Antimicrobial Susceptibilities of Neisseria gonorrhoeae *in Kigali, Rwanda, and Trends of Resistance Between 1986 and 2000*

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Background: **Plasmid-mediated and chromosomal-mediated resistance of *Neisseria gonorrhoeae* to penicillin, tetracycline, thiamphenicol, and trimethoprim-sulfamethoxazole has spread dramatically in Africa. Monitoring of antimicrobial susceptibility is a key element in the control of sexually transmitted diseases.**

Goal: **To document antimicrobial susceptibilities of gonococci isolated during the past 15 years in Kigali, Rwanda.**

Study Design: **Minimal inhibitory concentrations of recently collected gonococcal isolates of eight antimicrobials were determined. The results were compared with data collected for isolates obtained since 1986.**

Results: **In 1986, 35% of the gonococcal isolates were penicillinase-producing *N gonorrhoeae*. Tetracycline-resistant *N gonorrhoeae* appeared in 1989. The prevalence of penicillinase-producing *N gonorrhoeae* and tetracycline-resistant *N gonorrhoeae* increased significantly to 70.5% and 89.2%, respectively. Chromosomal resistance to penicillin, tetracycline, and thiamphenicol increased temporarily, then decreased significantly. Chromosomal resistance to trimethoprim-sulfamethoxazole appeared in 1988 and increased to 21.6%. All the isolates were susceptible to ceftriaxone, ciprofloxacin, spectinomycin, and kanamycin.**

Conclusions: **This study illustrated the rapidly increasing frequencies of penicillinase-producing *N gonorrhoeae* and tetracycline-resistant *N gonorrhoeae*. Chromosomal resistance to thiamphenicol and trimethoprim-sulfamethoxazole excludes these drugs as alternative treatment. Programs for antimicrobial susceptibility surveillance of *N gonorrhoeae* should urgently be established in Africa.**

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THE MAJORITY of sexually transmitted diseases (STDs) occur in developing countries, and Africa is the continent most affected. It is a gloomy reality that a high proportion of infected individuals either do not seek medical care or receive inappropriate or incomplete treatment for many different reasons: lacking awareness of asymptomatic patients, poorly trained healthcare providers in both the private and public sector, over-the-counter sale of drugs, poor compliance and lack of motivation to complete treatment, shortage of funds, and inefficient procurement of effective antibiotics in public health facilities.

Effective treatment of STDs is not only important for preventing severe medical complications and avoiding infection of sexual partners, but also for reducing the transmission of HIV. This was clearly shown by the landmark Mwanza study, which demonstrated that community-based low-cost syndromic management of genital infections reduced HIV transmission by 40%.[1] Major criticisms of such approaches are that community-based syndromic management has too little impact on the transmission of STDs within a community because most infected individuals are asymptomatic, and that such interventions are far beyond the capacities of primary healthcare services. An attractive alternative approach may be single-dose mass treatment of targeted core groups such as commercial sex workers and their clients, long-distance truck drivers, migrant workers, and military personnel. A major advantage of such mass treatment interventions is that they overcome problems of asymptomatic infections, poor compliance, and ineffective healthcare-seeking behavior. Whatever the treatment approach for STDs, monitoring antimicrobial resistance patterns of causative bacterial organisms will always be essential.

Low-level chromosomal-mediated resistance of *Neisseria*

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gonorrhoeae to penicillin, tetracycline, and chloramphenicol was described in 1975.[2] Plasmid-mediated resistance to penicillin caused by penicillinase-producing *N gonorrhoeae* (PPNG) strains emerged in 1976. Plasmid-mediated tetracycline-resistant *N gonorrhoeae* (TRNG) was first detected in 1985.[3,4] A rapid epidemic spread of PPNG in the eighties and of TRNG between 1988 and 1995 occurred in Africa.[5–20] Nevertheless, penicillin and tetracycline continued to be used for treatment of gonorrhea until quite recently.[13,17–21]

