International Journal of STD & AIDS 2001; 12: 89–93

ORIGINAL ARTICLE

Antimicrobial susceptibilities and plasmid patterns of *Neisseria gonorrhoeae* in Bénin

E van Dyck[1], **M Alary**[2], **A Guédou**[3], **S Abdellati**[1], **E Lafia**[4] and **S Anagonou**[3,4]

[1]*Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium,* [2]*Centre de Recherche, Centre Hospitalier affilié Universitaire de Québec, Québec, Canada,* [3]*Microbiologie, Centre National Hospitalier Universitaire, Cotonou, Bénin and* [4]*Programme National de Lutte contre le SIDA et les MST, Cotonou, Bénin*

Summary: This study describes antimicrobial susceptibility patterns of *Neisseria gonorrhoeae* isolates obtained from female sex workers in Cotonou, Bénin. All isolates were susceptible to spectinomycin, ceftriaxone and ciprofloxacin, and susceptible to moderately susceptible to kanamycin; 9.8% of isolates were resistant to thiamphenicol; 9%, 87.5% and 3.5% were susceptible, moderately susceptible, resistant to trimethoprim–sulfamethoxazole, respectively; 94.4% and 99.3% were resistant to penicillin and tetracycline, respectively. All isolates with a minimal inhibitory concentration of tetracycline of >8 mg/l carried the 'American type' *tet*M plasmid; 94% and 6% of penicillinase-producing isolates possessed a 3.2 MDa and a 4.4 MDa β-lactamase plasmid, respectively. Surveillance of antimicrobial susceptibility of *N. gonorrhoeae* isolates to currently used drugs in Africa should become part of sexually transmitted diseases (STDs) control programmes.

Keywords: *Neisseria gonorrhoeae*, **antimicrobial susceptibility, plasmids, Africa**

INTRODUCTION

Due to the very high levels of resistance to penicillin and tetracycline in Africa, these drugs have been abandoned as treatment for gonorrhoea for several years. The rapid spread of resistance to both penicillin and tetracycline has resulted mainly from the acquisition of parts of a transposable element.

In most African countries fluoroquinolones have become the treatment of choice for gonococcal infection. Although this drug can now be obtained at very low cost, the prices charged to patients for it still vary widely from one African country to another and it is not currently included on the essential drugs list of many countries. Very efficient alternative drugs, spectinomycin and third-generation cephalosporins, are much less popular. Unfortunately, these drugs are quite expensive and, even when available, not affordable for many patients in developing countries. As cheap alternatives, in 1994 the World Health Organization (WHO) has recommended the use of kanamycin or sulfamethoxazole–trimethoprim (TMP–SMZ), but one year later kanamycin was replaced by the less toxic gentamycin on the WHO essential drug list[1,2].

In Asia where fluoroquinolones had become widespread and popular drugs for different infectious diseases including gonorrhoea, resistant gonococci emerged soon after the introduction and use of these products, and very high levels of quinolone-resistant gonococci have been detected during the last decade in several Asian countries[3,4].

So far, by using standard reference techniques, quinolone-resistant gonococci have not yet been detected in Africa, maybe because surveillance for such resistance in Africa is very limited. For most sub-Saharan African countries there are no reliable recent data on antimicrobial susceptibility of *N. gonorrhoeae* isolates. Major obstacles are absence of monitoring programmes, lack of technical know how, and lack of financial resources. The absence of coherent control strategies and the lack of tools to evaluate the *in vitro* and *in vivo* efficacy of current antimicrobial products (change to resistance or emergence of new forms of resistance) is a major and serious problem in Africa. Permanent or intermittent monitoring is the only way to evaluate and adapt treatment guidelines.

Since the detection of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) in 1976, we have observed a rapid spread of these resistant gonococci through the African continent and PPNG

Correspondence to: Eddy van Dyck, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belguim
E-mail: evandyck@itg.be

prevalences have risen to extremely high levels. At the beginning of this epidemic, all African PPNG possessed the 3.2 MDa β-lactamase plasmid. A few years later PPNG carrying the 4.4 MDa plasmid were found in many African countries and have increased to high levels in South Africa[5,6]. In West Africa the 3.2 MDa plasmid still predominates[6–8].

