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First nationwide antimicrobial susceptibility surveillance for *Neisseria gonorrhoeae* in Brazil, 2015–16

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Objectives: Gonorrhoea and antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* are major public health concerns globally. Enhanced AMR surveillance for gonococci is essential worldwide; however, recent quality-assured gonococcal AMR surveillance in Latin America, including Brazil, has been limited. Our aims were to (i) establish the first nationwide gonococcal AMR surveillance, quality assured according to WHO standards, in Brazil, and (ii) describe the antimicrobial susceptibility of clinical gonococcal isolates collected from 2015 to 2016 in all five main regions (seven sentinel sites) of Brazil.

Methods: Gonococcal isolates from 550 men with urethral discharge were examined for susceptibility to ceftriaxone, cefixime, azithromycin, ciprofloxacin, benzylpenicillin and tetracycline using the agar dilution method, according to CLSI recommendations and quality assured according to WHO standards.

Results: The levels of resistance (intermediate susceptibility) to tetracycline, ciprofloxacin, benzylpenicillin and azithromycin were 61.6% (34.2%), 55.6% (0.5%), 37.1% (60.4%) and 6.9% (8.9%), respectively. All isolates were susceptible to ceftriaxone and cefixime using the US CLSI breakpoints. However, according to the European EUCAST cefixime breakpoints, 0.2% ($n = 1$) of isolates were cefixime resistant and 6.9% ($n = 38$) of isolates had a cefixime MIC bordering on resistance.

Conclusions: This study describes the first national surveillance of gonococcal AMR in Brazil, which was quality assured according to WHO standards. The high resistance to ciprofloxacin (which promptly informed a revision of the Brazilian sexually transmitted infection treatment guideline), emerging resistance to azithromycin and decreasing susceptibility to extended-spectrum cephalosporins necessitate continuous surveillance of gonococcal AMR and ideally treatment failures, and increased awareness when prescribing treatment in Brazil.

Introduction

Gonorrhoea is the second most prevalent bacterial sexually transmitted infection (STI) globally and, in 2012, the WHO estimated 78 million new cases globally of gonorrhoea in adults (16–49 years of age). Of these cases, 11 million were estimated in the WHO Region of the Americas.[1] The number of reported gonorrhoea cases is much lower owing to the lack of appropriate testing, diagnostics or reporting in many regions internationally.[2,3] Untreated gonorrhoea can result in severe complications, e.g. pelvic inflammatory disease, ectopic pregnancy and infertility. Public health control of gonorrhoea completely relies on effective antimicrobial treatment, i.e. in combination with prevention efforts, adequate diagnostics, partner notification and epidemiological surveillance.[2–4]

Despite the fact that *Neisseria gonorrhoeae* initially was highly susceptible to many antimicrobials, it has shown a remarkable ability to acquire or develop antimicrobial resistance (AMR) to all drugs that have been used for gonorrhoea treatment over the last 70–80 years, e.g. sulphonamides, penicillins, 'first-generation' cephalosporins, tetracyclines, 'early generation' macrolides and fluoroquinolones.[2–6] The emergence of *in vitro* resistance and

clinical resistance, resulting in treatment failures, to the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone, the last remaining options for empirical monotherapy of gonorrhoea, has caused major concerns globally.[2–15] Consequently, the WHO published a global action plan to control the spread and impact of AMR in *N. gonorrhoeae*[7] and similar regional or national response plans were subsequently developed by the ECDC[8] and US CDC,[15] respectively. One of the key actions in these action/response plans was to enhance the quality-assured AMR surveillance nationally and internationally, to monitor AMR trends, identify emerging AMR and inform STI management guidelines.[7,8,15]

The gonococcal AMR surveillance in several regions globally, including Latin America and the Caribbean, has remained sporadic, limited or even absent in many countries, as well as having lacked a sufficient number of isolates, representativeness, epidemiological data and timely reporting of AMR data for currently recommended therapeutic antimicrobials. The reasons for this include lack of aetiological diagnostics and culture of gonococci owing to widely implemented syndromic management, lack of sustainable programmes for monitoring AMR in gonococci and lack of sufficient funding.[4,16–18] In Brazil, syndromic management of STIs has been widely implemented since it was initiated in 1993. For example, the 2015 Brazilian STI treatment guideline[19] recommends syndromic treatment for all men presenting with urethral discharge, when no laboratory diagnostic results are available. The recommended treatment when suspecting gonorrhoea is (i) at the national level, ciprofloxacin 500 mg single oral dose plus azithromycin 1 g single oral dose, or (ii) in São Paulo, Minas Gerais and Rio de Janeiro (where limited ciprofloxacin resistance data have been produced[20–22]), ceftriaxone 500 mg single dose intramuscularly plus azithromycin 1 g single oral dose.[19] The wide implementation of syndromic management has resulted in a lack of appropriate incidence figures for gonorrhoea and a lack of laboratory diagnostics, including culture, of gonococci in many settings. Accordingly, internationally published recent AMR data for gonococcal strains spreading in Brazil have been exceedingly limited and mainly generated in minor studies performed at a few Brazilian sites.[20–22] A national gonococcal AMR surveillance programme has remained lacking, despite three attempts to establish national laboratory networks with a focus on gonococcal AMR having been conducted since 1996.[23]

The aims of the present study were to (i) establish the first nationwide gonococcal AMR surveillance programme, quality assured according to WHO standards and quality controls, in Brazil, and (ii) describe the antimicrobial susceptibility of clinical *N. gonorrhoeae* isolates collected from 2015 to 2016 in seven sentinel surveillance sites representing all the five main regions of Brazil.