Current World Health Organization (WHO) recommendations for the treatment of gonorrhea include fluoroquinolones, third-generation cephalosporins, and spectinomycin.[22] Kanamycin, gentamycin, thiamphenicol, and trimethoprim-sulfamethoxazole (TMP-SMZ) are some of the cheaper drugs in Africa, and their use for the treatment of gonococcal infection is still widespread.[16,19,23–25] Reported *N gonorrhoeae* in vitro resistance levels to these antimicrobials in Africa show substantial country-to-country variations, but comparison of the results is extremely difficult because of major differences in laboratory techniques and in antimicrobial resistance breakpoints. Several of the susceptibility reports used unapproved or invalidated methods, resulting in unreliable data.[26]

Rwanda has a long tradition of *N gonorrhoeae* antimicrobial surveillance, and many data for isolates collected between 1975 and 1994 have been published.[15,27–32] Because of regional political instability in 1993 and a civil war in 1994, major population movements and migration occurred in Rwanda. In 1996, the Ministry of Health developed new treatment guidelines for infectious diseases, indicating norfloxacin as the general first-choice drug for gonorrhea and spectinomycin for pregnant women. Thiamphenicol was recommended as a useful cheap alternative. Despite these guidelines, TMP-SMZ continued to be a popular drug for use with diverse infectious diseases including gonorrhea. Because increasing numbers of gonorrhea treatment failures were reported during the 1990s, Family Health International decided in 1998 to support a study on antimicrobial resistance.

This report describes the in vitro antimicrobial susceptibility patterns of gonococci collected for the current study and presents the antimicrobial resistance trends between 1986 and 2000.

Methods

Consecutive clinical samples for this study were obtained between April 1999 and April 2000 from male adults with urethral syndromes and from neonates with purulent conjunctivitis presenting at the Center Bilyogo, a primary healthcare center in Kigali. After collection, specimens were inoculated directly onto modified Thayer Martin medium. The culture plates were transported to the Laboratoire National des Infections au Rétro-Virus, and 151 gonococcal isolates were identified presumptively by Gram stain and oxidase testing. Enriched subcultures of 139 isolates were suspended in skimmed milk, stored at −20 °C until the end of the study, and shipped to the Institute of Tropical Medicine, Antwerp. All the isolates were recovered, and identification was confirmed by sugar acidification and reactivity with monoclonal antibodies.

β-Lactamase production was tested with nitrocefin discs, and MICs of ceftriaxone, ciprofloxacin, kanamycin, penicillin, spectinomycin, tetracycline, thiamphenicol, and TMP-SMZ (1:19) were determined using an agar dilution technique. The bacterial inoculum size was 10^4 colony-forming units. The WHO gonococcal reference strains A to E and the American-type culture collection gonococcal strain 49226 were included. TMP-SMZ was tested on diagnostic sensitivity test agar (Oxoid Basingstoke, Hampshire, UK) with 5% lysed horse blood and 1% Kellogg's supplement.[33,34] All the other antimicrobial compounds were tested on a gonococcal agar base (BBL, Becton Dickinson, Sparks, MD) supplemented with 1% IsoVitaleX.[35]

The inoculated plates were incubated at 36 °C in 5% carbon dioxide with high humidity. The MICs were determined after 20 hours. The MIC breakpoints for determination of resistance were 2 mg/l or more for penicillin, tetracycline, and thiamphenicol; 0.5 mg/l or more for ceftriaxone; 1 mg/l or more for ciprofloxacin; 128 mg/l or more for spectinomycin and kanamycin; and 80 mg/l (range, 4–76 mg/l) or more for TMP-SMZ, according to National Committee for Clinical Laboratory Standards (NCCLS)-approved standards for *N gonorrhoeae.* If these were not available, then NCCLS criteria recommended for aerobically grown gram-negative bacteria, criteria found in WHO guidelines, or criteria used in other publications were used.[34,35,36] Plasmid-mediated resistance in isolates with MICs of 16 mg/l or more of tetracycline (TRNG) was determined, as described by Xia et al.[37]

For comparison of results from this study and from former antimicrobial susceptibility studies performed on consecutive isolates from Kigali, and for determination of resistance trends during the past 15 years, data previously published and tested by similar techniques in the authors' laboratory were used.[15,29–32]

Data Analysis

Frequencies of resistance were compared with the Yates corrected χ^2 or Fisher's exact test when appropriate.

