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Spectinomycin, gentamicin, and routine disc diffusion testing: An alternative for the treatment and monitoring of multidrug-resistant *Neisseria gonorrhoeae*?

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ABSTRACT

Introduction: *Neisseria gonorrhoeae* is a major concern of public health due to its extraordinary capacity to develop and acquire resistance to different antimicrobials used to treat gonorrhoea. Limited treatment options and uncontrolled transmission have raised the need to assess the antimicrobial susceptibility profile of the isolates and to establish affordable alternatives for laboratory diagnosis.

Objectives: This study aimed to (i) determine the susceptibility profile of 336 clinical isolates of *N. gonorrhoeae* to ceftriaxone, azithromycin, ciprofloxacin, spectinomycin and gentamicin by the gold standard agar dilution method; (ii) assess the agreement among agar dilution and disc diffusion results for ciprofloxacin, azithromycin, ceftriaxone, spectinomycin and gentamicin.

Results: All isolates were susceptible to ceftriaxone and spectinomycin. The levels of resistance to azithromycin and ciprofloxacin were 3.9% and 35.1%, respectively. Intermediate susceptibility to gentamicin was observed in 19.4% of isolates. There was 100% agreement between methods for spectinomycin and ceftriaxone, 99.7% for ciprofloxacin, and 85.7% for azithromycin. For gentamicin, there was 86.3% agreement between agar dilution and disc diffusion, resulting in intermediate susceptible by one method and susceptible by the other method, defined as minor errors. The discordance among agar dilution and disc diffusion results is acceptable for ciprofloxacin, ceftriaxone and spectinomycin as per CLSI M23-Ed4.

Conclusions: Spectinomycin and gentamicin can be considered in some cases as options for the treatment of gonorrhoea in Brazil. Disc diffusion can be an alternative method in routine testing with comparable accuracy to agar dilution.

1. Introduction

The resistance of *N. gonorrhoeae* to antimicrobials is a threat to global public health and has been a concern for WHO since 1990, when the Gonococcal Antimicrobial Surveillance Program (GASP) was created. Different classes of antimicrobials used to treat gonorrhoea have lost their efficiency over the years because of the emergence of resistance (Unemo and Shafer, 2014; Global progress report on HIV, 2021). The increasing reports of isolates with reduced susceptibility or resistance to the current dual therapy for gonococcal infections (extended-spectrum cephalosporin and azithromycin) has increased the concern over treatment effectiveness and the lack of therapeutic options (Bignell and Unemo, 2013; Fifer et al., 2016; Gianecini et al., 2016; Unemo et al., 2019; Workowski and Bolan, 2015).

In this scenario, it is of major importance to not only develop new antimicrobials but also monitor the susceptibility of circulating isolates to old antimicrobials (Lagacé-Wiens et al., 2017; Sanchez-Buso et al., 2019; Unemo et al., 2016a; Tacconelli and Magrini, 2013). In 2015,

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WHO recommended the assessment of *N. gonorrhoeae* susceptibility to some antimicrobials, including spectinomycin and gentamicin (WHO, 2015).

Spectinomycin was the first-line treatment for gonorrhoea in the Netherlands and South Korea in the 1980s (Easman et al., 1984; Stolz et al., 1975; Boslego et al., 1987). However, with the increase of antimicrobial resistance, its use as first-line monotherapy was abandoned (Unemo, 2015). Currently, resistance to spectinomycin is a rare occurrence worldwide, and some countries use this antimicrobial in cases of therapeutic failure (Unemo and Shafer, 2014; WHO, 2016; CDC, 2015).

Gentamicin has been considered a promising option for the treatment of gonorrhoea, especially in dual therapy (Dowell and Kirkcaldy, 2012). This aminoglycoside has been used in some African countries since 1993. The susceptibility profile of *N. gonorrhoeae* isolates to this antimicrobial has not changed considerably during this time. It is the first-line treatment in Malawi, where cure rates are greater than 95% (Brown et al., 2010; Chisholm et al., 2011).

One of the challenges of international surveillance of gonococcal resistance is the diversity of methods used to assess antimicrobial susceptibility. Nucleic acid amplification tests are not capable of determining antimicrobial susceptibility in *N. gonorrhoeae*. Determination of minimum inhibitory concentration (MIC) by agar dilution is considered the gold standard; although Etest is a good alternative for agar dilution method, it may underestimate MIC values (Chisholm et al., 2011). Agar dilution is too complex to be used in routine laboratories, and there are differences between CLSI (Clinical and Laboratory Standards Institute, 2021) and EUCAST (EUCAST, n.d.) guidelines for the interpretation of MIC values for this microorganism. On the other hand, the disc diffusion test, which is widely used in routine laboratories, is not recommended by EUCAST; CLSI guidelines define interpretation criteria for some antimicrobials only.

