

Comparative assessment of CDS, CLSI disc diffusion and Etest techniques for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*: a 6-year study

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To cite: Singh V, Bala M, Kakran M, *et al.* Comparative assessment of CDS, CLSI disc diffusion and Etest techniques for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*: a 6-year study. *BMJ Open* 2012;2:e000969. doi:10.1136/bmjopen-2012-000969

► Prepublication history for this paper is available online. To view these files please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2012-000969>).

Received 31 January 2012
Accepted 8 June 2012

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ABSTRACT

Background: A variety of techniques are available for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*.

Objective: The aim of this study was to find a cost-effective, reliable and easily applicable microbiological method to detect antimicrobial susceptibilities of *N. gonorrhoeae* in resource-poor countries.

Design: Prospective study.

Setting: Male and female STD clinic of Regional STD Teaching, Training and Research Centre, New Delhi, India.

Participants: *N. gonorrhoeae* isolates from all male and female patients presenting with acute gonococcal urethritis and cervical discharge.

Material and methods: A total of 295 consecutive *N. gonorrhoeae* isolates during 2005–2010 was used to compare the Clinical and Laboratory Standards Institute (CLSI) and CDS disc diffusion technique with Etest by performing antimicrobial susceptibility testing in parallel for penicillin, tetracycline, ceftriaxone, ciprofloxacin and spectinomycin. WHO reference strains were used as controls.

Results: CDS disc diffusion zones of inhibition showed that complete percentage agreement for penicillin, ciprofloxacin and tetracycline was high with their analogous Etest minimal inhibitory concentrations in comparison to CLSI disc diffusion technique, that is, 91.5%, 92.9% and 99.3% versus 87.5%, 88.5% and 74.9%, respectively. CDS results had less number of major and minor category discrepancies in comparison to CLSI and CDS method showed excellent correlation coefficient ($r=1$) with Etest for all five antimicrobial agents tested in comparison to CLSI ($r=0.92$). It was very poor ($r=0.61$) by CLSI method for tetracycline. The correlation coefficients between the two methods and the Etest were identical if tetracycline was removed from the CLSI analysis.

Conclusions: The CDS technique is an attractive alternative for *N. gonorrhoeae* susceptibility testing and is recommended for monitoring the antimicrobial susceptibility in less developed and resource-poor settings to facilitate enhanced antimicrobial resistance surveillance when the WHO Gonococcal Antimicrobial Surveillance Programme is undergoing expansion to meet the ongoing challenges of surveillance and control of gonococcal antimicrobial resistance.

ARTICLE SUMMARY

Article focus

- A variety of techniques are available for antimicrobial susceptibility testing of *N. gonorrhoeae*.
- The aim of this study is to find a cost-effective, reliable and easily applicable microbiological method to detect antimicrobial susceptibilities of *N. gonorrhoeae* in resource-poor countries.

Key messages

- This study highlighted that the CDS disc diffusion technique yielded excellent category agreement with their analogous Etest minimal inhibitory concentrations in comparison to CLSI technique.
- CDS offers the advantage for those laboratories that process small numbers of specimens.
- CDS technique is cost-effective, reliable and easily applicable microbiological method to detect antimicrobial susceptibilities of *N. gonorrhoeae* in resource-poor countries.
- This technique will facilitate enhanced antimicrobial resistance surveillance and direct and meaningful comparison of resistance data generated within different national and international laboratories.

Strengths and limitations of this study

- This is the first study to determine the comparability of CDS and CLSI disc diffusion method with Etest minimal inhibitory concentrations and to accurately and reproducibly assess *N. gonorrhoeae* susceptibilities for penicillin, tetracycline, ceftriaxone, ciprofloxacin and spectinomycin, commonly used for susceptibility testing.
- Susceptibility testing for cefixime, cefpodoxime, gentamicin and azithromycin was performed only by one method because of non-availability of interpretation criteria for these antimicrobials or limitation of resources. Therefore, comparison of above three techniques could not be carried out for these antibiotics.

INTRODUCTION

Gonorrhoea caused by *Neisseria gonorrhoeae* is one of the most common sexually transmitted infections and is a global health problem. Uncomplicated urogenital *N. gonorrhoeae* infections can lead to epididymitis in men and salpingitis in women, conditions that are associated with infertility. In some cases, localised infections can lead to haematogenic dissemination causing severe complications like sepsis, meningitis and endocarditis. The worldwide distribution of *N. gonorrhoeae* and the emergence of resistance to various antimicrobials emphasise the importance of antimicrobial resistance (AMR) surveillance of clinical gonococcal isolates.

