containing 10 mg/l tetracycline and confirmed by the presence of the TetM gene by PCR.

Ciprofloxacin resistance (MIC ≥1 mg/l) was detected for the first time in 2004. Resistance was detected in 7% of strains; having MIC values ≥1 mg/l. Ninety-three percent (131/141) remained fully sensitive whilst no isolates showed decreased susceptibility to the agent, i.e. MIC values were very low.

As reported in the study of 2004,[12] all isolates remained susceptible to cefoxitin, ceftriaxone, cefpodoxime and spectinomycin. Ceftriaxone proved to be the most active agent with MIC_{90} values of 0.004 mg/l.

Table 3: Distribution of penicillin and ciprofloxacin MICs for 51 TRNG and 90 non-TRNG in the year 2004

Antibiotic susceptibility (MIC mg/l)	[1]TRNG [no. (%)] (total isolates = 51)	Non-TRNG [no. (%)] (total isolates = 90)
Penicillin ([2]PPNG ≥4 mg/l)	20 (39)	11 (12)
Penicillin ([3]CMRNG ≥2 mg/l)	6 (12)	2 (2)
Penicillin (non-PPNG 0.125-1 mg/l)	25 (49)	63 (70)
Penicillin (non-PPNG ≤0.06 mg/l)	0 (0)	14 (16)
Cefoxitin (MIC ≤2 mg/l)	51 (100)	90 (100)
Ceftriaxone (MIC ≤0.25 mg/l)	51 (100)	90 (100)
Cefpodoxime (MIC ≤0.25 mg/l)	51 (100)	90 (100)
Spectinomycin (MIC ≤64 mg/l)	51 (100)	90 (100)
Ciprofloxacin (MIC ≥1 mg/l)	10 (20)	0 (0)
Ciprofloxacin (MIC 0.06-0.5 mg/l)	0 (0)	0 (0)
Ciprofloxacin (MIC ≥0.03 mg/l)	41 (80)	90 (100)
Ofloxacin (MIC ≥1 mg/l)	10 (20)	0 (0)
Ofloxacin (MIC 0.06-0.05 mg/l)	16 (31)	19 (21)
Ofloxacin ((MIC ≤0.03 mg/l)	25 (49)	71 (79)

[1]TRNG: Plasmid-mediated tetracycline-resistant *N. gonorrhoeae*
[2]PPNG: Penicillinase-producing *N. gonorrhoeae.*
[3]CMRNG: Chromosomally mediated resistant *N. gonorrhoeae*

Thirty-six of the 141 (26%) isolates had MIC values of 64 mg/l for spectinomycin, i.e. only one dilution away from the cut off for resistance (Table 3).

The distribution of penicillin, cefoxitin, ceftriaxone, cefpodoxime, spectinomycin, ciprofloxacin and ofloxacin MICs among 51 TRNG and 90 non-TRNG is shown in Table 3. Twenty TRNG (39%) and 11 non-TRNG (12%) produced ß-lactamase (PPNG ≥4 mg/l); six TRNG (12%) and two non-TRNG (2%) were chromosomally resistant to penicillin (CMRNG ≥2 mg/l); 25 TRNG (49%) and 63 non-TRNG (70%) were of intermediate resistance to penicillin (0.125-1 mg/l), and no TRNG and 14 (16%) non-TRNG were fully susceptible to penicillin (≤0.06 mg/l). All isolates were susceptible to cefoxitin (MIC ≤2 mg/l), ceftriaxone (MIC ≤0.25 mg/l), cefpodoxime (MIC ≤0.25 mg/l) and spectinomycin (MIC ≤6 4mg/l). Ten (20%) TRNG were resistant to both ciprofloxacin and ofloxacin.

The plasmid profile for the PPNG isolates obtained in 2004 is shown in Table 4. There was an equal distribution of the 3.2 MDa and 4.4 MDa plasmids. All PPNG strains were found to carry the 2.6 MDa cryptic plasmid. The 25.2 MDa TetM plasmid was present in 11% of PPNG strains.

Table 4: Plasmid profiles of PPNG strains in the city of Pretoria in 2004 (n=22)

Plasmid content (MDa)	No. of strains	%
3.2	0	0
3.2 + 2.6	3	14
3.2 + 2.6 + 24.5	8	36
4.4	0	0
4.4 + 2.6	3	14
4.4 + 2.6 + 24.5	8	36

Discussion

In view of the ongoing resistance of the gonococcal isolates to penicillin and tetracycline, the South African Department of Health advocated ciprofloxacin (500 mg stat dose) as first-line therapy for uncomplicated gonococcal urethritis as part of syndromic management in 1996.[1] During this period, quinolone-resistant gonococci (QRNG) with MICs ≥1 mg/l isolates to ciprofloxacin had been reported from different parts of the world, notably the Far East, South-East Asia, Australia, UK and USA.[15,16]

Antimicrobial surveillance of gonococcal isolates from various centres in South Africa indicated that the isolates were susceptible to ciprofloxacin.[6,7] More recently, the first ciprofloxacin-resistant *N. gonorrhoeae* isolates were detected in KwaZulu-Natal.[8] Subsequent to the KwaZulu-Natal publication, there were reports of quinolone resistance from other centres in South Africa.[9]