The WHO Region of the Americas has the second-highest estimated gonorrhoea incidence worldwide: 23 cases per 1000 women and 32 per 1000 men. The Brazilian GASP reported a high level of resistance of *N. gonorrhoeae* to penicillin, tetracycline, and ciprofloxacin. These results supported the use of dual therapy (ceftriaxone + azithromycin) in the country from 2017 onwards (Bazzo et al., 2018). However, there are limited Brazilian data on the susceptibility profile of *N. gonorrhoeae* isolates to spectinomycin and gentamicin (Belda Junior et al., 2002; Belda Junior et al., 2007; Dillon et al., 2001; Costa et al., 2013).

This study, supported by the Brazilian GASP, aimed to (i) evaluate the susceptibility of *N. gonorrhoeae* isolates to spectinomycin and gentamicin using the agar dilution method in accordance with WHO quality standards and (ii) compare the gold standard method and disc diffusion for assessment of *N. gonorrhoeae* susceptibility to spectinomycin, gentamicin, ciprofloxacin, ceftriaxone, and azithromycin.

2. Methods

2.1. Clinical isolates

A total of 336 consecutive, non-duplicate clinical isolates of *N. gonorrhoeae* collected in Santa Catarina and São Paulo States, Brazil, between 2003 and 2016 (44 in 2003, 8 in 2004, 38 in 2005, 32 in 2006, 3 in 2007, 1 in 2008, 4 in 2009, 10 in 2010, 19 in 2011, 12 in 2012, 49 in 2013, 26 in 2014, 41 in 2015, and 49 in 2016) were analysed in this study.

234 (69.6%) *N. gonorrhoeae* isolates were recovered from males samples, 36 (10.7%) from females and 66 (19.7%) with unknown origin. Urethral discharge sample corresponded to 69.6% (234), vaginal discharge 10.7% (36), urine 18.2% (61) and extragenital sites 1.5% (5). Isolates were stored at −80 °C in trypticase soy broth containing 20% glycerol, and were subcultured on chocolate agar (Laborclin, Curitiba, Brazil) for 18 to 24 h at 35 °C under a 5% CO2 atmosphere in an incubator prior to testing. *N. gonorrhoeae* isolates were identified by automated analysis (VITEK 2 system, BioMérieux, Marcy-L'Étoile, France) and MALDI-TOF MS (BioMérieux, Marcy-L'Étoile, France), according to the manufacturer's instructions (Biomerieux, 2011; Biomerieux, 2016).

2.2. Agar dilution method

The MICs of spectinomycin (Sigma–Aldrich, China), gentamicin (Sigma–Aldrich, China), ciprofloxacin (Sigma–Aldrich, USA), ceftriaxone (Sigma–Aldrich, Israel), and azithromycin (Sigma–Aldrich, Israel) were determined by the agar dilution method, following US CLSI M07–10 recommendations (2015). Plates were prepared with Difco GC medium base (Becton Dickinson, Sparks, MD, USA) supplemented with 1% Vitox (Oxoid Ltd., Basingstoke, UK). Plates were prepared by mixing 22.5 mL of medium and 2.5 mL of each antimicrobial dilution and pouring into 90 mm diameter sterile Petri dishes. A bacterial suspension of 0.5 McFarland of each isolate was prepared in Muller Hinton Broth (Becton Dickinson, Sparks, MD, USA). With the aid of a Steers replicator, the *N. gonorrhoeae* isolates were stamped in the prepared plates and were incubated for 20 to 24 h at 35 °C under a 5% CO_2 atmosphere in an incubator. Isolates were classified as susceptible, intermediate susceptible, or resistant according to CLSI (Clinical and Laboratory Standards Institute, 2021) and EUCAST (EUCAST, n.d.) clinical breakpoints and gentamicin susceptibility criteria proposed by Brown and colleagues (Brown et al., 2010). MIC_{50} and MIC_{90} values were also determined. The CLSI gonococcal reference strain ATCC 49226 and 3 of the 14 WHO gonococcal reference strains (WHO F, G, K, L, M, N, O, P, U, V, W, X, Y, and Z) (Unemo et al., 2016b) were used in each round as quality controls. Each one of the 14 WHO strains was used at least three times during the experiments. Essential agreement (±1 MIC $\log_2$ dilution) was required between test and reference (Unemo et al., 2016b) MIC values for quality control strains.