Results

During the past 15 years, 1509 *N gonorrhoeae* isolates, secured from the Centre Bilyogo and from the Centre Hospitalier de Kigali, were obtained for antimicrobial susceptibility testing. The 139 isolates tested in this study were

TABLE 1. Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* Isolates Collected Between 1986 and 2000

Antimicrobial Agent	MIC %	No. of Isolates Tested			
		1986–1988 (n = 634)	1989–1991 (n = 513)	1992–1994 (n = 223)	1999–2000 (n = 139)
Penicillin*	50	0.50	1	0.5	0.125
	90	2	4	2	1
Tetracycline†	50	1	2	1	1
	90	4	4	4	2
Thiamphenicol	50	1	2	1	0.50
	90	2	2	2	1
Kanamycin	50	16	32	16	32
	90	32	32	32	32
Spectinomycin	50	32	32	16	32
	90	32	32	32	32
Ceftriaxone	50	0.004	0.008	0.004	0.004
	90	0.015	0.015	0.015	0.008
Ciprofloxacin	50	0.004	0.008	0.004	0.004
	90	0.015	0.015	0.015	0.008
TMP-SMZ (1/19)	50	1 /19	1 /19	1 /19	1 /19
	90	1 /19	2 /38	4 /76	4 /76

*Non-PPNG only: 1986–1988 (n = 394), 1989–1991 (n = 291), 1992–1994 (n = 98), 1999–2000 (n = 41).
†Non-TRNG only: 1986–1988 (n = 634), 1989–1991 (n = 484), 1992–1994 (n = 122), 1999–2000 (n = 15).
TMP-SMZ = trimethprim-sulfamethoxazole; PPNG = penicillinase-producing *N gonorrhoeae*; TRNG = tetracycline-resistant *N gonorrhoeae*.

collected in 1999 and 2000. All were susceptible to ceftriaxone (MICs, 0.002–0.060 mg/l), ciprofloxacin (MICs, 0.002–0.015 mg/l), kanamycin (MICs, 2–32 mg/l), and spectinomycin (MICs 16–32 mg/l). The MICs of thiamphenicol varied from 0.125 mg/l to 2 mg/l, 11 isolates (7.9%) had MICs of 2 mg/l and were considered resistant. The MICs of TMP-SMZ varied from 0.125/2.375 mg/l to 8/152 mg/l, 30 (21.5%) isolates had MICs 4/76 mg/l or greater and were considered resistant.

A very high number of the gonococcal isolates showed plasmid-mediated resistance: 98 (70.5%) were PPNG and 124 (89.2%) were TRNG. All 41 non-PPNG isolates were susceptible to penicillin and 3 of 15 (20%) non-TRNG isolates showed chromosomal resistance to tetracycline (MICs, 2 mg/l). Overall, only two isolates were susceptible to both penicillin and tetracycline.

For analysis of susceptibility data over time, the isolates tested in this study were compared with past isolates grouped by periods of 3 years: 634 for 1986 to 1988, 513 for 1989 to 1991, and 223 for 1992 to 1994. The MIC_{50} and MIC_{90} results of the four periods are shown in Table 1. For penicillin and tetracycline, results are presented for non-PPNG and non-TRNG, respectively. For penicillin, tetracycline, kanamycin, spectinomycin, ceftriaxone, and ciprofloxacin, the MIC_{50} and MIC_{90} values remained similar and did not change significantly by one dilution only. For thiamphenicol, the MIC_{50} decreased by two dilutions between 1989–1991 and 1999–2000, indicating a significant improvement in susceptibility. The MIC_{90} of TMP-SMZ increased by two dilutions between 1989–1991 and 1999–2000, indicating a significant increase in resistance. All 1509 isolates were susceptible to kanamycin, spectinomycin, ceftriaxone, and ciprofloxacin.

