influence of tropical climate conditions on drug content, *in vitro* dissolution and oral bioavailability of different formulations of two essential drugs marketed in Tanzania, the drug content and drug release from all the tested ciprofloxacin formulations were within USP-24 requirements and remained stable during storage at simulated tropical conditions. Oral bioavailability was also not influenced by tropical conditions.[3] The stability of essential drugs (ampicillin, benzylpenicillin, phenoxymethylpenicillin and tetracycline) was not affected during shipment to the tropics after being exposed to much higher temperatures and humidity than recommended by the manufacturer, with temperatures recorded within packs ranging from −3.5 to 42.4°C and humidity ranging from 20% to 88%.[4] A controlled longitudinal study on the quality and stability of essential drugs in rural Zimbabwe showed that even under the most adverse tropical conditions, clinically relevant instability of these agents is rare.[5]

Evidence of potency is an important aspect to consider whilst procuring data about consumption of antimicrobials in developing countries. However, in order to achieve this, one would have to establish potency for each drug on the market, a difficult, unrealistic and expensive exercise, considering the limited funding for research in developing countries. Otherwise, assumptions would be based on random sampling of imported or locally manufactured drugs. Accurate information relating to national antibiotic consumption would be essential in order to extrapolate the impact of these storage issues.

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Emergence of *Neisseria meningitidis* with decreased susceptibility to ciprofloxacin in Argentina

Alejandra Corso[1]*, Diego Faccone[1], Mariana Miranda[1], Marisa Rodriguez[1], Mabel Regueira[1], Cristina Carranza[2], Cecilia Vencina[3], Julio A. Vazquez[4] and Marcelo Galas[1]

[1]*Servicio Antimicrobianos, Dpto Bacteriología, Instituto Nacional de Enfermedades Infecciosas (INEI)–ANLIS 'Dr Carlos G. Malbrán', Av. Velez Sarsfield 563 (1281), Buenos Aires;* [2]*Hospital Area Cipolleti, Rio Negro;* [3]*Hospital Sor María Ludovica, La Plata, Buenos Aires, Argentina;* [4]*Reference Laboratory for Meningococci. Servicio de Bacteriología. Instituto de Salud Carlos III, Majadahonda, Spain*

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*Corresponding author. Tel/Fax: +54-11-4303-2812; E-mail: acorso@anlis.gov.ar

Sir,

To date, only three *Neisseria meningitidis* clinical isolates showing decreased susceptibility to ciprofloxacin (DSC) have been reported in France (1999), Australia (2000) and Spain (2003).[1] The mechanisms of resistance were mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene, resulting in amino acid substitutions Asp-95 → Gly, Asp-95 → Asn and Thr-91 → Ile, respectively.[1]

During 1997–2003, 873 meningococcal strains from invasive disease were submitted to the National Reference Laboratory (INEI) as part of the National Surveillance Programme for serogroup and antimicrobial resistance in *N. meningitidis*. In 2002, we detected one strain (M5191) with DSC (MIC 0.12 mg/L), isolated from the CSF of a 63-year-old woman at Hospital Area Cipolleti, Rio Negro Province. The woman suffered from diabetes and chronic urinary tract infections, and she had been previously treated with antibiotics, including fluoroquinolones. During 2003 a second strain (M5507) with DSC (MIC 0.06 mg/L) was isolated from both CSF and blood, in a 1-year-old child from Hospital Sor María Ludovica, La Plata, Buenos Aires Province. In this case, the patient had not been previously exposed to any antibiotic. Treatment with ceftriaxone resulted in a good clinical outcome in both cases. All other meningococci examined were susceptible to ciprofloxacin (MICs ≤0.015 mg/L).

Serogroup and serotype/serosubtype were determined by slide agglutination and ELISA, respectively. *N. meningitidis* M5191 was typed as Y:non-typeable:P1.5 and M5507 as B:1:P1.non-subtypeable. Determination of MICs by agar dilution and disc diffusion testing were both performed using Mueller–Hinton agar supplemented with 5% sheep blood, with incubation for 24 h at 35°C in air containing 5% CO_2. *N. meningitidis* EMGM-2, EMGM-10 and EMGM-13 were used as control strains.[2] The MICs for M5191 and M5507 were (mg/L): penicillin, 0.03/0.12; ampicillin, 0.06/0.25; ceftriaxone, 0.001/0.002; rifampicin,

Table 1. MICs (mg/L) of ciprofloxacin and nalidixic acid in the presence or absence of 6.25 mg/L reserpine

Strain	CIP	CIP + RES	NAL	NAL + RES
M5191	0.12	0.004	64	0.25
M5507	0.06	0.06	64	64
EMGM-2	0.004	0.004	0.5	0.25
EMGM-10	0.004	0.004	0.5	0.25
EMGM-13	0.004	0.004	0.5	0.25

CIP, ciprofloxacin; RES, reserpine; NAL, nalidixic acid.