2.3. Disc diffusion method

Disc diffusion assays were performed according to CLSI M02-A12 (2015) (Clinical and Laboratory Standards Institute, 2015) on GC medium (Becton Dickinson, Sparks, MD, USA) supplemented with 1% Vitox (Oxoid Ltd., Basingstoke, UK). Plates were prepared by pouring 25 mL of medium into 90 mm diameter sterile Petri dishes (depth of 4 mm). A bacterial suspension of 0.5 McFarland in Muller Hinton Broth (Becton Dickinson, Sparks, MD, USA) of each isolate was prepared and plated confluently using a swab. Antimicrobial discs (Oxoid England) containing 100 μg spectinomycin, 10 μg gentamicin, 5 μg ciprofloxacin, 30 μg ceftriaxone, or 15 μg azithromycin were used. Plates were incubated for 20 to 24 h at 35 °C under a 5% CO_2 atmosphere in an incubator. Zone diameter breakpoints for ceftriaxone, spectinomycin, azithromycin, ciprofloxacin followed CLSI M100 (2021) guidelines (Clinical and Laboratory Standards Institute, 2021), and for gentamicin followed the criteria proposed by Bala and colleagues (Bala et al., 2016). The gonococcal reference strain ATCC 49226 was used as quality control.

2.4. Comparison of agar dilution and disc diffusion results

Susceptibility profiles obtained by agar dilution and disc diffusion were compared by plotting the diameter of the zone of inhibition (mm) as a function of MIC values (mg/L) according to susceptibility categories (susceptible, intermediate, and resistant).

Discrepancies among agar dilution (gold standard) and disc diffusion results were categorised as very major, major, and minor. Errors were considered very major if the isolate was found resistant by the reference method but susceptible by disc diffusion. Major errors were defined as susceptible by agar dilution and resistant by disc diffusion. Minor errors were those determined as intermediate susceptible by one method and resistant or susceptible by the other. Discrepancy was assessed according to CLSI M23-Ed4 (2016) (Clinical and Laboratory Standards Institute, 2016) Acceptable discrepancy rates for MIC values within 1 $\log_2$ dilution (non-borderline isolates) were as follows: minor errors <40%, major

errors <10%, and very major errors <10%. For discrepancies equal to or greater than ±2 $\log_2$ dilutions (non-borderline isolates), acceptance was defined as minor errors <5%, major errors <2%, and very major errors <2% (Clinical and Laboratory Standards Institute, 2016).

2.5. Reproducibility

The 14 WHO gonococcal reference strains were used to evaluate the reproducibility of the disc diffusion method. Tests were performed in triplicate on three different days. Categorical agreement was calculated by dividing the number of concordant results by the total number of samples tested. Agreement was expected to be equal to or greater than 95% (Clark et al., 2009).

3. Results

3.1. Spectinomycin

All isolates ($n = 336$) were susceptible to spectinomycin (MIC of 4 to 32 mg/L) when considering CLSI (S ≤ 32, $I = 64$, $R \geq 128$ mg/L) and EUCAST (S ≤ 64 and $R > 64$ mg/L) breakpoints. Disc diffusion results agreed with those of the gold standard with CLSI breakpoints (S ≥ 18, I = 15–17, $R \leq 14$ mm) (Table 1).

3.2. Gentamicin

By applying the criteria proposed by Brown et al. (2010) to assess the susceptibility profile of isolates to gentamicin (S ≤ 4, I = 8–16, $R \geq 32$ mg/L) by agar dilution, we found that 80.6% of isolates were susceptible and 19.4% were intermediate susceptible (MIC = 8 mg/L). Disc diffusion, using the breakpoints suggested by Bala et al. (2016) (S ≥ 16, I = 13–15, $R \leq 12$ mm), showed that 80.6% of isolates were susceptible and 19.4% were intermediate. No gentamicin-resistant isolates were detected by either method. Although the number of isolates with intermediate susceptibility was the same by both methods, the methods agreed for only 64.6% (42/65) of isolates. The overall agreement between methods was 86.3%, with 13.7% of minor errors (46 isolates, of which 45 were borderline-susceptible and 1 was non-borderline) (Table 2).

3.3. Ciprofloxacin

Ciprofloxacin MIC ranged from 0.001 to ≥32 mg/L. Using both CLSI (S ≤ 0.06, I = 0.12–0.50, $R \geq 1$ mg/L) and EUCAST (S ≤ 0.03, $I = 0.06$, and $R > 0.06$ mg/L) criteria, we observed resistance to ciprofloxacin in 35.1% of isolates and intermediate susceptibility in 0.6% (Table 3). The agreement between methods was 99.7%, with a minor error of 0.3%.

Table 2
Relationship between gentamicin MIC and zone diameters for 336 clinical isolates of *Neisseria gonorrhoeae*. The *y*-axis shows the tested drug concentrations, and the *x*-axis the zone of inhibition diameters. Horizontal lines represent MIC breakpoints proposed by Brown et al. (2010). Vertical lines represent zone diameter breakpoints proposed by Bala et al. (2016).

