Original Article

Comparison of Two Disc Diffusion Methods with Minimum Inhibitory Concentration for Antimicrobial Susceptibility Testing of *Neisseria Gonorrhoeae* Isolates

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Abstract

Background: A few studies are available comparing either minimum inhibitory concentration (MIC) values with the Clinical and Laboratory Standards Institute (CLSI) disc diffusion method or MIC with the Australian Gonococcal Surveillance Program (AGSP) method. **Aim**: This study was conducted with the aim to identify the most feasible and cost-effective method for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. **Materials and Methods:** Antimicrobial susceptibility testing of *N. gonorrhoeae* was performed using, in parallel, the E-test for MIC determination and disc diffusion by CLSI and AGSP techniques, and were compared. Susceptibility to penicillin, ciprofloxacin, tetracycline, ceftriaxone and spectinomycin and cefixime were determined by CSLI and AGSP method and Kappa statistics used to analyse the data with SPSS software. **Results:** All isolates were susceptible to ceftriaxone and spectinomycin by three methods. Ninety-nine (99%) strains were resistant to ciprofloxacin, while 1% showed intermediate susceptibility to ciprofloxacin by all methods. Statistically, there was a moderate level of agreement between the methods for penicillin. **Conclusion:** All three methods gave reproducible results. Although the media used in the disc diffusion by the AGSP method is easy and cheap to prepare, the CLSI method of disc diffusion testing is recommended for susceptibility testing of gonococcal isolates because of its feasibility and 100% accuracy, with MIC by E-test as the reference method.

Keywords: Antimicrobial susceptibility testing, MIC, *Neisseria gonorrhoeae*

Introduction

An important and essential measure for monitoring the effectiveness of recommended drugs for the treatment of gonorrhoea is surveillance of the *in vitro* antimicrobial susceptibilities of clinical isolates of *Neisseria gonorrhoeae*.[1] In many countries, baseline data on the antimicrobial susceptibility of gonococcal isolates have not been collected. In the absence of such data, antibiotics to which gonococcal isolates are resistant may continue to be used. The establishment of such baseline data is especially important when syndromic approaches are used to diagnose and treat gonorrhoea.[2] The antimicrobial susceptibility pattern of *N. gonorrhoeae* may change rapidly, especially in areas where ineffective treatment regimens are applied.[3]

There are no universally accepted guidelines for testing the antimicrobial susceptibility of *N. gonorrhoeae* by a disc diffusion method, but different techniques are in practice, like the Clinical and Laboratory Standards Institute (CLSI)[4] method, the Australian Gonococcal Surveillance Program (AGSP) method[5] and the British Society for Antimicrobial Chemotherapy (BSAC) method.[6] The recommended procedure for antimicrobial susceptibility testing of gonococci is a determination of the minimum inhibitory concentration (MIC by agar dilution or E-test).[4] A few studies are available comparing either MIC values with the CLSI disc diffusion method or MIC values with the AGSP method.[7-9] A comparison between all the three *in vitro* susceptibility methods has not been reported so far. In the present study, antimicrobial susceptibility testing of *N. gonorrhoeae* was performed by using, in parallel, the E-test for MIC

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determination (the gold standard) and disc diffusion by the CLSI and AGSP techniques. All the three methods were compared to determine the agreement between them and to determine which method could be the most feasible and cost-effective for antimicrobial susceptibility testing of *N. gonorrhoeae*.

Materials and Methods

Gonococcal strains

This study was conducted at the Department of Microbiology, Maulana Azad Medical College, New Delhi. A total of 100 consecutive *N. gonorrhoeae* strains were isolated from 89 (86.4%) of 103 men with urethritis, five (12.8%) of 39 women with endocervicitis and six (46.1%) of 13 sexual contacts of these patients.