Materials and methods

Surveillance programme, sentinel sites and sampling

This first nationwide cross-sectional surveillance to monitor the AMR of clinical gonococcal isolates in Brazil was initiated and co-ordinated by the Department of STIs, AIDS and Viral Hepatitis at the Brazilian Ministry of Health, in collaboration with the Molecular Biology, Microbiology and Serology Laboratory (LBMMS) at the Federal University of Santa Catarina, Florianópolis, Brazil. Seven sentinel sites that appropriately represent all the five main geographic regions of Brazil were, after site visits and standardization, selected for sample collection. The Brazilian regions (sentinel sites) were: North (Alfredo da Mata Tropical Dermatology and Venereology Foundation, Manaus, Amazonas); Northeast (Specialized State Center in Diagnosis, Care and Research, Salvador, Bahia); Center-West (Asa Sul Polyclinic, Brasilia, Distrito Federal); Southeast (STI/AIDS Reference and Training Center, São Paulo, São Paulo and Belo Horizonte Municipal Health Secretariat, Belo Horizonte, Minas Gerais); and South (Clinical Analysis Department, University Hospital, Federal University of Santa Catarina, the São José Municipal Health Department, Florianópolis, Santa Catarina and Sanitary Dermatology Outpatient Clinic, Porto Alegre, Rio Grande do Sul).

Men aged ≥18 years with urethral discharge were, after informed consent, included in the study from October 2015 to December 2016. Individuals that had not had their sexual debut, partners of index cases, victims of suspected sexual abuse, men with complicated urogenital infection and men using systemic antimicrobials <1 week before sample collection were excluded. All men were treated for male urethral discharge according to the 2015 Brazilian STI treatment guideline;[19] unfortunately, no complete and/or reliable clinical and epidemiological data for a sufficient number of the patients were available for the present study.

Urethral discharge specimens were sampled using urethral swabs and placed in Amies transport medium (Copan, Brescia, Italy). Within ∼6 h the samples were inoculated on Thayer–Martin medium and Chocolate Agar (Laborclin, Pinhais, Brazil) and incubated at 35±1°C in a humid 5% CO_2-enriched atmosphere for 24 h. If no growth was observed after 24 h, the agar plates were reincubated for an additional 24 h. Suspected *N. gonorrhoeae* colonies were preserved in BHI broth supplemented with 20% glycerol at −80°C prior to shipment to the reference laboratory LBMMS. At LBMMS, species verification was performed based on characteristic colony morphology, Gram-stained microscopy, oxidase and catalase tests, and VITEK 2 (bioMérieux, Marcy-l'Étoile, France) and VITEK mass spectrometry MALDI-TOF (bioMérieux), according to the manufacturer's instructions.

Antimicrobial susceptibility testing

The MICs of ceftriaxone, cefixime, azithromycin, ciprofloxacin, benzylpenicillin and tetracycline were determined by the agar dilution method, in accordance with recommendations from the US CLSI,[24] on Difco GC medium agar base (Becton, Dickinson, and Company, Sparks, MD, USA) supplemented with 1% Vitox (Oxoid Ltd., Basingstoke, UK). For confirmation of high MICs of ceftriaxone, cefixime and azithromycin, the Etest (bioMérieux) was used in accordance with the manufacturer's instructions. The MICs were interpreted as susceptible, intermediate susceptible and resistant using the clinical SIR breakpoints stated by the CLSI.[24] For azithromycin, for which CLSI does not state any interpretative criteria, both the clinical breakpoints recommended by the EUCAST (www.eucast.org) and the epidemiological cut-off value defined by CLSI[24] were applied. The clinical breakpoints (susceptible, resistant) were as follows: ceftriaxone and cefixime (MIC ≤0.25 mg/L, not stated), azithromycin (MIC ≤0.25 mg/L, ≥1.0 mg/L), ciprofloxacin (MIC ≤0.064 mg/L, ≥1.0 mg/L), benzylpenicillin (MIC ≤0.064 mg/L, ≥2.0 mg/L) and tetracycline (MIC ≤0.25 mg/L, ≥2.0 mg/L). For quality controls, the CLSI gonococcal reference strain ATCC 49226 and three of the eight 2008 WHO gonococcal reference strains (WHO F, G, K, L, M, N, O and P were alternated in batches of three)[25,26] were used in each MIC determination. Essential agreement (±1 MIC $\log_2$ dilution) between the measured MICs and reference MIC values[24,26] for the quality control strains was required in the testing. β-Lactamase production was identified with nitrocefin discs (Becton Dickinson, Le Pont de Claix, France). For the final quality assurance of the MIC determination, a representative selection (every 15th isolate) of the Brazilian gonococcal isolates was retested at the WHO Collaborating Centre for Gonorrhoea and other STIs, Sweden. The essential agreement (±1 MIC $\log_2$ dilution) for each antimicrobial between the originally produced MICs and MICs in retesting was >95%, which meant the antimicrobial susceptibility testing performed in Brazil was quality assured.