Zone of inhibition diameter (mm)	1	2	4	8	16	32
23		2				
22		2	1			
21		7	1			
20	4	14	12			
19		16	14	1		
18	1	16	43	2		
17		8	48	6		
16		3	56	14		
15		2	19	39		
14		1	1	3		
13						
12						
≤11						
MIC (mg/L)						

3.4. Azithromycin

Both CLSI and EUCAST advise the use of azithromycin in combination with another antimicrobial agent having an epidemiological cut-off or susceptibility breakpoint of ≤1 mg/L. On the basis of this criterion, 13 isolates (3.9%) were found to be resistant to azithromycin. The breakpoint for disc diffusion was ≤30 mm, considering the CLSI breakpoint, resulting in the identification of 61 resistant isolates. The agreement between methods was 85.7%. However, because of the lack of intermediate susceptibility, these isolates were classified as major errors (14.3%) (Table 4).

3.5. Ceftriaxone

All isolates were susceptible to ceftriaxone (MIC = 0.001–0.125 mg/L), considering both EUCAST (S ≤ 0.125 and $R > 0.125$ mg/L) and CLSI (S ≤ 0.25 mg/L) breakpoints. Disc diffusion results agreed with those of the reference method, considering the CLSI breakpoint (S ≥ 35 mm) (Table 5).

3.6. Reproducibility

A reproducibility test was performed for azithromycin using CLSI/EUCAST criteria for agar dilution and CLSI criteria for disc diffusion and

Table 1
Relationship between spectinomycin MIC and zone diameters for 336 clinical isolates of *Neisseria gonorrhoeae*. The *y*-axis shows the tested drug concentrations, and the *x*-axis the zone of inhibition diameters. Solid horizontal lines represent CLSI MIC breakpoints. The dashed horizontal line represents the EUCAST MIC breakpoint. Vertical lines indicate CLSI zone diameter breakpoints.

Zone of inhibition diameter (mm)	4	8	16	32	64	128	256	512	≥1024
≥30	4	19	8						
29		12	4						
28	2	10	31						
27		11	31	1					
26		3	43	4					
25		3	37	8					
24	2	2	47	3					
23		1	27	2					
20–22			18	2					
19		1							
18									
17									
15									
≤14									
MIC (mg/L)									

Table 3
Relationship between ciprofloxacin MIC and zone diameters for 336 clinical isolates of *Neisseria gonorrhoeae*. The *y*-axis shows the tested drug concentrations, and the *x*-axis the zone of inhibition diameters. Solid horizontal lines represent CLSI MIC breakpoints. Dashed horizontal lines represent EUCAST MIC breakpoints. Vertical lines indicate CLSI zone diameter breakpoints.

		0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32
	≥55		4	4	5												
	51–54	1	13	42	9												
	46–50	2	23	55	19	4	1										
	43–45		4	15	3	2											
	42			4	1												
	41			4	1												
	40																
Zone of inhibition diameter (mm)	38								1								
	35								1								
	28												1				
	27											1			1		
	26											2					
	21–25											2	4	2			
	11–20											1	3	36	17	4	1
	≤10													2	6	33	2
								MIC (mg/L)									

Table 4
Relationship between azithromycin MIC and zone diameters for 336 clinical isolates of *Neisseria gonorrhoeae*. The *y*-axis shows the tested drug concentrations, and the *x*-axis the zone of inhibition diameters. Horizontal lines represent CLSI/EUCAST MIC breakpoints. Vertical lines indicate CLSI zone diameter breakpoints.

		0.03	0.06	0.125	0.25	0.5	1	2	>4
	≥40	15	19	12	4				
	37–39	9	22	15	7				
	34–36	4	26	17	28	3			
	33	1	2	7	8	2			
	32		10	5	9	2			
	31		3	6	5				
Zone of inhibition diameter (mm)	30		8	12	12	1			
	29			6	5	2			
	28		3	8	16	4			
	27							1	
	26						1		
	25						1	1	
	21–24						2	2	4
	18–20							2	3
						MIC (mg/L)			

Table 5
Relationship between ceftriaxone MIC and zone diameters for 336 clinical isolates of *Neisseria gonorrhoeae*. The *y*-axis shows the tested drug concentrations, and the *x*-axis the zone of inhibition diameters. Solid horizontal lines represent CLSI MIC breakpoints. The dashed horizontal line represents the EUCAST MIC breakpoint. Vertical lines indicate CLSI zone diameter breakpoints.

		0.0005	0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1
	≥55			3	5	6							
	50–54		2	7	18	29	7	2	1				
	45–49		3	4	26	47	35	6	3				
	44				6	13	8	2					
	43			2	8	9	4	4	1				
	42				2	7	5	1	1				
	41			1	2	9	11	3	2				
Zone of inhibition diameter (mm)	40		1	1	1	5	1		2				
	39				1	2	4	2					
	38				1	3							
	37				1		4		1				
	36				1		2						
	35				1					1			
	34												
	33												
							MIC (mg/L)						

82.5% categorical agreement was obtained. For spectinomycin CLSI/EUCAST criteria for agar dilution and CLSI criteria for disc diffusion), the categorical agreement was 100%. A categorical agreement of 93% was obtained for gentamicin using criteria proposed by Brown et al. (2010) for agar dilution and those proposed by Bala et al. (2016) for disc diffusion.