Ciprofloxacin resistance has emerged for the first time in Pretoria, with 7% of the isolates showing MIC values of ≥1 mg/l. There has been a sudden appearance of these quinolone-resistant isolates indicating importation of resistant isolates and not a gradual step-wise development of resistance.[6,9,12] The rate of ciprofloxacin resistance detected in the 2004 survey in Pretoria is lower than that detected in Johannesburg (16%) [9] and in Durban (42%).[17]

The emergence of these isolates has serious implications for the national guidelines for the management of sexually transmitted infections, and these were updated in 2008 by the Department of Health, recommending cefixime as first-line choice for the treatment of gonorrhoea. Furthermore, the Centers for Disease Control and Prevention (CDC) have also made changes in their therapeutic guidelines for the USA.[18]

PPNG strains were first identified in 1977 in South Africa when cases were described in Johannesburg[18] and Durban.[20] Since then, increased PPNG strains have been reported from various centres in South Africa,[20] with the highest rates (30%) in 1999/2000 from KwaZulu-Natal.[6] PPNG strains were first detected in Pretoria in 1984, with a prevalence rate of 4%, and these have increased gradually to 16% in 2004. CMRNG has followed a similar trend with rates of 16%, indicating that both chromosomal and plasmid-mediated resistance to penicillin appear to be entrenched in the local population.

Profiling of the penicillinase plasmids revealed equal distribution of the 3.2 MDa ('African') and 4.4 MDa ('Asian') plasmids, as described previously by Chalkley et al in their study of isolates from 1995.[4] In other South African studies, the African plasmid was more prominent than the Asian plasmid,[3,21] but this was not observed in the 2004 Pretoria study.[12] Furthermore, plasmids other than the 3.2 MDa and 4.4 MDa, were not found, including the novel 2.2 MDa penicillinase plasmid detected previously in Durban.[20] Dissemination of the penicillinase plasmid can be facilitated by the presence of the 24.5 MDa conjugative plasmid. The carriage of the 24.5 MDa conjugative plasmid, together with the 3.2 MDa 'African' plasmid or the 4.4 MDa 'Asian' plasmid, indicates that there is potential for these plasmids to continue spreading.

There was a dramatic increase in the number of strains showing high-level resistance to tetracycline (TRNG) between 1992 (0%) and 2004[12] (36%), indicating the emergence of TRNGs in the Pretoria region. Fifty-one percent of gonococcal isolates in 1998/1999 in KwaZulu-Natal were found to be TRNGs.[6] Low tetracycline resistance (CMTRNG) (MIC 2-8 mg/l) among Pretoria strains was detected in only 4/44 (9%) of the isolates in the 1992 study, compared to 24/141 (18%) in 2004. Higher rates of tetracycline resistance (CMTRNG) (29%-45%) have been documented in KwaZulu-Natal.[6]

Tetracycline and tetracycline derivatives are not the drugs of choice for treating gonorrhoea; these are the recommended drugs for the treatment of chlamydial and ureaplasmal infections. Since differentiation between gonorrhoea and chlamydial infection is not made when syndromic management is applied, patients with gonorrhoea are exposed to this group of drugs. Furthermore, this group of antibiotics is administered for up to a week, thus exerting selective pressure on *N. gonorrhoeae,* leading to development of chromosomally mediated resistance to tetracycline. This probably reflects on the decreasing prevalence of tetracycline susceptible isolates in South Africa.

Gonococcal isolates have remained fully susceptible to the cephalosporins over the 20-year period. The MIC_{50} values for ceftriaxone among non PPNG isolates in both 1992 and 2004 have remained the same (MIC 0.015 mg/l). Ceftriaxone is one of the agents recommended for the treatment of infection with *N. gonorrhoeae* in pregnancy, neonates or in those who fail to respond to treatment with ciprofloxacin. Resistance to ceftriaxone has not been detected in South Africa. Recently, however, a slight increase in MIC values has been reported in gonococcal isolates from KwaZulu-Natal.[22]

Although resistance to spectinomycin has not been documented in southern Africa, gradual increase in MICs have been observed in the 20-year period in the Pretoria region with a wide MIC range of 4-64 mg/l. This phenomenon has also been observed in gonococcal isolates in KwaZulu-Natal.[6] It should be noted that MIC values are bordering close to the breakpoint for resistance and will require clinical evaluation studies to demonstrate therapeutic efficacy.

In view of the emergence of quinolone resistance amongst gonococcal isolates in South Africa, there is an urgent need to use alternative antimicrobial agents for therapy. The options available include the cephalosporins (viz. ceftriaxone, cefixime and cefpodoxime), as well as spectinomycin. All the isolates were susceptible to these antimicrobial agents, with cefixime not being tested. Cefixime is also an antimicrobial agent prescribed by the CDC[18] for the treatment of gonococcal infections.

The emergence of ciprofloxacin as well as high-level tetracycline resistance (TRNG) was seen in the Pretoria region for the first time in 2004. This emphasises the importance of the recent change in the management protocols for persons with sexually transmitted infections. However, ongoing surveillance is essential as strains from KwaZulu-Natal are already showing small increases in MIC values for ceftriaxone. Overall, continued surveillance of antimicrobial susceptibilities to commonly used antibiotics and determining MIC values of *N. gonorrhoeae* isolates are absolutely necessary if successful therapeutic outcomes are to be achieved for symptomatic and asymptomatic infected individuals.

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