Antimicrobial susceptibility testing (AST) may be carried out by a variety of techniques. The most commonly used methods have been the high-content disc diffusion method first described by Bauer *et al*,¹ and later modified by the National Committee for Clinical Laboratory Standards (NCCLS),² the broth micro-dilution technique as described by the NCCLS,³ the agar dilution method described by Ericsson and Sherris,⁴ and adapted by the NCCLS,³ more recently Etest (AB Biodisk, Solna, Sweden), a modification of the disc diffusion and the agar dilution methods mentioned previously. The Etest is an impervious carrier with a continuous gradient of antimicrobial agent applied to one side of the strip. The test is performed in the same manner as the disc diffusion test but determines a minimal inhibitory concentration (MIC) of antimicrobial agent rather than a category result based on a zone size. The Calibrated Dichotomous Sensitivity (CDS) test is a method for determining the antibiotic susceptibility of micro-organisms using agar disc diffusion and was developed in 1969 by Sydney M. Bell assisted by members of the Department of Microbiology, The Prince of Wales Hospital, Sydney, Australia. It was introduced into laboratory practice in Australia in an effort to correct the unsatisfactory results of antibiotic susceptibility testing in Australia that were obtained from surveys conducted by The Royal College of Pathologists of Australia in the late 1960s and early 1970s.⁵

The recent emergence of *N. gonorrhoeae* isolates with decreased susceptibility and resistance to the currently recommended treatment guidelines of the Centres for Disease Control and Prevention, including extended-spectrum cephalosporins (ESC), has further established the necessity for a standardised, reliable, economical, less labour intensive and reproducible susceptibility testing method.^{6–8} Previous studies with the Etest determined that results correlated well with the reference agar dilution method and that it is a useful guide for determining chemotherapy against many organisms,⁹ including gonococci.^{9–11} The CDS disc diffusion technique is recommended by WHO Collaborating Centre for STD, Sydney, Australia, and was regarded as the only practical and affordable means of phenotypic susceptibility testing.¹² It was found to be cost-effective and more feasible during an External Quality Assurance Scheme

(EQAS) in routine diagnostic laboratories in developing countries like India.¹² Earlier, this method was evaluated in comparison to Etest for only three antibiotics (ciprofloxacin, penicillin and ceftriaxone) and that also on a limited number of isolates.¹³ Moreover, tetracycline and spectinomycin (an alternative drug of choice for treatment of gonorrhoea) were not tested in the earlier study of 2005. Recently, CDS technique has been recommended in less-resourced settings for detection of decreased susceptibility to oral ESCs using cefpodoxime disc,¹⁴ and interpretation criteria for azithromycin by CDS have been established in September 2011. The purpose of this study was to determine the comparability of the Etest, CDS and Clinical and Laboratory Standards Institute (CLSI) disc diffusion technique, formerly NCCLS, to accurately and reproducibly assess *N. gonorrhoeae* susceptibilities for five antibiotics commonly used for susceptibility testing, which included penicillin, tetracycline, ceftriaxone, spectinomycin and ciprofloxacin. The aim was also to assess the feasibility of recommending CDS technique for its use in developing and resource-poor countries. To our knowledge, this is the first study to compare the two disc diffusion methods with Etest MICs testing for above five antibiotics.

MATERIALS AND METHODS

N. gonorrhoeae isolates

A total of 295 *N. gonorrhoeae* isolates from all male and female patients presenting with acute gonococcal urethritis and cervical discharge, respectively, to the Regional STD Teaching, Training and Research Centre, Safdarjung Hospital, New Delhi, India, from January 2005 to December 2010 was included in the study. The strains were consecutive and non-repetitive. Methods used for isolation and identification of *N. gonorrhoeae* have been described previously.¹⁵

β -Lactamase testing

N. gonorrhoeae isolates were tested for β -lactamase production by the chromogenic cephalosporin method using nitrocefim freeze-dried powder (Oxoid, Hampshire, UK) or nitrocefim slide (Becton, Dickinson and Company, Sparks, Maryland, USA).¹⁵

Antimicrobial susceptibility testing

Inoculum preparation

The inoculum was prepared from 18 to 24 h pure culture on chocolate agar medium and a homogenised suspension was prepared in 5 ml of sterile saline solution and turbidity was adjusted to an equivalent of 0.5 McFarland standard. Same suspension was used for the following three methods of AST within 15 min. All the following three susceptibility tests were run simultaneously on same day.