The first high level tetracycline-resistant gonococci (TRNG) carrying a 25.2 *tet*M MDa plasmid in Africa were detected in the Democratic Republic of Congo (former Zaire) in 1988[9]. These TRNG have spread from Central Africa to all countries of the continent[5,7,10,11]. All African TRNG characterized so far belonged to the 'American' type *tet*M gene[6,8].

The aim of this study was to document the *in vitro* antimicrobial susceptibilities and to characterize plasmid patterns of gonococcal isolates obtained from female sex workers in Cotonou, Bénin.

MATERIALS AND METHODS

Female sex workers were enrolled in a prospective study on the STD/HIV protective role of a vaginal microbicide. A clinical examination was performed every month and clinical specimens were collected for HIV and STD laboratory testing. Cultures on modified Thayer–Martin medium (MTM) for detecting *N. gonorrhoeae* were performed on cervical samples. Typical colonies grown on MTM were presumptively identified as gonococci by Gram stain and oxidase reactivity. β-lactamase production was tested by use of nitrocefin disks. Overnight subcultures on non-selective chocolate medium were suspended in skimmed milk, frozen at -20°C and sent on dry ice to the Institute of Tropical Medicine, Antwerp for further testing: 143 of 170 (84%) isolates obtained between January 1998 and September 1999 arrived in a viable state in Antwerp, where confirmatory identification based on sugar acidification and reactivity with monoclonal antibodies was performed.

Minimal inhibitory concentrations (MICs) of ceftriaxone, ciprofloxacin, kanamycin, penicillin, spectinomycin, thiamphenicol, trimethoprim–sulfamethoxazole (1:19) (TMP–SMZ) and tetracycline were determined with an agar dilution technique. WHO gonococcal (GC) reference strains A–E and American Type Culture Collection (ATCC) GC strain 49226 were included. TMP–SMZ was tested on diagnostic sensitivity test agar with 5% lysed horse blood and 1% Kellogg's supplement[12,13]. All other antimicrobial compounds were tested on GC agar base supplemented with 1% IsoVitaleX™ [14]. Inoculated plates were incubated at 36°C in 5% carbon dioxide with high humidity. The MICs were determined after 20 h.

Plasmid profiles were determined according to previously described procedures with minor modifications[15]. Gonococci were cultured overnight on blood agar. One loopful (10 μl) of growth was suspended in Eppendorf microcentrifuge tubes containing 250 μl of a lysing solution[15]. After vigorous vortexing, the suspensions were heated at 80°C for 20 min. After cooling on ice, the lysed suspensions were mixed with 250 μl phenol-chloroform (50:50, v/v) and vortexed. After centrifugation at 12,000 rev/min for 10 min at 4°C, the upper aqueous phase was used for gel electrophoresis. A panel of 6 GC strains showing different plasmid patterns was used as control.

For the detection and typing of the 25.2 MDa *tet*M plasmids, a polymerase chain reaction (PCR) was performed using primers described by Xia *et al.*[16]. Colonies of TRNG, grown overnight on blood agar were suspended in distilled water to a density of 0.5 McFarland unit ($2–3 \times 10^8$ bacteria per ml). Five microlitres of 1 N NaOH were added to 95 μl of bacterial suspensions and the mixtures were heated at 95°C for 15 min. After cooling to room temperature, 10 μl of 1 M Tris-HCl, pH 8.0 were added. Five microlitres of the bacterial lysates were added to 20 μl of PCR mixtures containing 200 μM of each deoxynucleoside triphosphate, $1 \times$ PCR buffer (50 μM KCl, plus 10 μM Tris-HCl, pH 8.3), 2 mM $MgCl_2$, 0.3 μM of each primer, and 2 units of Taq polymerase (Perkin Elmer Cetus, Norwalk, Connecticut, USA). The amplification was performed in a thermal cycler (Gene Amp™ 9600, Perkin Elmer). After an initial denaturation step at 94°C for 3 min, the temperature was cycled at 94°C for 30 s, 44°C for 30 s, and 72°C for 1 min, for a total of 30 cycles, with a final extension step at 72°C for 7 min. PCR was followed by gelelectrophoresis on 2% agarose for 10 min. Gels were stained with ethidium bromide.

Three GC control strains (*tet*M negative containing a 24.5 MDa plasmid, 'Dutch' type *tet*M, 'American' type *tet*M) were included.