Table 1. Antimicrobial susceptibility of clinical *N. gonorrhoeae* isolates ($n = 550$) cultured across Brazil from October 2015 to December 2016

	Breakpoints (mg/L)[a]			MIC (mg/L)			Percentage of isolates (%)		
Antimicrobial	S	I	R	MIC_{50}	MIC_{90}	Range	S	I	R
Tetracycline	≤0.25	0.5–1	≥2	2.0	32.0	0.125–>32	4.2	34.2	61.6
Ciprofloxacin	≤0.06	0.12–0.5	≥1	0.016	8.0	0.001–32	43.8	0.5	55.6
Benzylpenicillin	≤0.06	0.12–1	≥2	0.5	8.0	0.016–128	2.5	60.4	37.1
Azithromycin	≤0.25	0.5	≥1	0.06	0.5	0.03–8	84.2	8.9[b]	6.9[b]
Ceftriaxone	≤0.25			0.008	0.016	0.0005–0.125	100	NA	NA
Cefixime	≤0.25			0.008	0.06	0.0005–0.250	100	NA	NA

S, susceptible; I, intermediate susceptible; R, resistant; NA, not applicable; MIC_{50}, MIC of an antimicrobial inhibiting 50% of isolates; MIC_{90}, MIC of an antimicrobial inhibiting 90% of isolates.
[a]Agar dilution method and SIR breakpoints from the US CLSI were used.[24]
[b]Using the US CLSI ecological cut-off value for azithromycin,[24] 1.3% of isolates were considered non-wild-type.

Ethics approval

The study was approved by the Committee of Ethics in Research with Human Beings (CEPSH), Brazil; assent number 1.187.476 and CAAE 44891815.7.0000.0121.

Results

Neisseria gonorrhoeae isolates

In total, 584 suspected gonococcal isolates were sent to the reference laboratory LBMMS. Of these, 34 (5.8%) were not viable, were contaminated or were not identified as gonococci. Accordingly, 550 gonococcal isolates were included: 18.2% ($n = 100$) were from the North Region (Manaus); 18.9% ($n = 104$) from the Northeast Region (Salvador); 12.4% ($n = 68$) from the Center-West Region (Brasilia); 23.8% ($n = 131$) from the Southeast Region (103 from Belo Horizonte and 28 from São Paulo); and 26.7% ($n = 147$) from the South Region (74 from Florianópolis and 73 from Porto Alegre).

Antimicrobial susceptibility in N. gonorrhoeae isolates from Brazil, 2015–16

The antimicrobial susceptibility of all isolates ($n = 550$) is summarized in Table 1. Briefly, the rates of resistance (intermediate susceptibility) to tetracycline, ciprofloxacin and benzylpenicillin were 61.6% (34.2%), 55.6% (0.5%) and 37.1% (60.4%), respectively. The level of resistance and intermediate susceptibility to azithromycin was 6.9% and 8.9%, respectively (MIC range: 0.03–8 mg/L). Using the CLSI epidemiological cut-off value for azithromycin,[24] 1.3% of isolates were considered non-wild-type. All isolates were susceptible to ceftriaxone (MIC range: ≤0.002–0.125 mg/L) and cefixime (MIC range: ≤0.002–0.25 mg/L) using CLSI breakpoints[24] (Table 1). However, one isolate (from Brasilia) had a cefixime MIC of 0.25 mg/L and ceftriaxone MIC of 0.125 mg/L (confirmed by Etest). According to the European cefixime resistance breakpoint (MIC >0.125 mg/L; www.eucast.org), this isolate was cefixime resistant. Furthermore, 6.9% ($n = 38$) of all isolates had a cefixime MIC of 0.125 mg/L, which is bordering on resistance according to the European cefixime resistance breakpoint, and 11 (28.9%) of these 38 isolates were also resistant to azithromycin and ciprofloxacin. Two (0.4%) isolates also displayed a ceftriaxone MIC of 0.125 mg/L, which is bordering on resistance according to the European ceftriaxone resistance breakpoint (MIC >0.125 mg/L; www.eucast.org). The MIC distributions of ceftriaxone, cefixime, azithromycin and ciprofloxacin are presented in Figure 1. β-Lactamase was produced by 29.1% ($n = 160$) of isolates, and of these 160 isolates 73.1% ($n = 117$) showed high-level resistance to benzylpenicillin (MIC ≥8.0 mg/L).

Regarding resistance to multiple antimicrobials, 5.4% ($n = 30$) of isolates were resistant to both azithromycin and ciprofloxacin, and in total 3.3% ($n = 18$) of isolates were resistant to all examined antimicrobials except the ESCs, i.e. azithromycin, ciprofloxacin, benzylpenicillin and tetracycline.

Antimicrobial susceptibility in N. gonorrhoeae isolates cultured in the North, Northeast, Center-West, Southeast and South Regions of Brazil

The antimicrobial susceptibilities of gonococcal isolates cultured in the five main geographic regions of Brazil are summarized in Table 2. Briefly, high rates of resistance to tetracycline and benzylpenicillin were found in all five regions; ranging from 43.5% in the South Region to 80.0% in the North Region for tetracycline and from 23.1% in the South Region to 49.0% in the Northeast Region for benzylpenicillin. High resistance rates were also observed in all the regions for ciprofloxacin (77.9% in Center-West, 65.6% in Southeast, 55.8% in Northeast, 47.0% in North and 42.2% in South). The rates of resistance to azithromycin were lower, however, except for the South Region (4.1% resistance); all the other regions had resistance rates of ≥5% (9.9% in Southeast, 9.6% in Northeast, 5.9% in Center-West and 5.0% in North) (Table 2). Isolates with cefixime MICs bordering on resistance (MIC = 0.125 mg/L), according to the European cefixime resistance breakpoint (www.eucast.org), were found in all five regions (and all seven sentinel cities), ranging from 3.1% in the Southeast Region to 9% in the North Region (from 1.9% in Belo Horizonte to 10.8% in Florianópolis).