All discrepancies between agar dilution and disc diffusion were within 1 $\log_2$ dilution and 1–3 mm, respectively. There were found a total of nine minor errors for gentamicin (borderline) and 22 very major errors for azithromycin (13.5% borderline and 4.0% non-borderline.

4. Discussion

N. gonorrhoeae is known for its ability to develop resistance to various classes of antimicrobials, which is why monotherapy treatment is not indicated. The use of spectinomycin as first-line treatment in the 1980s was followed by an increase in antimicrobial resistance (Easman et al., 1984; Stolz et al., 1975; Boslego et al., 1987; Unemo, 2015). Currently, spectinomycin is not used in Brazil, and all isolates tested in our study were susceptible to the antimicrobial. Of the 20 isolates with borderline susceptibility (MIC = 32 mg/L), 65% were collected in 2003. Between 2000 and 2010, Latin American studies reported that less than 10% of isolates had a spectinomycin MIC of 64 mg/L (Belda Junior et al., 2002; Belda Junior et al., 2007; Starnino et al., 2012). One *N. gonorrhoeae* isolate was shown to have a high level of resistance (MIC ≥1024 mg/L) in Norway in 2010 (Unemo et al., 2013). From 2010 onward, studies conducted in Latin America, Europe, Africa, and Asia began to report that all *N. gonorrhoeae* isolates were susceptible to spectinomycin (Brown et al., 2010; Costa et al., 2013; Lee et al., 2015; Thakur et al., 2017). Spectinomycin resistance levels decreased over time, different from that of other antimicrobials formerly used to treat gonorrhoea.

In 2019, Brazilian guidelines began to recommend gentamicin (240 mg intramuscular) plus azithromycin (2 g per oral) as a second-line therapy for gonococcal retreatment (Protocolo, 2019). In our study, 80.6 and 19.4% of isolates were susceptible and intermediate susceptible to gentamicin, respectively, and 77.4% of MICs ranged from 4 to 8 mg/L. Intermediate susceptibility to gentamicin among Brazilian isolates was lower than that observed among isolates in Argentina, the United States of America, and Europe (69.2, 73, and 82.7%, respectively) (Chisholm et al., 2011; Gianecini et al., 2018; Mann et al., 2018). As also observed in the present study, various researchers found that the majority of *N. gonorrhoeae* isolates had a gentamicin MIC of 4 (susceptible) to 8 mg/L (intermediate) by the agar dilution method. Comparisons between the Etest and agar dilution for assessing gentamicin susceptibility revealed that the gradient diffusion method affords lower MIC values (Chisholm et al., 2011; Daly et al., 1997). Therefore, the Etest can lead to a bias when it classifies isolates as susceptible or borderline to gentamicin, which may hinder comparisons between studies.

The distribution of isolates with intermediate susceptibility to gentamicin varied over the years but did not tend towards a decrease in susceptibility. Thus, an increase in gentamicin resistance was not observed, as also reported in Malawi, where gentamicin has been used as the first-line treatment for more than 15 years. Gentamicin is an interesting alternative in the face of limited therapeutic options. In a clinical study comparing the efficacies of ceftriaxone and gentamicin, both in combination with azithromycin, ceftriaxone (the current therapy) eliminated the infection in 98% of cases, whereas gentamicin was effective in 91% of cases (Ross et al., 2017). Although gentamicin is not as effective as ceftriaxone in treating gonococcal infections, this antimicrobial could still be considered useful, particularly in dual therapy or in the absence of another therapeutic options. In addition, gentamicin is an inexpensive drug.

High susceptibility rates to ceftriaxone (100%) and azithromycin (96.1%) were observed. The 13 (3.9%) azithromycin-resistant isolates were evenly distributed over the years (0–2 isolates/year from 2003 to 2016). A surveillance study conducted by the Brazilian GASP investigated 550 isolates and found 100% susceptibility to ceftriaxone and 93.1% susceptibility to azithromycin (Bazzo et al., 2018). The first azithromycin-resistant isolates in Latin America were detected in the 1990s. In 2001, the first report of a highly resistant gonococcus strain (MIC >2048 mg/L) was made in Argentina (Galarza et al., 2009; Dillon et al., 2013), and then more reports were published worldwide (Palmer et al., 2008; Starnino and Stefanelli, 2009; Chisholm et al., 2010). In the present study, we did not observe isolates with high levels of resistance to azithromycin. Although resistance to ceftriaxone was not found, one isolate collected in 2016 showed a ceftriaxone MIC of 0.125 mg/L, indicative of a possible decrease in susceptibility to extended spectrum cephalosporins in the country. On the other hand, resistance to ciprofloxacin was high (35.1%), making its use unfeasible for empirical therapy for gonorrhoea.