RESULTS

Antimicrobial susceptibilities

After confirmatory testing all 143 isolates were GC. All isolates were susceptible to ceftriaxone (MICs 0.001–0.015 mg/l), ciprofloxacin (MICs 0.001–0.008 mg/l), spectinomycin (MICs 4.0–32 mg/l), and susceptible to moderately susceptible to kanamycin (MICs 8.0–32 mg/l). The MICs of thiamphenicol varied from 0.125–2.0 mg/l, 26 (9.8%) isolates showed a MIC of 2.0 mg/l, which is the breakpoint for thiamphenicol resistance. For TMP–SMZ (1:19) the MICs varied from 0.125/2.375–4.0/76 mg/l: 13 isolates (9%) were fully susceptible (MICs 0.125/2.375–0.25/4.75 mg/l), 125 isolates (87.5%) were moderately susceptible (MICs 0.5/9.5–2.0/38 mg/l), and 5 isolates (3.5%) showed a MIC of 4.0/76 mg/l, which is the breakpoint for TMP–SMZ resistance. One hundred and thirty-five isolates (94.4%) were PPNG with penicillin MICs of 2 to $\geqslant$64 mg/l, all 8 non-PPNG were susceptible to penicillin (MICs 0.03–1.0 mg/l). One hundred and thirty-nine (97.2%) isolates were TRNG (MICs 16 to $\geqslant$64 mg/l), one non-TRNG was

susceptible to tetracycline (MIC 0.25 mg/l), 3 non-TRNG showed chromosomal resistance (MIC 8.0 mg/l).

Plasmid profiles

Plasmid profiles of all 143 GC isolates were determined. As shown in Table 1, 127 PPNG possessed the 3.2 MDa and 8 PPNG possessed the 4.4 MDa β-lactamase plasmid. All 139 TRNG carried the 25.2 MDa *tet*M plasmid and all 139 TRNG were subjected to PCR of the downstream region of the incomplete *tet*M transposon and showed a 1600 base pair PCR fragment, corresponding to the 'American' restriction plasmid type. The 4 non-TRNG remained PCR negative, but carried the 24.5 MDa conjugative plasmid. Three of the non-TRNG were PPNG. One single non-PPNG/non-TRNG isolate was susceptible to penicillin (MIC 0.03 mg/l) and to tetracycline (MIC 0.25 mg/l).

DISCUSSION

This study documented extremely high levels of plasmid-mediated resistance of *N. gonorrhoeae* isolates to penicillin and tetracycline in Cotonou. Only one single GC isolate was susceptible to both penicillin and tetracycline. PPNG and TRNG levels in this study were the highest ever detected in Africa. In Bénin, self-medication with ampicillin and tetracycline and even use of these drugs as STD prophylaxis are very common practices[17]. Reliable data of gonococcal antimicrobial susceptibility in Africa are rare, and data of several studies that used non-recommended techniques are not comparable. Table 2 shows recent data on PPNG and TRNG prevalences in 14 African countries. In Cotonou all TRNG possessed the 'American' type *tet*M 25.2 MDa plasmid. The 'Dutch' type has not been observed yet in Africa[6,8]. Both 3.2 MDa and 4.4 MDa β-lactamase plasmids are present on the African continent. Unlike South Africa where the 4.4 MDa plasmid is predominant, the majority of PPNG in all other countries carry the 3.2 MDa plasmid, which was also the case in this study[5,6,8,10,21].

Single oral dose of 500 mg ciprofloxacin becomes more and more the first choice treatment for gonococcal infection in Africa. All GC isolates in Cotonou were susceptible to ciprofloxacin, although, in Bénin, this drug has been on the essential drugs list and recommended in the national STD management algorithms since 1994; its current retail price for patients attending health centres in Cotonou is approximately US$0.40. All other African studies that used recommended antimicrobial testing methods detected no resistance to fluoroquinolones[5,6,8–11,18,19,22,23]. In this study, as well as in all African studies, using recommended methods, all GC isolates were fully susceptible to third generation cephalosporins and to spectinomycin[5,6,8,10,18,19,22,23]. In many African countries, *N. gonorrhoeae in vitro* susceptibility to thiamphenicol or chloramphenicol has decreased continuously during the last decade[6,11,23,24]. In 2 recent studies no resistance to thiamphenicol/chloramphenicol was observed[7,19]. In this study we found 10% of GC isolates with an thiamphenicol MIC of 2 mg/l (2 mg/l is the resistance breakpoint). Based on current resistance levels, thiamphenicol/chloramphenicol are no longer used to treat gonococcal infection in Africa.