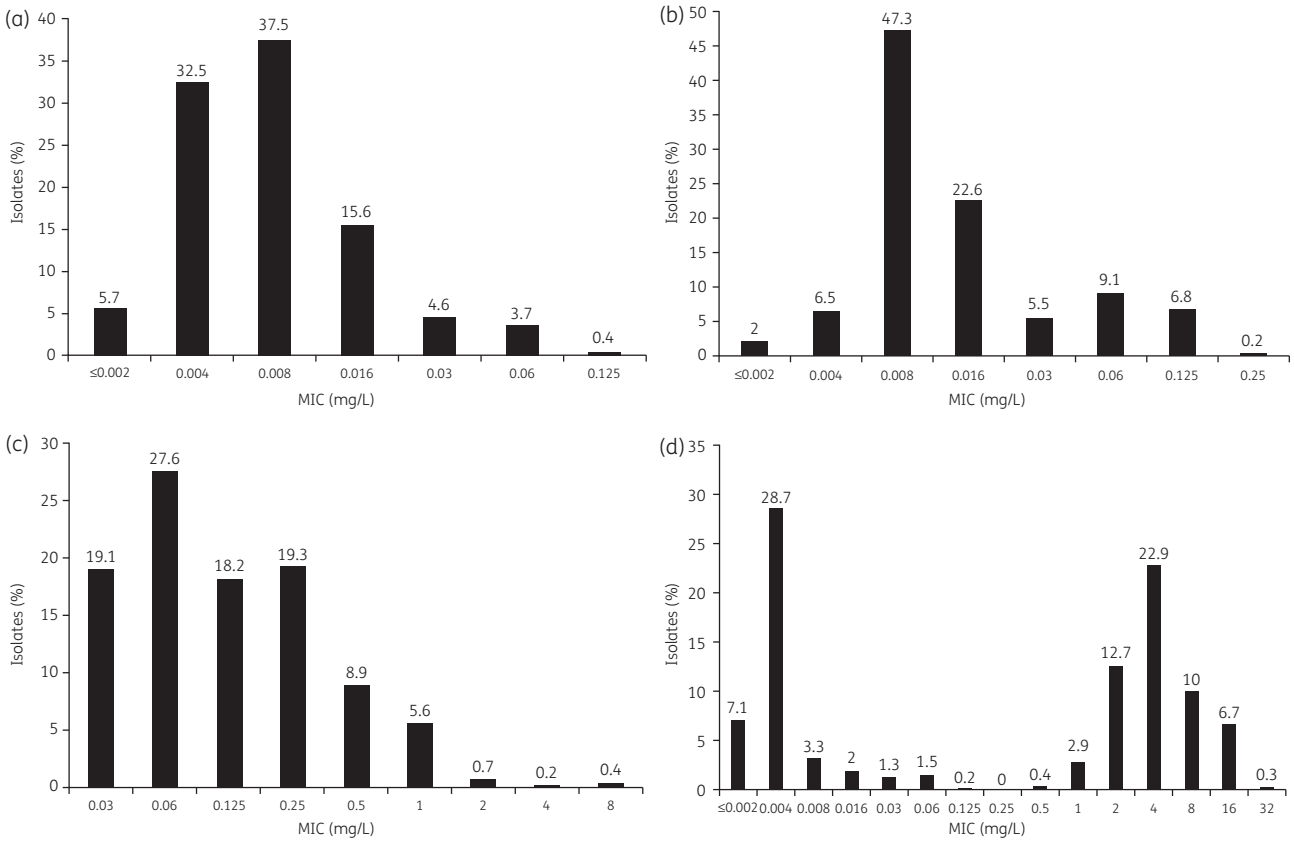

Figure 1. MIC distributions for ceftriaxone (a), cefixime (b), azithromycin (c) and ciprofloxacin (d) for clinical *N. gonorrhoeae* isolates ($n = 550$) cultured across Brazil from October 2015 to December 2016.

Discussion

This article describes the first national surveillance of AMR in *N. gonorrhoeae* in Brazil, which was quality assured according to WHO standards and quality controls.[7,25–27] Sentinel sites ($n = 7$) in the five main geographic regions of Brazil were established and standardized (sample collection, gonococcal culture and preservation of gonococcal strains) to obtain samples with national representativeness, and a reference laboratory, performing appropriate species verification and centralized AMR testing (agar dilution method) in accordance with CLSI and WHO recommendations,[4,7,24–27] was designated and quality assured. The AMR in clinical gonococcal isolates ($n = 550$) cultured from 2015 to 2016 in the five main regions of Brazil was examined.