Our results showed 100% agreement between the gold standard method and disc diffusion for spectinomycin and ceftriaxone; however, there were no resistant isolates to these antimicrobials. For ciprofloxacin, the agreement between methods was 99.7%, with only minor errors (0.3%). The agreement between methods was 85.7% for azithromycin, nevertheless, because of the lack of intermediate susceptibility, these isolates were classified as major errors (14.3%), with 9.2% for non-borderline, above the acceptable, according to CLSI criteria (<2% very major errors for non-borderline isolates) and 5.3% for borderline isolates, which is acceptable according to CLSI criteria (<10% very major errors for borderline isolates). For gentamicin, the agreement was 86.3%, lower than the target agreement of 95%. However, we did not verified major or very major errors, and minor errors (13.4% for borderline-susceptible and 0.3% for non-borderline isolates) were within CLSI limits (<40% for borderline-susceptible and < 5% for non-borderline isolates). Because of the large proportion (77.4%) of isolates with borderline MICs (4 and 8 mg/L), a high percentage of discrepancies was expected (CLSI M23) (Clinical and Laboratory Standards Institute, 2016). Thus, the disagreement rates of our quality-assured results were considered acceptable as per CLSI M23-Ed4 guidelines. In the current context of emergence of antimicrobial resistance, it is essential that clinical laboratories be able to reliably test *N. gonorrhoeae* susceptibility in daily routine. Whenever possible, the recommendation is to perform MIC by agar dilution or MIC Strip to determine the antimicrobial susceptibility profile. However, for small laboratories and low-income countries, the disk diffusion method could be an alternative for susceptibility testing.

In conclusion, resistance to spectinomycin or gentamicin in *N. gonorrhoeae* isolates were not observed, showing that these two antimicrobials are potential treatment options for current cases of therapeutic failure in Brazil. The disc diffusion method, combined with the breakpoints described by Bala et al. (2016) for gentamicin, affords comparable results to agar dilution and can be used by routine laboratories when the reference method is not readily available.

Author contributions

JMM, HMM, MAS, LG, TMS, FHB, and VCBA conducted the MIC, disc diffusion and bacterial identification. JMM, MCS, and MLB designed the project and wrote the manuscript. All authors reviewed the manuscript.

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Transparency declarations

The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Bala, M., Singh, V., Philipova, I., et al., 2016. Gentamicin in vitro activity and tentative gentamicin interpretation criteria for the CLSI and calibrated dichotomous sensitivity disc diffusion methods for *Neisseria gonorrhoeae*. J. Antimicrob. Chemother. 71, 1856–1859. https://doi.org/10.1093/jac/dkw102.

Bazzo, M.L., Golfetto, L., Gaspar, P.C., et al., 2018. First nationwide antimicrobial susceptibility surveillance for *Neisseria gonorrhoeae* in Brazil, 2015–16. J. Antimicrob. Chemother. 4–11. https://doi.org/10.1093/jac/dky090.

Belda Junior, W., Fagundes, L.J., De Góes Siqueira, F.L., Nico, M.M.S., 2002. Atividade in vitro de cinco drogas antimicrobianas contra *Neisseria gonorrhoeae*. An. Bras. Dermatol. 77, 661–667. https://doi.org/10.1590/S0365-05962002000600003.

Belda Junior, W., Velho, P.E.N.F., Fagundes, L.J., et al., 2007. Evaluation of the in vitro activity of six antimicrobial agents against *Neisseria gonorrhoeae*. Rev. Inst. Med. trop. 49, 55–58. https://doi.org/10.1590/S0036-46652007000100010.

Bignell, C., Unemo, M., 2013. European guideline on the diagnosis and treatment of gonorrhoeae in adults. Int. J. STD AIDS 24, 85–92. https://doi.org/10.1177/0956462412472837.

Biomerieux, 2011. VITEK MS: Prep Station - user manual. Marcy l'Étoile, França 1, 1–76.

Biomerieux, 2016. VITEK MS: MALDI-TOF MS - User manual. Marcy l'Étoile, França 3, 1–78.

Boslego, J.W., Tramont, E.C., Takafuji, E.T., et al., 1987. Effect of spectinomycin use on the prevalence of spectinomycin-resistant and penicillinase-producing *Neisseria gonorrhoeae*. N. Engl. J. Med. 317, 272–278. https://doi.org/10.1056/NEJM198707303170504.

Brown, L.B., Krysiak, R., Kamanga, G., et al., 2010. *Neisseria gonorrhoeae* antimicrobial susceptibility in Lilongwe, Malawi, 2007. Sex. Transm. Dis. 3, 169–172. https://doi.org/10.1097/OLQ.0b013e3181bf575c.

CDC, 2015. Sexually Transmitted Diseases Treatment Guidelines. Centers for Disease Control and Prevention. https://www.cdc.gov/std/tg2015/gonorrhea.htm.