For regions where first choice drugs are not affordable or not available for any reason, in 1994 the WHO recommends TMP–SMZ and kanamycin (later replaced by the less toxic gentamycin) as cheap alternatives for gonorrhoea treatment[1,2]. For several years there exists a big debate and polarization between research groups about the activity of kanamycin/gentamycin and TMP–SMZ. In this study all gonococcal isolates were susceptible to moderately susceptible to kanamycin and only 3.5% were resistant to TMP–SMZ. Our results for kanamycin are in agreement with all African studies using recommended methods and correct *in vitro* resistance breakpoints[6,8,20,24] but disagree

Table 1. Plasmid profiles of 143 Neisseria gonorrhoeae *isolates*

Resistance phenotype	No.	Plasmid profiles (MDa)
PPNG/TRNG	124	2.6, 3.2, 25.2
PPNG/TRNG	8	2.6, 4.4, 25.2
PPNG/non-TRNG	3	2.6, 3.2, 24.5
Non-PPNG/TRNG	7	2.6, 25.2
Non-PPNG/non-TRNG	1	2.6, 24.5

PPNG=penicillin-producing *N. gonorrhoeae*; TRNG=tetracycline-resistant *N. gonorrhoeae*

Table 2. Prevalence of penicillin-producing Neisseria gonorrhoeae *(PPNG) and tetracycline-resistant* N. gonorrhoeae *(TRNG) in Africa, 1991–1999*

Country	No. of GC isolates tested	% PPNG	% TRNG	Period	Ref.
Bénin	143	94	97	1998–99	This study
Cameroon	84	70	82	1996	(6)
Ethiopia	44	89	68	1996	(6)
Gambia	103	77	84	1994–95	(7)
Ghana	1187	94	0	1991–93	(18)
Ivory Coast	724	76	79	1992–96	(6)
Kenya	177	56	86	1995–96	(19)
Liberia	100	83	92	1993–95	(8)
Mozambique	151	65	21	1996	(6)
Rwanda	984	47	13	1988–94	(6)
Senegal	48	17	42	1997	(6)
South Africa	184	36	38	1996	(6)
Tanzania	130	50	35	1992	(10)
Zimbabwe	264	45	NT	1995–96	(20)

NT=not traced; GC=gonococci

with results from studies using non recommended methods which showed resistance prevalences of 17–23%[25,26]. For TMP–SMZ, African studies using recommended techniques detected resistance levels of 0–6%, where studies that used non recommended techniques detected resistance levels of 14% to >90%[6,7,19,20,23,26].

Mozambique is one of the few African countries still using single dose 2 g kanamycin i.m. as first-line treatment for gonococcal infection with cure rates of >90%[24]. In 1992–1993 a clinical trial with 5 different drugs for the treatment of men with gonococcal urethritis was performed in Malawi. Cure rates of laboratory confirmed gonococcal urethritis were 95% for gentamycin and 48% for TMP–SMZ[27]. Unfortunately, the investigators did not respect the WHO recommended treatment regimens: instead of injecting 80 mg i.m. 3 times every 8 h in one day, 240 mg gentamycin was injected as a single dose. The treatment of TMP–SMZ was even worse: instead of using the WHO recommended regimen of 10 tablets of TMP 80 mg/SMZ 400 mg orally, once daily for 3 days, patients were treated with a significant lower dose of TMP 320 mg/SMZ 1600 mg for 2 days (less than 27% of the recommended dose in 2 instead of 3 days)[1]. Another problem in that study was that for the TMP–SMZ treatment, patients' compliance was not measured. Cure rates of TMP–SMZ in that study were totally unreliable. It may be that patients' compliance for the WHO TMP–SMZ recommended regimen was so bad that treatment of GC infection with this drug was unrealistic. In any case, because of country-to-country variability, kanamycin (or gentamycin) and TMP–SMZ should not be recommended for all African countries, without reliable baseline assessment, clinical trials, and regular surveillance.