The frequencies and evolutions of chromosomal-mediated resistance of *N gonorrhoeae* (CMRNG) to penicillin, tetracycline, thiamphenicol, and TMP-SMZ during the 15-year monitoring period are shown in Figure 1. For penicillin, the frequencies of resistance were 11.7%, 35.1%, 13.3%, and 0% for 1986–1988, 1989–1991, 1992–1994, and 1999–2000, respectively. The increase of resistance between 1986 and 1991 was statistically significant ($P < 0.00001$), as was the decrease of resistance between 1991 and 2000 ($P = 0.0001$). The frequencies of resistance to tetracycline were 33.9%, 54.5%, 41.9%, and 20% for 1986–1988, 1989–1991, 1992–1994, and 1999–2000, respectively. A significant increase of resistance between 1986 and 1991 ($P < 0.00001$) was followed by a significant decrease of resistance between 1991 and 1994 ($P < 0.00001$). The further decrease of resistance between 1994 and 2000 was not significant. The prevalence of thiamphenicol-resistant isolates increased significantly from 41.8% in 1986–1988 to 52.6% in 1989–1991 ($P = 0.0003$). After 1991, the resistance decreased to 34.9% in 1992–1994 ($P = 0.00001$), and to 7.9% in 2000 ($P < 0.00001$). For the combination TMP-SMZ, resistance appeared in 1988 and increased gradually from 0.8% in 1986–1988 to 5.7% in 1989–1991 ($P < 0.00001$), to 13% in 1992–1994 ($P = 0.001$), and to 21.6% in 2000 ($P = 0.045$).

The evolution of plasmid-mediated resistance is shown in Figure 2. In 1986–1988, 37.6% of the isolates were PPNG. The frequency increased to 43.3% in 1989–1991 ($P = 0.07$), to 56.1% in 1992–1994 ($P = 0.002$), and to 70.5% in

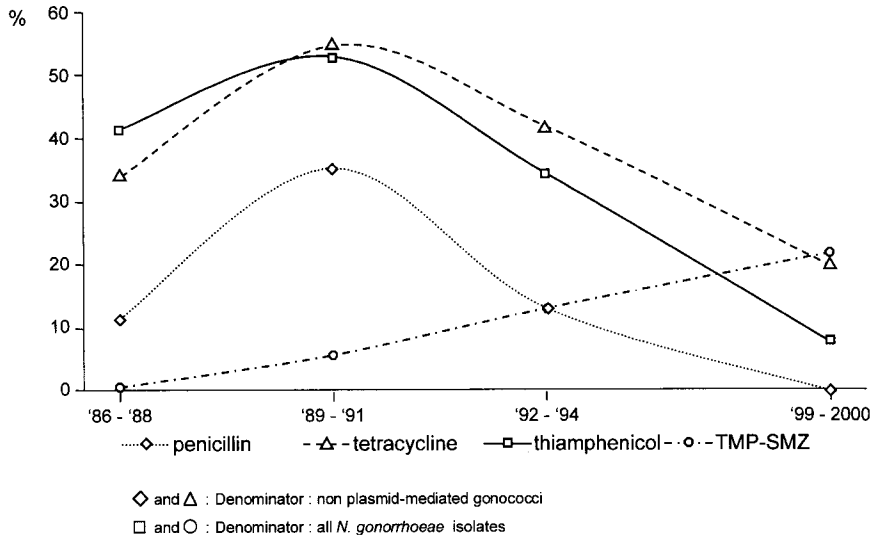

Fig. 1. Fluctuation of chromosomal-mediated resistance of *Neisseria gonorrhoeae* over time.

2000 ($P = 0.008$). The total resistance levels for penicillin (PPNG + CMRNG) increased significantly from 45.1% in 1986–1988 to 63.2% in 1989–1991 ($P < 0.00001$), did not vary between 1991 and 1994 (61.9%), and then increased further to 70.5% in 2000 ($P = 0.12$). In 1986–1988, there was no plasmid-mediated resistance to tetracycline. The first TRNG in Kigali were observed in 1989, and the prevalence for the period 1989 to 1991 was 5.7%. From 1989, a very rapid increase of TRNG was observed: to 45.3% in 1992–1994 ($P < 0.00001$), and to 89.2% in 2000 ($P < 0.00001$). The total prevalences of tetracycline resistant gonococci (TRNG + CMRNG) varied from 33.9% in 1986–1988 to 57.1% in 1989–1991 ($P < 0.00001$), to 61.4% in 1992–1994 ($P = 0.31$), and to 91.4% in 2000 ($P < 0.00001$).