Chisholm, S.A., Dave, J., Ison, C.A., 2010. High-level azithromycin resistance occurs in *Neisseria gonorrhoeae* as a result of a single point mutation in the 23S rRNA genes. Antimicrob. Agents Chemother. 54, 3812–3816. https://doi.org/10.1128/AAC.00309-10.

Chisholm, S.A., Quaye, N., Cole, M.J., et al., 2011. An evaluation of gentamicin susceptibility of *Neisseria gonorrhoeae* isolates in Europe. J. Antimicrob. Chemother. 66, 592–595. https://doi.org/10.1093/jac/dkq476.

Clark, R.B., Lewinski, M.A., Loeffelholz, M.J., et al., 2009. Cumitech 31A, Verification and Validation of Procedures in the Clinical Microbiology Laboratory. ASM Press, Washington, DC. https://doi.org/10.1016/S0196-4399(97)83919-0.

Clinical and Laboratory Standards Institute, 2015. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard - Twelfth Edition M02-A12. CLSI, Wayne, PA, USA.

Clinical and Laboratory Standards Institute, 2016. Development of in Vitro Susceptibility Testing Criteria and Quality Control Parameters M23Ed4. CLSI, Wayne, PA, USA.

Clinical and Laboratory Standards Institute, 2021. Performance Standards for Antimicrobial Susceptibility Testing M100-S31. CLSI, Wayne, PA, USA.

Costa, L.M.B., Pedroso, E.R.P., Vieira Neto, V.S., et al., 2013. 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. 46, 304–309. https://doi.org/10.1590/0037-8682-0009-2013.

Daly, C.C., Hoffman, I., Hobbs, M., et al., 1997. Development of an antimicrobial susceptibility surveillance system for *Neisseria gonorrhoeae* in Malawi: comparison of methods. J. Clin. Microbiol. 35, 2985–2988. https://doi.org/10.1128/JCM.35.11.2985-2988.1997.

Dillon, J.A.R., Rubabaza, J.P.A., Benzaken, A.S., et al., 2001. Reduced susceptibility to azithromycin and high percentages of penicillin and tetracycline resistance in *Neisseria gonorrhoeae* isolates from Manaus Brazil, 1998. Sex. Transm. Dis. (28), 521–526. https://doi.org/10.1097/00007435-200109000-00008.

Dillon, J.R., Trecker, M.A., Thakur, S.D., 2013. Two decades of the gonococcal antimicrobial surveillance program in South America and the Caribbean: challenges and opportunities. Sex. Transm. Infect. 89, 36–41. https://doi.org/10.1136/sextrans-2012-050905.

Dowell, D., Kirkcaldy, R.D., 2012. Effectiveness of gentamicin for gonorrhoea treatment: systematic review and meta-analysis. Sex. Transm. Infect. 0, 1–6. https://doi.org/10.1136/sextrans-2012-050604.

Easman, C., Forster, G., Walker, G., et al., 1984. Spectinomycin as initial treatment for gonorrhoea. Br. Med. J. 289, 1032–1034. https://doi.org/10.1136/bmj.289.6451.1032.

EUCAST. European Committee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_2.0_120221.pdf.

Fifer, H., Natarajan, U., Jones, L., et al., 2016. Failure of dual antimicrobial therapy in treatment of gonorrhoeae. N. Engl. J. Med. 374, 2503–2506. https://doi.org/10.1056/NEJMc1512757.

Galarza, P.G., Alcalá, B., Salcedo, C., et al., 2009. Emergence of high level azithromycin-resistant *Neisseria gonorrhoeae* strain isolated in Argentina. Sex. Transm. Dis. 36, 787–788. https://doi.org/10.1097/OLQ.0b013e3181b61bb1.

Gianecini, R., Oviedo, C., Stafforini, G., et al., 2016. Neisseria gonorrhoeae resistant to ceftriaxone and cefixime, Argentina. Emerg. Infect. Dis. 22, 1139–1141. https://doi.org/10.3201/eid2206.152091.

Gianecini, R., Oviedo, C., Irazu, L., et al., 2018. Comparison of disk diffusion and agar dilution methods for gentamicin susceptibility testing of *Neisseria gonorrhoeae*. Diagnostic Microbiol. Infect. Dis. 91, 299–304. https://doi.org/10.1016/j.diagmicrobio.2018.03.005.

Global progress report on HIV, 2021. viral hepatitis and sexually transmitted infections. Available at: https://www.who.int/publications/i/item/9789240027077.

Lagacé-Wiens, P.R.S., Adam, H.J., Laing, N.M., et al., 2017. 315 antimicrobial susceptibility of clinical isolates of *Neisseria gonorrhoeae* to alternative antimicrobials with therapeutic potential. J. Antimicrob. Chemother. 1–5. https://doi.org/10.1093/jac/dkx147.