In 1995, the WHO has stated that every member country should have a national bacteriology reference laboratory where local resistance patterns could be monitored[2]. Most African countries cannot maintain such reference centres because of lack of financial and logistic resources. Another important problem is the lack of training and experience with testing fastidious bacteria like *N. gonorrhoeae*, so reliability of data cannot be guaranteed. A more realistic approach is that African STD reference laboratories should have the possibility of referring GC clinical isolates to an international STD reference centre instead of relying on individual laboratory and clinical observations. Loss of isolates is a serious problem when transporting GC isolates to overseas laboratories. To avoid this problem, transfer of technology should be encouraged. Many African GC susceptibility studies do not meet recommended standard methods and do not respect correct MIC resistance breakpoints. There is a real need to develop standard surveillance guidelines throughout Africa. Only then can susceptibility data be compared between countries. Instead of recommending the quite difficult agar dilution MIC method, it might be better to recommend and introduce the E-test MIC susceptibility testing system[28,29]. Utilization of this simpler technique can be achieved with the help of international donors.

Resistance to used drugs can be expected to emerge and spread rapidly, as was the case with penicillin and tetracycline worldwide, and with fluoroquinolones in Asia. Given the fact that the latter class of drugs is now widely recommended for the treatment of GC in Africa, continued surveillance of the efficacy of currently-used drugs, which is very important *per se*, becomes of paramount importance in Africa.

Acknowledgements: This study was partly sponsored by an UNAIDS grant.

We are very grateful to Continental Pharma, Pharmacia and Upjohn, Roche Pharmaceuticals, Pfizer, Bayer and Inpharzam for free supply of antimicrobial standards.

We are also very grateful to L Mukenge for clinical examination and specimen collection and to K Janssens for secretarial work.

References

1 WHO. *Management of Sexually Transmitted Diseases 1994*. WHO/GPA/TEM/94.1

2 WHO. The use of essential drugs. *Sixth Report of the WHO Expert Committee Technical Report Series No. 850*, 1995:29–30

3 Tapsall J. Surveillance of antibiotic resistance in *Neisseria gonorrhoeae* and implications for the therapy of gonorrhoea. *Int J STD AIDS* 1995;**6**:233–6

4 Tanaka M, Naito S, Nakayama H, Kobayashi I. Antimicrobial susceptibility of *Neisseria gonorrhoeae* in Fukuoka City, Japan, in the early 1980s and 1997–1998: emergence of high-level fluoroquinolone resistance. *Antimicrob Agents Chemother* 1999;**43**:722–3

5 Chenia H, Pillay B, Hoosen A, Pillay D. Antibiotic susceptibility patterns and plasmid profiles of penicillinase-producing *Neisseria gonorrhoeae* strains in Durban, South Africa, 1990–1993. *Sex Transm Dis* 1997;**24**:18–22

6 Van Dyck E, Thiam A, Crabbé F, Laga M. Plasmids of penicillinase-producing and tetracycline resistant *N. gonorrhoeae* in Africa (Abstract O119). *12th International Society of Sexually Transmitted Diseases Research*, Sevilla, Spain, 1997

7 Adegbola R, Sabally S, Corrah T, West B, Mabey D. Increasing prevalence of penicillinase-producing *Neisseria gonorrhoeae* and the emergence of high-level, plasmid-mediated gonococcal isolates in The Gambia. *Trop Med Intern Health* 1997;**2**:428–32

8 Guyot A, Jarrett B, Sanvee L, Dore D. Antimicrobial resistance of *Neisseria gonorrhoeae* in Liberia. *Trans R Soc Trop Med Hyg* 1998;**92**:670–4

9 Van Dyck E, Rossau R, Duhamel M, *et al*. Antimicrobial susceptibility of *Neisseria gonorrhoeae* in Zaire: high level plasmid mediated tetracycline resistance in Central Africa. *Genitourin Med* 1992;**68**:111–16

10 West B, Changalucha J, Grosskurth H, *et al*. Antimicrobial susceptibility, auxotype and plasmid content of *Neisseria gonorrhoeae* in Northern Tanzania: emergence of high level plasmid mediated tetracycline resistance. *Genitourin Med* 1995;**71**:9–12