The samples were inoculated directly onto the selective Modified Thayer Martin (MTM) medium and incubated at 35-36.5ºC in moist air containing 5% CO_2 for 24-72 h. A candle jar was used for carbon dioxide production. The colonies suspected to be *N. gonorrhoeae* were presumptively identified by Gram stain and oxidase and superoxal tests.[10] Confirmation of identity after subculture on chocolate agar was based on the rapid carbohydrate utilization test (RCUT). Gonococcal isolates were stored at −70ºC in tryptic soy broth (Difco Laboratories, Detroit, MI, USA) containing 20% glycerol.[10]

Antimicrobial susceptibility testing

All gonococcal isolates were examined for susceptibility to penicillin, ciprofloxacin, tetracycline, ceftriaxone and spectinomycin by the disc diffusion method of CLSI and the AGSP [Table 1].

MICs to all antibiotics were determined by the E-test. The E-test was performed as specified in the manufacturer's (AB Biodisk) product package insert. World Health Organization strains A to E and *N. gonorrhoeae* ATCC 49226 were included as quality control. The interpretive criteria for all antibiotics except azithromycin were as recommended by the CLSI; criteria for interpretation of azithromycin were recommended by the Neisseria Reference Laboratory (NRL) at CDC.

β-lactamase production was assayed using nitrocefin discs (BBL Cefinase; Becton Dickinson, Harayana, India).

Table 1: Disc potency of used antibiotics for antimicrobial susceptibility testing by the CLSI and AGSP methods

Antimicrobial agents	CLSI	AGSP
Penicillin	10 IU	0.5
Tetracycline	30 μg	10
Ceftriaxone	30 μg	0.5
Spectinomycin	100 μg	100
Ciprofloxacin	5 μg	1

CLSI: Clinical and laboratory standards institute, AGSP: Australian gonococcal surveillance program

Statistical analysis

Data management and statistical analyses were performed using statistical software SPSS version 13.0. (Chicago Illinois, USA). Linear regression analysis was carried out to correlate the MICs by E-test and disc diffusion methods.

Kappa statistics and percent agreement were applied to determine the agreement between the methods.

Results

All isolates were found to be susceptible to ceftriaxone and spectinomycin by three methods. Ninety-nine (99%) strains were resistant to ciprofloxacin (QRNG), while one (1%) showed intermediate susceptibility to ciprofloxacin by all methods [Tables 2 and 3]. There was complete agreement between the CLSI, AGSP and MIC methods for ceftriaxone, spectinomycin and ciprofloxacin (kappa = 1).

For penicillin, one (1%), 67 (67%) and 32 (32%) isolates were interpreted as susceptible, intermediate susceptible and resistant, respectively, by the CLSI and MIC methods [Tables 2-4], while one (1%), 59 (59%) and 40 (40%) isolates were interpreted as susceptible, less sensitive and resistant, respectively, by the AGSP method. Eight isolates were less susceptible to penicillin by CLSI and MIC whereas they were resistant by AGSP [Table 5]. Statistically, there was a moderate level of agreement between the two methods for penicillin (kappa = 0.83).

Of the penicillin-resistant strains, 17 were found to be penicillinase-producing *N. gonorrhoeae* (PPNG) isolates. Multi-drug resistance (resistance to three or more antibiotics) was observed in 10 gonococcal isolates.

Discussion

We observed that only 1% of the isolates were sensitive to ciprofloxacin and penicillin, while 46% were sensitive to tetracycline. All isolates were sensitive to ceftriaxone and spectinomycin, which can therefore be used as the first-line drug for syndromic management of urethritis.

Our findings show complete agreement between the CLSI, AGSP and MIC methods for ceftriaxone, spectinomycin and ciprofloxacin. All the isolates (100%) were susceptible to ceftriaxone and spectinomycin, and 99% were resistant to ciprofloxacin by the three methods. However, for penicillin, using MIC by E-test as the gold standard, more isolates were labeled as being resistant by AGSP. Because interpretative criteria for azithromycin are not available by CLSI and AGSP, comparisons could not be done for aithromycin.

