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Reduced susceptibility to cefixime but not ceftriaxone: an uncertain perspective for the treatment of gonorrhoea in Brazil

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Sir,

Resistance to extended-spectrum cephalosporins (ESC) in *Neisseria gonorrhoeae* has been described in different countries and is an important cause for concern regarding the successful treatment of gonorrhoea [1]. Modifications of the penicillin-binding protein 2

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(penA) gene are the main ESC resistance determinants in gonococcus [2]. Specific mosaic *penA* alleles are associated with cefixime minimum inhibitory concentrations (MICs) equivalent to the Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoint for cefixime (MIC ≤ 0.25 mg/L), which characterises reduced susceptibility to cefixime (Cef^{RS}) [3]. Thus, despite no longer being recommended as a first-line treatment for gonococcal infections (http://www.cdc.gov/std/tg2015/gonorrhea.htm), cefixime is considered a sentinel drug associated with the evolution of ESC resistance in *N. gonorrhoeae* [3]. Additional mutations in mosaic alleles, including A501P in *penA* XXXIV, and A311V, T316P and T483S in *penA* X, lead to high-level ESC resistance [2,4].

Brazil currently recommends ciprofloxacin combined with azithromycin for gonorrhoea therapy in the vast majority of its territory, excluding some states where ciprofloxacin should be replaced with ceftriaxone owing to high documented resistance rates. This recommendation includes Rio de Janeiro and is based on small studies because Brazil lacks a surveillance programme for gonococcal resistance [5].

Based on the importance of ESC resistance surveillance in *N. gonorrhoeae* worldwide, this retrospective study aimed to investigate the occurrence of Cef^{RS} strains in Rio de Janeiro, which may represent a high-risk phenotype that might spread within the population following the introduction of ceftriaxone.

Neisseria gonorrhoeae isolates obtained from patients with acute gonorrhoea were sent to the Laboratory for Investigation in Medical Microbiology (LIMM, Brazil) by public and private healthcare facilities in Rio de Janeiro between 2006 and 2015. A total of 116 isolates were included in the study without screening, and isolate identification was confirmed using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) (Bruker Biotyper 3.1; Bruker Daltonics, Billerica, MA). Patient data included specimen type, sex and age. Ceftriaxone, cefixime, penicillin, tetracycline and ciprofloxacin MICs were determined by agar dilution according to CLSI guidelines, and azithromycin MICs were determined by Etest (bioMérieux, Rio de Janeiro, Brazil). Cef^{RS} isolates were characterised according to associated resistance mechanisms and whole-genome sequence (WGS)-based strain typing.

WGS was conducted using Illumina HiSeq and MiSeq platforms (Illumina Inc., San Diego, CA). The obtained data were assembled using CLC Genomics Workbench 7 (https://www.qiagenbioinformatics.com/products/clc-genomics-workbench/), and the software was also used to investigate mutations associated with resistance to cefixime *(penA),*penicillin *(ponA, porB* and *rps)*, tetracycline *(porB* and S10 ribosomal protein) and fluoroquinolones *(gyrA* and *parC)* by aligning the translated proteins with reference sequences. Moreover, CLC Genomics Workbench 7 was also used to extract gene sequences for multilocus sequence typing (MLST) (https://pubmlst.org/neisseria/) and to identify plasmids conferring resistance to penicillin (Asia, Africa and Toronto/Rio) and tetracycline (Dutch and American) via the in silico alignment of primer sequences targeting these plasmids.

Of the 116 *N. gonorrhoeae* isolates, 7 (6.0%) exhibited Cef^{RS}, however reduced susceptibility to ceftriaxone was not detected. These isolates were collected between 2010 and 2015 from male and female patients ranging in age from 24 to 59 years. Six of the

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Cef^{RS} isolates encoded the mosaic *penA* XXXIV allele and one encoded the *penA* XII allele. All seven isolates were resistant to penicillin, tetracycline and ciprofloxacin, and these patterns were associated with chromosomal mutations in *ponA, porB, rpsJ, gyrA* and *parC* (detected in six isolates) or with the combination of a Toronto/Rio β-lactamase plasmid, a *tetM* American plasmid, and *gyrA* and *parC* mutations (detected in one isolate). Moreover, two Cef^{RS} isolates exhibited azithromycin MICs of >2 mg/L (Table 1). The seven Cef^{RS} isolates were assigned to ST1901, which is a sequence type associated with increased ESC resistance in North America, Asia and Europe that also contains the mosaic *penA* XXXIV allele [1–3].