Briefly, high resistance levels to tetracycline (61.6%; from 43.5% to 80.0% in the different regions) and benzylpenicillin (37.1%; from 23.1% to 49.0%) were found across Brazil. Despite the fact that the levels of resistance to tetracycline and benzylpenicillin were higher in the present national surveillance, these results are mainly in concordance with the internationally published data from the few recent minor regional Brazilian studies,[20–22] and other studies from Latin America and globally.[2–7,9,16–18] Some studies in Latin America have indicated a decrease in resistance (mainly in plasmid-mediated resistance) to benzylpenicillin and tetracycline over the years.[16,17] However, the present study shows that the levels of resistance to benzylpenicillin (37.1%), including chromosomal and plasmid-mediated (29.1% of isolates were β-lactamase producing), and tetracycline (61.6%) have remained very high in Brazil, which is in broad agreement with AMR data worldwide.[2–4,6,7,9] In addition, the level of resistance to ciprofloxacin (55.6%; from 42.2% to 77.9% in the different regions) was very high in Brazil. This is a significantly higher resistance level than the resistance levels of 2.4% reported for isolates from Manaus, Amazonas in 2009,[28] and 21.4% for isolates in Belo Horizonte, Minas Gerais in 2011–12.[20] Accordingly, resistance to ciprofloxacin appears to have rapidly increased across Brazil and is now in line with the ciprofloxacin resistance levels reported in, e.g. many countries in North America, Europe and Asia.[4,29–32] As empirical therapy, in the absence of any AMR data, is most frequently administered for gonorrhoea, the WHO recommends that gonorrhoea treatment should be ≥95% effective and, accordingly, <5% of gonococcal isolates should be resistant to the antimicrobial used for first-line empirical treatment.[4,7,27] The present study provides strong evidence that benzylpenicillin, tetracycline and ciprofloxacin should definitely not be used in the syndromic treatment of urethral discharge and vaginal discharge when gonorrhoea is suspected, or in the empirical treatment of aetiologically diagnosed gonorrhoea in any regions of Brazil. Thus, the results of the present study have informed a revision of the 2015 Brazilian STI treatment guideline,[19] in which ciprofloxacin 500 mg single oral dose plus azithromycin 1 g single oral dose will be discontinued and only ceftriaxone 500 mg single dose intramuscularly plus

Table 2. Antimicrobial susceptibility[a] of clinical *N. gonorrhoeae* isolates ($n = 550$) from the five main geographic regions of Brazil, 2015–16

	Region											
	North		Northeast		Center-West		Southeast		South		Total	
Antimicrobial	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Tetracycline												
susceptible	3	3.0	1	1.0	5	7.4	4	3.1	10	6.8	23	4.2
intermediate	17	17.0	25	24.0	27	39.7	46	35.1	73	49.7	188	34.2
resistant	80	80.0	78	75.0	36	52.9	81	61.8	64	43.5	339	61.6
Ciprofloxacin												
susceptible	53	53.0	46	44.2	14	20.6	44	33.6	84	57.1	241	43.8
intermediate	0	0	0	0	1	1.5	1	0.8	1	0.7	3	0.5
resistant	47	47.0	58	55.8	53	77.9	86	65.6	62	42.2	306	55.6
Benzylpenicillin												
susceptible	5	5.0	0	0	3	4.4	1	0.8	5	3.4	14	2.5
intermediate	62	62.0	53	51.0	37	54.4	72	55.0	108	73.5	332	60.4
resistant	33	33.0	51	49.0	28	41.2	58	44.3	34	23.1	204	37.1
Azithromycin												
susceptible	90	90.0	84	80.8	54	79.4	109	83.2	126	85.7	463	84.2
intermediate	5	5.0	10	9.6	10	14.7	9	6.9	15	10.2	49	8.9
resistant	5	5.0	10	9.6	4	5.9	13	9.9	6	4.1	38	6.9
Ceftriaxone												
susceptible	100	100.0	104	100.0	68	100.0	131	100.0	147	100.0	550	100.0
reduced susceptible	0	0	0	0	0	0	0	0	0	0	0	0
Cefixime												
susceptible	100	100.0	104	100.0	68	100.0	131	100.0	147	100.0	550	100.0
reduced susceptible	0	0	0	0	0	0	0	0	0	0	0	0

S, susceptible; I, intermediate susceptible; R, resistant.

[a]Agar dilution method and SIR breakpoints, with the exception of azithromycin, from the US CLSI were used.[24] For azithromycin, the SIR breakpoints recommended by the EUCAST (www.eucast.org) were applied. Breakpoints (S, R) were as follows: ceftriaxone and cefixime (≤0.25 mg/L, not stated); azithromycin (≤0.25 mg/L, ≥1.0 mg/L); ciprofloxacin (≤0.064 mg/L, ≥1.0 mg/L); benzylpenicillin (≤0.064 mg/L, ≥2.0 mg/L); and tetracycline (≤0.25 mg/L, ≥2.0 mg/L).

azithromycin 1 g single oral dose will be recommended in the future in all regions of Brazil.[33] This national recommendation of dual antimicrobial first-line therapy with ceftriaxone plus azithromycin is in concordance with treatment guidelines in the USA,[34] Canada,[35] Europe[36] and Australia[37] and by the WHO in 2016.[38] Nevertheless, some countries have continued to recommend ceftriaxone monotherapy.[9,39]

For azithromycin, which is included in the dual antimicrobial therapy recommended in Brazil and internationally,[19,34–38] the resistance (intermediate susceptibility) in Brazil was 6.9% (8.9%) at national level. With the exception of the South Region (4.1%), the azithromycin resistance was ≥5% in all the other regions (5.0%–9.9%). However, 82% of the azithromycin-resistant isolates had an MIC of 1 mg/L, i.e. just above the resistance breakpoint (MIC >0.5 mg/L), and the highest azithromycin MIC in any isolate was 8 mg/L ($n = 2$). A lower level of resistance to azithromycin (4.5%) was reported in a recent Brazilian study from Belo Horizonte, Minas Gerais.[20] It is imperative to monitor closely the resistance to azithromycin both in Brazil and countries in Latin America (where limited azithromycin susceptibility surveillance has been performed[4]) and globally, where the resistance also has increased in recent years.[4,29,30,40,41] Nevertheless, with the exception of the sustained transmission in England,[42,43] mainly sporadic isolates with high-level resistance to azithromycin (MIC ≥256 mg/L) have been identified in countries worldwide,[2,3,9,41,44,45] including one isolate in Argentina.[46]