In India, to the best of our knowledge, our study is the first to compare two disc diffusion methods with MIC. Otherwise, few published reports have compared the E-test with the agar

Table 2: Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates by the CLSI and AGSP methods (*n*=100)

Antibiotic disc	AGSP no. (%)			CLSI no. (%)		
	S	LS	R	S	I	R
Penicillin	1 (1)	59 (59)	40 (40)	1 (1)	67 (67)	32 (32)
Tetracycline**	NON-TRNG 80 (80)	TRNG 20 (20)		46 (46)	23 (23)	31 (31)
Ciprofloxacin*	0	1 (1)	99 (99)	0	1 (1)	99 (99)
Ceftriaxone	100 (100)	0	0	100 (100)	0	0
Spectinomycin	100 (100)	0	0	100 (100)	0	0

S: Susceptible, I: Intermediate susceptible, R: Resistant, *Quinolone testing is performed with a combination of both nalidixic acid (30 μg) and ciprofloxacin (1 μg) discs. The category of susceptible, less sensitive or resistant for ciprofloxacin is determined by considering the annular radius measurements obtained with both antibiotic discs. Ciprofloxacin (R<6, LS≥6, S≥6); nalidixic acid (R=0, LS=0, S≥6), **Used to screen for high-level tetracycline resistance only. CLSI: Clinical and laboratory standards institute, AGSP: Australian gonococcal surveillance program

Table 3: Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates by the E-test for MIC determination (*n*=100)

Antibiotics	Range (μg/mL)	MIC_{50}* (μg/mL)	MIC_{90}** (μg/mL)	S	I	R
Penicillin	0.023-6	0.5	3	1	67	32***
Tetracycline	0.094-64	0.38	24	46	23	31****
Ciprofloxacin	0.38-32	2	12	0	1	99
Ceftriaxone	0.002-0.023	0.006	0.012	100	0	0
Spectinomycin	1-8	6	8	100	0	0
Azithromycin	0.016-0.25	0.064	0.19	100	0	0

S: Susceptible, I: Intermediate susceptible, R: Resistant, *MIC_{50}: The MICs at which 50% of the gonococcal isolates tested were inhibited, **MIC_{90}: The MICs at which 90% of the gonococcal isolates tested were inhibited, ***Penicillinase producing *N. gonorrhoeae* (PPNG): 17 (17%), ****High-level plasmid-mediated tetracycline resistance *N. gonorrhoeae* (TRNG): 20 (20%). MIC: Minimum inhibitory concentration

Table 4: Antimicrobial resistance of 100 isolates of *Neisseria gonorrhoeae* by the CLSI, AGSP and E-test methods

Antibiotics	No. (%) of resistant		
	AGSP	CLSI	E-test
Penicillin	40 (40)	32 (32)	32 (32)
Tetracycline*	20 (20)*	31 (31)	31 (31)
Ciprofloxacin	99 (99)	99 (99)	99 (99)
Ceftriaxone	0 (0)	0 (0)	0 (0)
Spectinomycin	0 (0)	0 (0)	0 (0)

*TRNG only, AGSP: Australian gonococcal surveillance programs, CLSI: Clinical and laboratory standards institute

Table 5: Comparison of antimicrobial susceptibility of *Neisseria gonorrhoeae* isolates by the AGSP and CLSI, and E-test methods

	Category	E-test			Total	Kappa value
		S	I	R		
AGSP						
Penicillin	S	1	-	-	1	0.83
	I	-	59	-	59	
	R	-	8	32	40	
Tetracycline	Not TRNG	46	23	11	80	Cannot be calculated
	TRNG	-	-	20	20	
CLSI						
Penicillin	S	1	-	-	1	1
	I	-	67	-	67	
	R	-	-	32	32	
Tetracycline	S	46	-	-	46	1
	I	-	23	-	23	
	R	-	-	31	31	

100% agreement in results for ciprofloxacin, ceftriaxone, spectinomycin (Kappa=1). AGSP: Australian gonococcal surveillance programs, CLSI: Clinical and laboratory standards institute

disc diffusion method by CSLI or AGSP for antimicrobial susceptibility testing of *N. gonorrhoeae*. Bala, *et al.*[7] compared the results of two methods of susceptibility testing, MIC by E-test with disc diffusion by the AGSP method, in *N. gonorrhoeae* isolates. They also observed a moderate level of agreement for penicillin between and 96.9% agreement for ceftriaxone. Another study conducted in the United States[8] compared disc diffusion by CSLI, E-test and reference agar dilution method for macrolide azalides. They reported that all methods were accurate, and 100% agreement between the three methods was observed.