Lee, H., Unemo, M., Kim, H.J., et al., 2015. Emergence of decreased susceptibility and resistance to extendedspectrum cephalosporins in *Neisseria gonorrhoeae* in Korea. J. Antimicrob. Chemother. 70, 2536–2542. https://doi.org/10.1093/jac/dkv146.

Mann, L.M., Kirkcaldy, R.D., Papp, J.R., et al., 2018. Susceptibility of *Neisseria gonorrhoeae* to gentamicin-gonococcal isolate surveillance project, 2015-2016. Sex. Transm. Dis. 45, 96–98. https://doi.org/10.1097/OLQ.0000000000000693.

Palmer, H.M., Young, H., Winter, A., et al., 2008. Emergence and spread of azithromycin-resistant *Neisseria gonorrhoeae* in Scotland. J. Antimicrob. Chemother. 62, 490–494. https://doi.org/10.1093/jac/dkn235.

Protocolo, 2019. clínico e diretrizes terapêuticas para atenção integral às pessoas com infecções sexualmente transmissíveis (IST). PCDT, Brasília, DF, Brasil.

Ross, J.D.C., Harding, J., Duley, L., et al., 2017. The efficacy and safety of gentamicin for the treatment of genital, pharyngeal and rectal gonorrhoea: a randomised controlled trial. Sex. Transm. Infect. 93, 93–94. https://doi.org/10.1136/sextrans-2017-053264.106.

Sanchez-Buso, L., et al., 2019. The impact of antimicrobials on gonococcal evolution. Nat. Microbiol. 4, 1941–1950. https://doi.org/10.1038/s41564-019-0501-y.

Starnino, S., Stefanelli, P., 2009. Azithromycin-resistant *Neisseria gonorrhoeae* strains recently isolated in Italy. J. Antimicrob. Chemother. 63, 1200–1204. https://doi.org/10.1093/jac/dkp118.

Starnino, S., Galarza, P., Carvalho, M.E.T., et al., 2012. 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 ceftriaxona. Sex. Transm. Dis. 39, 813–821. https://doi.org/10.1097/OLQ.0b013e3182631c9f.

Stolz, E., Zwartt, H., Michelt, M., 1975. Activity of eight antimicrobial agents in vitro against *N. gonorrhoeae*. Br. J. Vener. Dis 257–264. https://doi.org/10.1136/sti.51.4.257.

Tacconelli, E., Magrini, N., 2013. WHO (2017) Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics, Vol. 43. Cadernos de Pesquisa, Geneva, Switzerland, pp. 348–365. http://www.cdc.gov/drugresistance/threat-report-2013/.

Thakur, S.D., Araya, P., Borthagaray, G., et al., 2017. Resistance to ceftriaxone and azithromycin in *Neisseria gonorrhoeae* isolates from 7 countries of South America and the Caribbean: 2010–2011. Sex. Transm. Dis. 44, 157–160. https://doi.org/10.1097/OLQ.0000000000000587.

Unemo, M., 2015. Current and future antimicrobial treatment of gonorrhoea - the rapidly evolving *Neisseria gonorrhoeae* continues to challenge. BMC Infect. Dis. 364, 15. https://doi.org/10.1186/s12879-015-1029-2.

Unemo, M., Shafer, W.M., 2014. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin. Microbiol. Rev. 27, 587–613. https://doi.org/10.1128/CMR.00010-14.

Unemo, M., Golparian, D., Skogen, V., al., 2013. *Neisseria gonorrhoeae* strain with high-level resistance to spectinomycin due to a novel resistance mechanism (mutated ribosomal protein S5) verified in Norway. Antimicrob. Agents Chemother. 57, 1057–1061. https://doi.org/10.1128/AAC.01775-12.

Unemo, M., Del Rio, C., Shafer, W.M., 2016a. Antimicrobial resistance expressed by *Neisseria gonorrhoeae*: a major global public health problem in the 21st century. Microbiol. Spectr. 4, 32. https://doi.org/10.1128/microbiolspec. EI10-0009-2015.

Unemo, M., Golparian, D., Sánchez-Busó, L., et al., 2016b. The novel 2016 WHO *Neisseria gonorrhoeae* reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. J. Antimicrob. Chemother. 71, 3096–3108. https://doi.org/10.1093/jac/dkw288.

Unemo, M., Seifert, H.S., Hook, E.W., Hawkes, S., Ndowa, F., Dillon, J.R., 2019. Gonorréia. Nat. Rev. Dis. Primers. 5, 79. https://doi.org/10.1038/s41572-019-0128-6.

WHO, 2015. Global antimicrobial resistance surveillance system. World Heal. Organ. 1, 1–44.

WHO, 2016. WHO guidelines for the treatment of *Neisseria gonorrhoeae*. World Heal. Organ. 1–55.

Workowski, K.A., Bolan, G.A., 2015. Sexually transmitted diseases treatment guidelines. MMWR 64, 1–138.