The emergence of *in vitro* and clinical resistance, including treatment failures, to the ESCs cefixime and ceftriaxone, the last options for empirical monotherapy of gonorrhoea, has caused major concerns globally.[2–15,30,34–38] In the present study, according to the US CLSI resistance breakpoints,[24] all Brazilian isolates were susceptible (MIC ≤0.25 mg/L) to both cefixime and ceftriaxone, which is included in the dual antimicrobial therapy recommended in Brazil and internationally.[19,34–38] However, according to the European cefixime resistance breakpoint (MIC >0.125 mg/L; www.eucast.org) one (0.2%) isolate from Brasilia (Center-West Region) was resistant to cefixime and 38 (6.9%) additional isolates had a cefixime MIC of 0.125 mg/L, which is bordering on resistance according to the European resistance breakpoint, with concomitant resistance to both azithromycin and ciprofloxacin in 28.9% ($n = 11$) of isolates. Cefixime is not available in Brazil. Accordingly, the decreasing cefixime susceptibility is likely due to the importation of gonococcal strains with higher cefixime MICs from other countries that have managed to establish domestic spread, but an

intra-country emergence and/or selection of decreased susceptibility to cefixime by the use of other cephalosporins in Brazil cannot be excluded. Nevertheless, because cefixime and other less potent oral ESCs have never been widely used for the treatment of gonorrhoea in Brazil, relatively low selection pressure for emergence and spread of ESC resistance in the country is likely. Additionally, two (0.4%) isolates displayed a ceftriaxone MIC of 0.125 mg/L, which is bordering on resistance according to the European ceftriaxone resistance breakpoint (MIC >0.125 mg/L; www.eucast.org). To date, no resistance to ceftriaxone or cefixime in Brazil has been internationally or nationally published,[20–22,28] but a limited number of isolates from only a few states in Brazil has been tested prior to the present national study. In Latin America, isolates with decreased susceptibility or resistance to ESCs have been sporadically reported in countries neighbouring Brazil, e.g. Argentina, Bolivia (only disc diffusion results that have not been verified by MIC determination) and Uruguay, but also in Chile and Cuba.[4,12,16,17,47] In Latin America, it is essential to enhance substantially the quality-assured surveillance of gonococcal AMR in general and particularly to azithromycin and ESCs, for which the surveillance has been highly limited.

In general, an overuse and misuse of antimicrobials, caused by self-medication, unrestricted access (over-the-counter selling without prescription), inappropriate selection, suboptimal quality and dosing, are likely to be important contributors to AMR emergence in many bacterial species, including gonococci, in Latin America and the Caribbean, as well as globally.[3,7] Since 2011, antimicrobials can only be sold by prescription in Brazil. This is an important first step to ensure the prudent use of antimicrobials and mitigate emergence of resistance in gonococci. However, a quality assured, representative and continuous gonococcal AMR surveillance system, ideally also surveying treatment failures using recommended therapies at least at some sentinel sites, is also essential to monitor the resistance trends, identify emerging resistance and inform regular updates of the Brazilian STI treatment guideline. The established Brazilian Gonococcal Antimicrobial Surveillance Programme (GASP), described in the present study, might help to catalyse enhanced and sustained national and regional GASP networks, which are important for Latin America, where longitudinal, quality assured AMR data outside Argentina and Chile have been limited.[16–18] Owing to the wide implementation of syndromic management of STIs in Latin America and elsewhere, skills for sample collection, sample transportation and gonococcal culturing (including preservation of gonococcal strains) have been lost among many healthcare providers and laboratory staff, and additional and continual training is imperative. In Brazil, initiatives to further improve GASP, e.g. continued training of healthcare providers and laboratory staff, increasing the number of examined gonococcal isolates, representativeness (geographic and for different risk groups and anatomical sites, i.e. including isolates also from females and from extra-genital sites, e.g. the pharynx and rectum), collection and linkage to improved clinical and epidemiological data, survey of treatment failures, updating to the 2016 WHO gonococcal reference strains[26] for quality control and molecular epidemiological examinations using whole-genome sequencing are considered for future surveillance.

In conclusion, the present study describes the first national surveillance of AMR in *N. gonorrhoeae* in Brazil, which was quality assured according to WHO standards and quality controls.[7,25–27] The high resistance to ciprofloxacin identified has directly informed a revision of the 2015 Brazilian STI treatment guideline,[19] in which ciprofloxacin is excluded in the syndromic treatment and for aetiologically diagnosed gonorrhoea.[33] In particular, the high resistance to ciprofloxacin, emerging resistance to azithromycin and decreasing susceptibility to ESCs necessitate continuous surveillance of gonococcal AMR, and ideally treatment failures, as well as increased awareness and care when administering treatment for gonorrhoea in Brazil. In addition, sustained national and international support, including political and financial commitment, is essential to establish new GASPs and/or strengthen the existing GASPs, in Brazil and other countries globally. Ultimately, novel antimicrobial agents, with new mechanisms of action and, ideally, a vaccine for the sustainable effective management of gonorrhoea is crucial.[4,48–50]

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Transparency declarations

None to declare.

References

1 Newman L, Rowley J, Vander Hoorn S *et al*. Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting. *PLoS One* 2015; **10**: e0143304.

2 Unemo M, Del Rio C, Shafer WM. Antimicrobial resistance expressed by *Neisseria gonorrhoeae*: a major global public health problem in the 21st century. *Microbiol Spectr* 2016; **4**: doi:10.1128/microbiolspec.EI10-0009-2015.

3 Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 2014; **27**: 587–613.