A limitation of this study is the type of collection investigated, which is unsuitable for prevalence estimations. Nevertheless, we determined that multidrug-resistant CefRS *N. gonorrhoeae* have been isolated in Rio de Janeiro since 2010, including two isolates exhibiting azithromycin resistance. Although no outbreaks related to similar strains have been described in Brazil, these results highlight the presence of highly relevant resistance phenotypes, which might pose a significant threat to the treatment of gonorrhoea in Rio de Janeiro.

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References

[1]. Unemo M, Shafer WM. Future treatment of gonorrhea—novel emerging drugs are essential and in progress? Expert Opin Emerg Drugs 2015;20:357–60. [PubMed: 25907334]

[2]. Unemo M, Golparian D, Nicholas R, Ohnishi M, Gallay A, Sednaoui P. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoea* in France: novel *penA* mosaic allele in a successful international clone causes treatment failure. Antimicrob Agents Chemother 2012;56:1273–80. [PubMed: 22155830]

[3]. Grad YH, Kirkcaldy RD, Trees D, Dordel J, Harris SR, Goldstein E, et al. Genomic epidemiology of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime in the USA: a retrospective observational study. Lancet Infect Dis 2014;14:220–6. [PubMed: 24462211]

[4]. Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K, et al. Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrob Agents Chemother 2011;55:3538–45. [PubMed: 21576437]

[5]. CONITEC. Protocolo Clínico e Diretrizes Terapêuticas Infecções Sexualmente Transmissíveis, 2015 Brasília, DF, Brazil: Ministerio da Saude http://conitec.gov.br/images/Relatorios/2015/Relatorio_PCDT-IST_final.pdf. [Accessed 10 June 2016].

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Table 1

Characteristics of seven *Neisseria gonorrhoeae* isolates assigned to ST1901 by multilocus sequence typing (MLST) with reduced susceptibility to cefixime from Rio de Janeiro, Brazil.

Year	Specimen type	Sex	Age (years)	MIC (mg/L)[a]						Resistance profile[b]
				CEF	CRO	PEN	TET	CIP	AZM	
2010	Vaginal	F	32	0.25	0.03	2	2	8	0.5	CMRP/CMRT/CRNG
2011	Urine	M	Unknown	0.25	0.06	4	4	16	0.25	CMRP/CMRT/CRNG
2013	Urine	M	24	0.25	0.06	4	4	8	0.38	CMRP/CMRT/CRNG
2013	Urethral	M	54	0.25	0.06	8	2	16	1.5	PPNG/TRNG/CRNG
2013	Cervix	F	58	0.25	0.06	4	4	16	16	CMRP/CMRT/CRNG/AZRNG
2014	Vaginal	F	59	0.25	0.06	4	4	16	8	CMRP/CMRT/CRNG/AZRNG
2015	Urethral	M	49	0.25	0.06	2	2	8	0.38	CMRP/CMRT/CRNG

MIC, minimum inhibitory concentration; CEF, cefixime; CRO, ceftriaxone; PEN, penicillin; TET, tetracycline; CIP, ciprofloxacin; AZM, azithromycin; CLSI, Clinical and Laboratory Standard Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CDC, US Centers for Disease Control and Prevention.

[a]Resistance breakpoints according to the CLSI/EUCAST are as follows: CEF, >0.25 */>0.125; CRO, >0.25 */>0.125; PEN, ≥2/≥1; TET, ≥2/≥1; CIP, ≥1/≥0.5; and AZM, >0.5 **. * The CLSI does not determine resistance breakpoint for CEF and CRO; it considers <0.25 mg/L as susceptible. However, the CDC Gonococcal Isolate Surveillance Project defines MIC ≥ 0.25 mg/L and MIC ≥ 0.125 mg/L as alert values for CEF and CRO resistance, respectively. ** The CLSI does not determine breakpoints for AZM. However, in the CDC Gonococcal Isolate Surveillance Project, an MIC ≥ 2 mg/L is defined as an alert value for resistance.

[b]CMRP, chromosomally mediated resistance to penicillin; CMRT, chromosomally mediated resistance to tetracycline; CRNG, ciprofloxacin-resistant *N. gonorrhoeae*; PPNG, penicillinase-producing *N. gonorrhoeae*; TRNG, plasmid-mediated tetracycline resistance; AZRNG, azithromycin-resistant *N. gonorrhoeae*.