Discussion

This study detected increasing levels of plasmid-mediated *N gonorrhoeae* between 1986 and 2000, and documented the evolution of chromosomal-mediated resistance between 1986 and 2000 in Kigali, Rwanda. Increasing levels of plasmid-mediated resistance to penicillin and tetracycline during the 1990s have been documented for several African countries.[5,14–20] Chromosomal resistance of *N gonorrhoeae* to penicillin in African countries, including Rwanda, has been described since the 1970s.[28,38–41] Between 1978 and 1984, PPNG appeared in Rwanda, probably imported from neighboring Kenya, a country with extensive commercial relations with Rwanda, and where the first PPNG was isolated in 1981.[42] Chromosomal resistance to tetracycline and thiamphenicol were observed in Rwanda among gonococcal isolates from 1978, and TRNG appeared in 1989.[15,28] Since then, increasing levels of plasmid-mediated resistance to penicillin and tetracycline and varying levels of chromosomal-mediated resistance to penicillin, tetracycline, and thiamphenicol have been described.[15,31,32]

All 1509 gonococcal isolates described in this report were susceptible to ceftriaxone, ciprofloxacin, and spectinomycin, the *N gonorrhoeae* treatment drugs recommended by

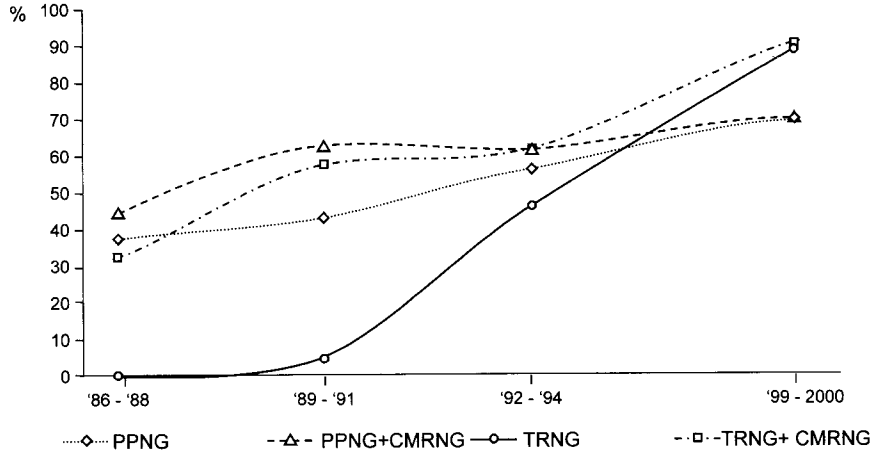

Fig. 2. Increasing resistance of *Neisseria gonorrhoeae* to penicillin and to tetracycline over time. PPNG, penicillinase producing *N gonorrhoeae*; CMRNG, chromosomal-mediated resistant *N gonorrhoeae*; TRNG, tetracycline resistant *N gonorrhoeae*.

WHO.[22] None of the African studies, using appropriate antimicrobial susceptibility test methods, have so far detected resistance to ceftriaxone, ciprofloxacin, or spectinomycin.[5,13–16,20,26,31,32,43,44]

All the isolates were susceptible to kanamycin. This finding compares with the results of other African studies using similar susceptibility testing methods and resistance breakpoints,[23,24,26] but differs from the results of studies using other, unapproved techniques, in which resistance prevalences to kanamycin of 17% to 23% were observed.[45,46] Kanamycin still is used in Africa for the treatment of gonorrhea.[23,24]

The current study showed that the frequency of penicillin and tetracycline CMRNG increased during the late 1980s, then decreased substantially during the 1990s. This evolution, however, should be interpreted with caution because prevalences of CMRNG were defined among nonplasmid-mediated isolates, and chromosomal resistance is masked by high-level resistance in PPNG and TRNG, creating a bias if the prevalence of plasmid-mediated resistance is different between CMRNG and non-CMRNG.