Table 2. Disc diffusion assay of *N. meningitidis* strains using ciprofloxacin or nalidixic acid discs

	Inhibition zones of *N. meningitidis* strains (mm)				
Disc	M5191	M5507	EMGM-2	EMGM-10	EMGM-13
Ciprofloxacin	31	35	42	42	38
Nalidixic acid	7	6	33	34	34

0.008/0.008; chloramphenicol, 0.5/0.5; and tetracycline, 0.12/0.12, respectively. Both meningococci showed resistance to nalidixic acid (MICs 64 mg/L).

Sequencing of QRDRs in *gyrA* and *parC*,[3] and *gyrB* and *parE*,[4] was performed by standard methods. As described for the Australian isolate, *N. meningitidis* M5507 contained a mutation in the *gyrA* gene that resulted in the amino acid substitution Asp-95 → Asn. No mutations were detected in the QRDR of the *parC* gene in M5507. Unexpectedly, no mutations were detected in *N. meningitidis* M5191 when the four QRDRs were analysed.

The major mechanisms of fluoroquinolone resistance identified in bacterial strains are chromosomal mutations in DNA gyrase and topoisomerase IV, and overexpression of endogenous efflux pumps.[5] Therefore, we phenotypically assessed the possibility of increased efflux of quinolones in this strain, by determining ciprofloxacin MICs with and without 6.25 mg/L of reserpine (an inhibitor of multidrug efflux pumps). In *N. meningitidis* M5191, the addition of reserpine significantly reduced the ciprofloxacin and nalidixic acid MICs 30-fold and 256-fold, respectively, reaching the same levels as those for the susceptible control strains (Table 1). Reserpine alone had no effect on growth or colony morphology. Although accumulation and efflux have not been specifically investigated here, the absence of mutations in the QRDRs of *gyrA*, *parC*, *gyrB* and *parE* genes analysed and the reduction in quinolone MICs in the presence of reserpine, strongly suggest that an efflux mechanism is responsible for DSC in *N. meningitidis* M5191. In fact, the sequence of the *mtrRCDE* gene complex from this strain showed a deletion affecting most of the *mtrR* gene (data not shown). Although some authors have reported that this pump appears less active in meningococci than in gonococci,[6] the deletion detected in M5191 might be involved in the expression of the efflux system. Alternatively, we cannot discard a combination of mechanisms conferring DSC.

Nalidixic acid has been used to detect DSC in *Neisseria gonorrhoeae*.[7] Therefore, we evaluated nalidixic acid (30 μg) and ciprofloxacin (5 μg) by the disc diffusion method, to detect DSC in meningococci. *N. meningitidis* with DSC showed zones of inhibition of 6–7 mm with nalidixic acid and of 31–35 mm with ciprofloxacin (Table 2). In contrast, susceptible strains displayed bigger zones: 33–34 mm for nalidixic acid and 38–42 mm for ciprofloxacin. Thus, the nalidixic acid disc seems to be a useful screen for DSC, independent of the resistance mechanism involved. The lack of clear guidelines for susceptibility breakpoints in this species would impair the detection of emerging resistance mechanisms. Therefore, we propose the inclusion of a 30 μg disc in the antibiogram, in order to detect the emergence of DSC in *N. meningitidis* isolates. When reduced zones of inhibition are noted, the MIC of ciprofloxacin should be determined in order to confirm the DSC and monitor trends in susceptibility to this antimicrobial agent in meningococcal strains.

Rifampicin is commonly used as chemoprophylaxis to eradicate meningococci carriage. However, there is a tendency to replace rifampicin by ciprofloxacin in adults, because it can be used in single doses, whereas rifampicin requires twice-daily administration for 2 days. To date, we cannot attribute the use of ciprofloxacin in meningococci chemoprophylaxis as a cause of the emergence of DSC, but it seems reasonable to speculate that fluoroquinolone consumption in the community, for a range of infections such as urinary tract infections or community-acquired respiratory infections, may be, in part, responsible for the emergence of fluoroquinolones resistance.

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