4 Wi T, Lahra MM, Ndowa F *et al*. Antimicrobial resistance in *Neisseria gonorrhoeae*: global surveillance and a call for international collaborative action. *PLoS Med* 2017; **14**: e1002344.

5 Bolan GA, Sparling PF, Wasserheit JN. The emerging threat of untreatable gonococcal infection. *N Engl J Med* 2012; **366**: 485–7.

6 Lewis DA. Global resistance of *Neisseria gonorrhoeae*. When theory becomes reality. *Curr Opin Infect Dis* 2014; **27**: 62–7.

7 World Health Organization (WHO), Department of Reproductive Health and Research. *Global Action Plan to Control the Spread and Impact Of Antimicrobial Resistance in Neisseria gonorrhoeae*. 2012. http://www.who.int/reproductivehealth/publications/rtis/9789241503501.

8 European Centre for Disease Prevention and Control. *Response Plan to Control and Manage the Threat of Multidrug-Resistant Gonorrhoea in Europe*. 2012. http://www.ecdc.europa.eu/en/publications/Publications/1206-ECDC-MDR-gonorrhoea-response-plan.pdf.

9 Unemo M. Current and future antimicrobial treatment of gonorrhoea—the rapidly evolving *Neisseria gonorrhoeae* continues to challenge. *BMC Infect Dis* 2015; **15**: 364.

10 Ohnishi M, Golparian D, Shimuta 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.

11 Unemo M, Golparian D, Nicholas R *et al*. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel *penA* mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012; **56**: 1273–80.

12 Gianecini R, Oviedo C, Stafforini G *et al*. *Neisseria gonorrhoeae* resistant to ceftriaxone and cefixime, Argentina. *Emerg Infect Dis* 2016; **22**: 1139–41.

13 Lahra MM, Ryder N, Whiley DM. A new multidrug-resistant strain of *Neisseria gonorrhoeae* in Australia. *N Engl J Med* 2014; **371**: 1850–1.

14 Allen VG, Mitterni L, Seah C *et al*. *Neisseria gonorrhoeae* treatment failure and susceptibility to cefixime in Toronto, Canada. *JAMA* 2013; **309**: 163–70.

15 Centers for Disease Control and Prevention (CDC). *Cephalosporin-resistant Neisseria gonorrhoeae public health response plan*. 2012. https://www.cdc.gov/std/treatment/ceph-r-responseplanjuly30-2012.pdf.

16 Starnino S, Galarza P, Carvallo ME *et al*. Retrospective analysis of antimicrobial susceptibility trends (2000-2009) in *Neisseria gonorrhoeae* isolates from countries in Latin America and the Caribbean shows evolving resistance to ciprofloxacin, azithromycin and decreased susceptibility to ceftriaxone. *Sex Transm Dis* 2012; **39**: 813–21.

17 Dillon JA, Trecker MA, Thakur SD *et al*. Two decades of the gonococcal antimicrobial surveillance program in South America and the Caribbean: challenges and opportunities. *Sex Transm Infect* 2013; **89** Suppl 4: iv36–41.

18 Thakur SD, Araya P, Borthagaray G *et al*. Resistance to ceftriaxone and azithromycin in *Neisseria gonorrhoeae* isolates from 7 countries of South America and the Caribbean: 2010-2011. *Sex Transm Dis* 2017; **44**: 157–60.

19 Brasil Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de DST, Aids e Hepatites Virais. *Protocolo Clínico e Diretrizes Terapêuticas para Atenção Integral às Pessoas com Infecções Sexualmente Transmissíveis*. Brasília: Ministério da Saúde, 2015. In Portuguese.

20 Costa LMB, Pedroso ERP, Vieira Neto V *et al*. Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates from patients attending a public referral center for sexually transmitted diseases in Belo Horizonte, State of Minas Gerais, Brazil. *Rev Soc Bras Med Trop* 2013; **46**: 304–9.

21 Ferreira WA, Ferreira CM, Naveca FG *et al*. Molecular epidemiology of β-lactamase-producing *Neisseria gonorrhoeae* strains in Manaus, AM, Brazil. *Sex Transm Dis* 2013; **40**: 469–72.

22 Uehara AA, Amorin EL, Ferreira Mde F *et al*. Molecular characterization of quinolone-resistant *Neisseria gonorrhoeae* isolates from Brazil. *J Clin Microbiol* 2011; **49**: 4208–12.

23 Costa-Lourenço APRD, Barros Dos Santos KT, Moreira BM *et al*. Antimicrobial resistance in *Neisseria gonorrhoeae*: history, molecular mechanisms and epidemiological aspects of an emerging global threat. *Braz J Microbiol* 2017; **48**: 617–28.

24 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Eighth Informational Supplement M100-S28*. CLSI, Wayne, PA, USA, 2018.

25 Unemo M, Fasth O, Fredlund H *et al*. Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. *J Antimicrob Chemother* 2009; **63**: 1142–51.

26 Unemo M, Golparian D, Sánchez-Busó L *et al*. The novel 2016 WHO *Neisseria gonorrhoeae* reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. *J Antimicrob Chemother* 2016; **71**: 3096–108.

27 Unemo M, Ison CA. Gonorrhoea. In: M Unemo, R Ballard, C Ison *et al*., eds. *Laboratory Diagnosis of Sexually Transmitted Infections, Including Human Immunodeficiency Virus*. Geneva: World Health Organization, 2013; 21–54. http://apps.who.int/iris/bitstream/10665/85343/1/9789241505840_eng.pdf.