11 Van Dyck E, Crabbé F, Nzila N, *et al*. Increasing resistance of *Neisseria gonorrhoeae* in West and Central Africa: consequences on therapy of gonococcal infection. *Sex Transm Dis* 1997;**24**:32–7

12 Doern G, Jones R. Antimicrobial susceptibility testing of *Haemophilus ducreyi*, *Branhamella catarrhalis*, and *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1998;**32**:1747–53

13 Van Dyck E, Meheus A, Piot P. Gonorrhoea. In: World Health Organization, ed. *Laboratory Diagnosis of Sexually Transmitted Diseases*. Geneva: WHO, 1999:1–21

14 NCCLS. Performance Standards for Antimicrobial Susceptibility Testing. *9th Informational Suppl* 1999;**19**:M100–S9

15 Kado C, Liu S. Rapid procedure for detection and isolation of large and small plasmids. *J Bacteriol* 1981;**145**:1365–73

16 Xia M, Pang Y, Roberts M. Detection of two groups of 25.2 MDa *Tet*M plasmids by polymerase chain reaction of the downstream region. *Molec Cell Probes* 1995;**9**:327–32

17 Lowndes CM, Gnintoungbé C, Alary M, Bédard E, Jossou P, Anagonou S. Clients of prostitutes in Benin: health-seeking behaviour for STDs (Abstract 040). *13th International Society for Sexually Transmitted Diseases Research*, Denver, USA, 1999

18 Addy P. Susceptibility patterns of *Neisseria gonorrhoeae* isolated at the Komfo, Anokye Teaching Hospital, Ghana to commonly prescribed antimicrobial agents. *E Afr Med J* 1994;**71**:368–72

19 Claeys G, Taelman H, Gichangi P, Tyndall M, Ombette J, Verschraegen G. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from men with urethritis in Kenya. *Sex Transm Inf* 1998;**74**:294–5

20 Mason P, Gwanzura L, Latif A, Marowa E, Ray S, Katzenstein D. Antimicrobial resistance in gonococci isolated from patients and from commercial sex workers in Harare, Zimbabwe. *Int J Antimicrob Agents* 1998;**9**:175–9

21 Ison C, Roope N, Dangor Y, Radebe F, Ballard R. Antimicrobial susceptibilities and serotyping of *Neisseria gonorrhoeae* in southern Africa: influence of geographic source of infection. *Epidemiol Infect* 1993;**110**:297–305

22 Van Dyck E, Laga M, Manoka A, Behets F, Piot P. Epidemic spread of plasmid-mediated tetracycline resistant *Neisseria gonorrhoeae* in Zaire. *Int J STD AIDS* 1995;**6**:345–7

23 Bogaerts J, Van Dyck E, Mukantabana B, Munyabikali J, Martinez Tello W. Auxotypes, serovars, and trends of antimicrobial resistance of *Neisseria gonorrhoeae* in Kigali, Rwanda (1985–93). *Sex Transm Inf* 1998;**74**:205–9

24 De Hulsters B, Noya A, Barreto A, Bastos R. Temporal changes in gonococcal antibiotic resistance in Maputo, Mozambique (Abstract A526). *IXth International Conference on AIDS and STD in Africa*, Kampala, Uganda, 1995

25 Roux R, Dosso M, Herouin P. Traitement de l'uréthrite aigue non compliquée à Abidjan. *Med Afr Noire* 1991;**38**:459–62

26 Meless H, Abegaze B. Drug susceptibility of *Neisseria gonorrhoeae* from patients attending clinics for sexually transmitted diseases in Addis Ababa. *E Afr Med J* 1997;**74**:447–9

27 Lule G, Behets F, Hoffman I, *et al*. STD/HIV control in Malawi and the search for affordable and effective urethritis therapy: a first field evaluation. *Genitourin Med* 1994;**70**:384–8

28 Van Dyck E, Smet H, Piot P. Comparison of E test with agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1994;**32**:1586–8

29 Biedenbach D, Jones R. Comparative assessment of E test for testing susceptibilities of *Neisseria gonorrhoeae* to penicillin, tetracycline, ceftriaxone, cefotaxime, and ciprofloxacin: investigation using 510 (K) review criteria, recommended by the Food and Drug Administration. *J Clin Microbiol* 1996;**34**:3214–17

(Accepted 30 September 2000)