The continual spread and ongoing emergence of resistance among *N. gonorrhoeae* isolates require that an accurate and simple method be performed to determine antimicrobial susceptibilities. We found that both disc diffusion methods showed a high degree of agreement with MIC values. All the three methods gave reproducible results. However, media used in disc diffusion by the AGSP method is easy and less expensive to prepare when compared with CSLI and E-test [Table 6]. In addition, we can apply six discs per plate in the AGSP method; therefore, making the method overall more cost-effective. Although AGSP method is more economical, its feasibility depends on the availability of antimicrobial discs of low potency, and it requires more accuracy in measuring the inhibition zones. All the three methods can be easily performed in any routine diagnostic laboratory, but we recommend the CSLI method of disc diffusion for susceptibility testing of gonococcal isolates, because, firstly, it has 100% agreement with the E-test as the reference method for all the antibiotics, including penicillin and tetracycline; secondly, high potency

Table 6: Comparison of procedures involved in the AGSP, CLSI and E-test methods

Procedures	AGSP	CLSI	E-test
Media			
Components	Columbia agar+ 10% sheep blood	GC agar base+ growth supplement+ hemoglobin	GC agar base+ growth supplement+ hemoglobin
Cost	Cheap	Expensive	Expensive
Ease of preparation	Easy	Difficult	Difficult
Discs or strip/ plate (100 mm)	6 discs	4 discs	1 strip

AGSP: Australian gonococcal surveillance program, CLSI: Clinical and laboratory standards institute

discs are easily available; and thirdly, it is technically less demanding in measuring the inhibition zones. Further studies on monitoring antimicrobial resistance of *N. gonorrhoeae* against all antibiotics, including azithromycin, and newer antimicrobial agents used in the treatment of gonorrhea need to be conducted and the best method of antimicrobial suscepitibility testing for gonococcus needs to be identified.

Hence, the CSLI method of disc diffusion is the recommended method due to its higher feasibility and accuracy than the AGSP method.

References

1. Dyck EV, Smet H, Plot P. Comparison of E-test with agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. J Clin Microbiol 1994;32:1586-8.
2. Sosa J, Ramrez-arcos S, Rubin M, Hui L, Rafael L, Alina L, *et al.* High percentages of resistance to tetracycline and penicillin and reduced susceptibility to azithromycin characterize the majority of strain types of *N.gonorrhoeae* isolates in Cuba, 1995-1998. Sex Transm Dis 2003;30:443-8.
3. Lind I. Present status of antimicrobial resistance of *N. gonorrhoeae*. Eur J Sex Transm Dis 1986;3:185-9.
4. Clinical Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—twenty second informational supplement E: Approved Standard M100-S22. Wayne, PA, USA: NCCLS; 2012.
5. Tapsall JW. Antimicrobial resistance in *Neisseria gonorrhoeae* WHO/CDS/CSR/DRS/2001.3. Geneva: World Health Organization; 2001.
6. Andrews JM; BSAC Working Party on Susceptibility Testing. BSAC standardized disc susceptibility testing method (version 8). J Antimicrob Chemother 2009;64:454-89.
7. Bala M, Ray K, Gupta SM. Comparison of disc diffusion results with MIC value for antimicrobial susceptibility testing of *N.gonorrhoeae*. Indian J Med Res 2005;122:48-51.
8. Mehaffey PC, Putnam SD, Barrett MS, Jones RN. Evaluation of *in vitro* spectra of activity of azithromycin, clarithromycin and erythromycin tested against strains of *N.gonorrhoeae* by reference agar dilution, disc diffusion and E test methods. J Clin Microbiol 1996;34:479-81.
9. Bhuiyan BU, Rahman M, Miah MR, Nahar S, Islam N, Ahmed M, *et al.* Antimicrobial susceptibility and plasmid contents of *N.gonorrhoeae* isolates from CSW in Dhaka, Bangladesh. J Clin Microbiol 1999;37:1130-6.
10. Janda W, Koneman E, Procop G, Schreckenberger P, Woods G. 'Koneman's color atlas and textbook of diagnostic microbiology. Winn W, Allen S, editors. 6th ed. 566.

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