28 Ferreira WA, Vasconcelos WS, Gomes JS *et al*. Susceptibility of *Neisseria gonorrhoeae* to gentamicin, chloramphenicol and other antibiotics in Manaus, Amazon, Brazil. *J bras Doenças Sex Transm* 2015; **27**: 3. In Portuguese.

29 Kirkcaldy RD, Harvey A, Papp JR *et al*. *Neisseria gonorrhoeae* antimicrobial susceptibility surveillance—the Gonococcal Isolate Surveillance Project, 27 sites, United States, 2014. *MMWR Surveill Summ* 2016; **65**: 1–19.

30 Cole MJ, Spiteri G, Jacobsson S *et al*. Overall low extended-spectrum cephalosporin resistance but high azithromycin resistance in *Neisseria gonorrhoeae* in 24 European countries, 2015. *BMC Infect Dis* 2017; **17**: 617.

31 Bala M, Kakran M, Singh V *et al*. Monitoring antimicrobial resistance in *Neisseria gonorrhoeae* in selected countries of the WHO South-East Asia Region between 2009 and 2012: a retrospective analysis. *Sex Transm Infect* 2013; **89** Suppl 4: iv28–35.

32 Lahra MM, Lo YR, Whiley DM. Gonococcal antimicrobial resistance in the Western Pacific Region. *Sex Transm Infect* 2013; **89** Suppl 4: iv19–23.

33 Depto Vigilância, Prevenção E Controle Das Infecções Sexualmente Transmissíveis Do HIV/Aidse Das Srtvn 701 Bloco D—Bairro Asa Norte, Brasília/DF, CEP 70719040 Site. NOTA INFORMATIVA N◦ 6-SEI/2017-COVIG/CGVP/.DIAHV/SVS/MS. In Portuguese.

34 Workowski KA, Bolan GA. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep* 2015; **64**: 1–137.

35 Public Health Agency of Canada. *Canadian Guidelines on Sexually Transmitted Infections. Gonococcal Infections Chapter*. 2013. www.phac-aspc.gc.ca/std-mts/sti-its/cgsti-ldcits/assets/pdf/section-5-6-eng.pdf.

36 Bignell C, Unemo M. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. *Int J STD AIDS* 2013; **24**: 85–92.

37 Australasian Sexual Health Alliance (ASHA). *Australian STI Management Guidelines for Use in Primary Care*. www.sti.guidelines.org.au/sexually-transmissible-infections/gonorrhoea#management.

38 World Health Organization (WHO), Department of Reproductive Health and Research. WHO *Guidelines for the Treatment of Neisseria gonorrhoeae*. 2016. http://www.who.int/reproductivehealth/publications/rtis/gonorrhoea-treatment-guidelines/en/.

39 World Health Organization. *Gonococcal Antimicrobial Resistance in the Western Pacific Region*. Manila, 2017. http://iris.wpro.who.int/bitstream/handle/10665.1/13688/WPR-2017-DCD-001-eng.pdf.

40 Martin I, Sawatzky P, Liu G *et al*. Decline in decreased cephalosporin susceptibility and increase in azithromycin resistance in *Neisseria gonorrhoeae*, Canada. *Emerg Infect Dis* 2016; **22**: 65–7.

41 Jacobsson S, Golparian D, Cole M *et al*. WGS analysis and molecular resistance mechanisms of azithromycin-resistant (MIC>2 mg/L) *Neisseria gonorrhoeae* isolates in Europe from 2009 to 2014. *J Antimicrob Chemother* 2016; **71**: 3109–16.

42 Public Health England (PHE). *Surveillance of Antimicrobial Resistance in Neisseria gonorrhoeae in England and Wales Key Findings from the*

Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP). 2017. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/651636/GRASP_Report_2017.pdf.

43 Chisholm SA, Wilson J, Alexander S *et al*. An outbreak of high-level azithromycin resistant *Neisseria gonorrhoeae* in England. *Sex Transm Infect* 2016; **92**: 365–7.

44 Katz AR, Komeya AY, Soge OO *et al*. *Neisseria gonorrhoeae* with high-level resistance to azithromycin: case report of the first isolate identified in the United States. *Clin Infect Dis* 2012; **54**: 841–3.

45 Demczuk W, Martin I, Peterson S *et al*. Genomic epidemiology and molecular resistance mechanisms of azithromycin-resistant *Neisseria gonorrhoeae* in Canada from 1997 to 2014. *J Clin Microbiol* 2016; **54**: 1304–13.

46 Galarza PG, Alcalá B, Salcedo C *et al*. Emergence of high level azithromycin-resistant *Neisseria gonorrhoeae* strain isolated in Argentina. *Sex Transm Dis* 2009; **36**: 787–8.

47 Gianecini R, Romero MLM, Oviedo C *et al*. Emergence and spread of *Neisseria gonorrhoeae* isolates with decreased susceptibility to extended-spectrum cephalosporins in Argentina, 2009 to 2013. *Sex Transm Dis* 2017; **44**: 351–5.

48 Alirol E, Wi TE, Bala M *et al*. Multidrug-resistant gonorrhea: a research and development roadmap to discover new medicines. *PLoS Med* 2017; **14**: e1002366.

49 Unemo M, Bradshaw CS, Hocking JS *et al*. Sexually transmitted infections: challenges ahead. *Lancet Infect Dis* 2017; **17**: e235–79.

50 Unemo M, Jensen JS. Antimicrobial-resistant sexually transmitted infections: gonorrhoea and *Mycoplasma genitalium*. *Nat Rev Urol* 2017; **14**: 139–52.