The trend of chromosomal resistance to thiamphenicol was quite similar to the evolution of chromosomal resistance to penicillin and tetracycline. Decreasing resistance between 1991 and 2000 may suggest a reduction in the use of these drugs during the 1990s. In two recent African studies, no resistance to thiamphenicol was observed,[17,45] whereas in other recent studies, resistance prevalences varied between 10% and 55%.[15,23,26]

For TMP-SMZ, a first resistant gonococcal isolate was detected in 1988. Since then, a gradual significant increase of resistant isolates has been observed, resulting in a frequency of 21.6% in 2000. Some studies detected resistance levels in Africa between 0% and 6%, whereas other studies, using different, unapproved techniques, reported resistance levels between 14% and more than 90%.[14,17,24–26,44,45]

By analyzing the different African *N gonorrhoeae* susceptibility studies, it can be clearly observed that resistance levels to thiamphenicol and TMP-SMZ vary from country to country, and that these drugs should not be recommended without reliable baseline susceptibility assessment, clinical trials, and regular surveillance.

Resistance to all used drugs can be expected to emerge, sometimes followed by rapid spread, as with penicillin and tetracycline worldwide, and with the fluoroquinolones in Asia.[47–49] The long-term efficacy of the quinolones for the treatment of gonorrhea is questionable. There is growing concern that the quinolones may follow the path of penicillin and tetracycline, and that most gonococci will become resistant in the near future.[50] In vitro manipulation of microorganisms resulting in chromosomal-resistant mutants suggests that resistance may arise in clinical isolates. Zhanel et al[51] have shown that serial exposure of bacteria to fluoroquinolones can result in multidrug-resistant mutants, including cross-resistance to third-generation cephalosporins.

Given the fact that the quinolones now are widely used for the treatment of gonorrhea in Africa, continued surveillance over the efficacy of these and other currently used drugs has become of paramount importance, and should become a critical key component of public health initiatives in Africa. The increasing resistance of gonococci in developing countries has to be considered a problem of global importance: While the incidence of gonorrhea has decreased dramatically in Western Europe, infections with resistant gonococci acquired abroad, often in Asia and Africa, have become more frequent.

Surveillance over the antimicrobial susceptibility of *N gonorrhoeae* in Africa has been restricted largely to sporadic sentinel studies or to irregular pointed prevalence studies because of unavailable or insufficient national resources. Consequently, the extent of antimicrobial resistance in many regions is not known.

In a 1995 report on essential drugs, WHO stated that every member country should have a national bacteriology reference laboratory in which local antimicrobial resistance patterns can be monitored.[52] Most African countries, however, cannot maintain or sustain such reference centers because of other health priorities, insufficient financial and logistic resources, and inadequate expertise in testing fastidious organisms such as *N gonorrhoeae* to guarantee reliable data. Instead of relying on clinical observations and laboratory results of poor quality, it seems more realistic to establish collaboration between African STD laboratories and international STD reference centers for training and quality assurance, or for testing of referred gonococcal clinical isolates. Storage and transport of gonococci to overseas laboratories may result in loss of isolates. It would be preferable to encourage efforts to build capacity and transfer technology, not only to avoid transport problems for isolates, but mainly to strengthen laboratory networks, to standardize susceptibility testing techniques and resistance breakpoints, and to coordinate data reporting.

Differences in culture media for susceptibility testing can significantly affect MIC results for several antibiotics.[53–55] Instead of using the quite difficult agar dilution MIC technique, it might be better to introduce and generalize the E-test strip MIC susceptibility system, which has been proved equivalent to the agar dilution technique.[56,57] Reliable performance of this test also needs training and quality control measures, as shown by the rather poor results obtained under field conditions.[19]

The World Health Organization should urgently develop standard surveillance guidelines and increase efforts to establish programs to monitor resistance of drugs for treatment of gonorrhea through Africa. Only then can more and better susceptibility data be generated and

compared between countries and regional treatment recommendations adapted and updated.

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