CDS disc diffusion technique

Antibiotic susceptibility testing of all the 295 *N. gonorrhoeae* isolates was performed by the CDS technique on

chocolate agar plates (Columbia agar base; HiMedia Laboratories Pvt. Ltd, Mumbai, India) with low concentration antibiotic discs (Oxoid). Six antibiotics with concentrations recommended, that is, penicillin (0.5 IU), tetracycline (10 µg), ceftriaxone (0.5 µg), ciprofloxacin (1 µg), spectinomycin (100 µg) and nalidixic acid (30 µg) were used as per standard methodology.¹⁶ The strains were interpreted as susceptible, less susceptible and resistant.¹⁶ Nalidixic acid was used only to identify isolates less susceptible to ciprofloxacin, and results of susceptibility to this antibiotic are not included.

CLSI disc diffusion method

Same inoculum was used for this method, which was performed using GC agar base (Oxoid Ltd, Hampshire, UK) with 1% isovitalex or vitamino growth supplement (HiMedia Laboratories Pvt. Ltd) with higher disc concentration (HiMedia Laboratories Pvt. Ltd) recommended by CLSI, that is, penicillin (10 IU), tetracycline (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), spectinomycin (100 µg) and nalidixic acid (30 µg) were used.¹⁷ The strains were defined as susceptible, less susceptible and resistant.¹⁷

Etest MIC determination

The MICs of all the isolates for penicillin, tetracycline, ciprofloxacin, spectinomycin and ceftriaxone were determined by the Etest method (AB Biodisk) on GC agar base with 1% isovitalex or vitamino growth supplement. The Etest was performed as specified in the manufacturer's product package insert. The strains were defined as susceptible, less susceptible and resistant.¹⁷ The Etest method was selected as the reference method for comparison of results of CDS and CLSI disc diffusion techniques.

Control strains

N. gonorrhoeae WHO reference strains C, G, K, L, O and Q were used as controls for disc diffusion and Etest MIC testing. The WHO control strains K and L with decreased susceptibility to ESC were included in 2010 as these became available to this centre only by the end of 2009.¹⁸ The reproducibility of control strains tested for Etest, CDS and CLSI disc diffusion method was >95%. This centre participated in EQAS of gonococcal AST from 2001 to 2010 conducted by the Neisseria Reference Laboratory, WHO Collaborating Centre for STD, Sydney, Australia. EQAS results every year showed almost 100% agreement with the reference laboratory expected results, except some disagreement of results was observed for one strain for ceftriaxone in 2002, 2007, 2008, 2010 and for one strain for penicillin in 2004 and 2010. On repeat testing, 100% agreement was observed.

Statistical analysis

Discrepancies were differentiated into three categories, minor (susceptible 'S' interpreted as less sensitive 'LS', 'LS' as 'S' or resistant 'R' and 'R' as 'LS'), major ('S'

interpreted as 'R') and very major ('R' interpreted as 'S'), which are defined by US Department of Health and Human Services Food and Drug Administration.¹⁹ Complete and essential per cent agreement between the reference and test method was evaluated. The complete per cent agreement is the percentage of isolates tested by the test method that gave the same category as those tested by the reference method. The essential per cent agreement value is the percentage of agreement obtained between the reference and test method when minor discrepancies are ignored.¹⁹ Pearson's correlation coefficient (*r* values) was generated for each antimicrobial agent indexed by susceptibility test method. Statistical correlation result was considered perfect for the correlation coefficient (*r* value) of 1.00, desirable for ≥0.90 and acceptable for ≥0.80.

RESULTS

The incidence of susceptible, less susceptible and resistant isolates differed following the performance of both the disc diffusion assays and Etest (table 1). Tables 2 and 3 show the comparison of discrepancies and agreement of CDS and CLSI disc diffusion method with Etest for five different antibiotics. In overall, the rates of discrepancies for different antibiotics differed in both the CDS and CLSI technique on comparison to Etest method. On comparison of CDS method with Etest, the highest discrepancy rate was observed for penicillin (8.5%) and lowest for spectinomycin (0) and overall complete agreement was 82.0%.

Highest discrepancy rate with the CLSI disc diffusion method was detected for tetracycline (25.1%). It was lowest for spectinomycin (0), and overall complete agreement was 49.5%. A total number of minor and major errors was 133 and 16, respectively, for CLSI disc diffusion method, while for CDS, they were only 51 and 2, respectively. Complete percentage agreement for penicillin, ciprofloxacin and tetracycline by CDS test was high in comparison to CLSI disc diffusion technique. It was found to be same for spectinomycin and ceftriaxone. Pearson's correlation coefficient was perfect for all the antibiotics by the CDS method in comparison to CLSI technique, that is, *r* value 1.00 vs 0.92. Moreover, it was very poor (0.61) by CLSI disc diffusion method for tetracycline. Results with individual antibiotics by the three techniques were as follows:

Penicillin

Out of 295 isolates, 34 (11.5%), 156 (52.9%) and 105 (35.6%) isolates were interpreted as susceptible, less susceptible and resistant, respectively, by the Etest method (table 1). On comparison of CDS and CLSI disc diffusion technique with Etest method, minor discrepancies were observed to be 8.5% and 12.5%, respectively, while no major or very major discrepancies were found (tables 2 and 3). The complete per cent agreement of penicillin for the CDS and CLSI method with Etest was 91.5% and 87.5%, respectively, and the essential

Table 1 Results of susceptibility testing of 295 *N. gonorrhoeae* isolates for five antibiotics by three techniques from 2005 to 2010

Method	Penicillin			Ciprofloxacin			Spectinomycin			Ceftriaxone			Tetracycline			Tetracycline		
	S	LS	R	S	LS	R	S	LS	R	S	LS	R	S	LS	R	TRNG	N-TRNG	
Etest	34 (11.5)	156 (52.9)	105 (35.6)	1 (0.3)	48 (16.3)	246 (83.4)	295 (100)	—	0	283 (95.9)	12 (4.1)	0	116 (39.3)	91 (30.8)	30 (10.2)	58 (19.7)	237 (80.3)	
CDS	30 (10.2)	151 (51.2)	114 (38.6)	1 (0.3)	27 (9.2)	267 (90.5)	295 (100)	—	0	282 (95.6)	13 (4.4)	0	—	—	—	60 (20.3)	235 (79.7)	
CLSI	21 (7.1)	157 (53.2)	117 (39.7)	1 (0.3)	64 (21.7)	230 (78.0)	295 (100)	0	0	283 (95.9)	12 (4.1)	0	80 (27.1)	103 (35.9)	64 (21.7)	48 (16.3)	247 (83.7)	

CLSI, Clinical and Laboratory Standards Institute; LS, less susceptible; N-TRNG, not-TRNG; R, resistant; S, susceptible; TRNG, tetracycline-resistant *N. gonorrhoeae*.

agreement for both was found as 100% (tables 2 and 3). Penicillin demonstrated high level of correlation coefficient (0.99) on comparison of both the methods. Antibiotic susceptibility by the Etest method revealed that 105 (35.6%) isolates were resistant to penicillin, out of which 95 (32.2%) were β -lactamase positive and 10 (3.4%) were having chromosomally mediated resistance. Out of remaining 190 (64.4%) isolates, 156 (52.9%) had reduced susceptibility to penicillin and 34 (11.5%) were susceptible to penicillin.

Ciprofloxacin

Only 1 (0.3%) isolate was found susceptible for ciprofloxacin with the Etest method. The number of less susceptible and resistant isolates were 48 (16.3%) and 246 (83.4%), respectively (table 1). Out of 246 resistant isolates, 102 (34.6%) were observed to have high-level resistance (MIC ≥ 4). On comparison of Etest method with CDS and CLSI disc diffusion methods, minor discrepancies were observed as 7.1% and 11.5%, respectively (tables 2 and 3). The complete agreement for both the CDS and CLSI was 92.9% and 88.5%, respectively. No major and very major discrepancies occurred. Person's correlation coefficient was excellent ($r=0.99$) for both the methods (tables 2 and 3).

Ceftriaxone

Out of 295 isolates, 283 (95.9%) were susceptible and remaining 12 (4.1%) were observed to have decreased susceptibility for ceftriaxone by the Etest method (table 1). On comparison of CDS and CLSI disc diffusion methods with the Etest method, 1.7% and 1.4% minor discrepancy were observed, respectively (tables 2 and 3). The complete per cent agreement for ceftriaxone was 98.3% and 98.6% for both the methods. Essential agreement for both the methods was 100% with the Etest method (tables 2 and 3). Pearson's correlation coefficient was perfect ($r=1$) for both the methods.

Spectinomycin

All the isolates were susceptible to spectinomycin by all the three methods (table 1). This resulted in 100% of essential and complete agreement on comparison of the CDS and CLSI method with the Etest method (tables 2 and 3).

Tetracycline

Out of 295 isolates, 58 (19.7%) were tetracycline-resistant *N. gonorrhoeae* (TRNG), while 237 (80.3%) were not-tetracycline-resistant *N. gonorrhoeae* (N-TRNG) by the Etest method (table 1). Out of 237 N-TRNG isolates, 116 (39.3%), 91 (30.8%) and 30 (10.2%) were observed as susceptible, less susceptible and resistant, respectively. On comparison of N-TRNG isolates by the CLSI disc diffusion technique with the Etest method, 5.4% and 19.7% major and minor discrepancies were found, respectively (table 3). The complete and essential percentage agreement for the CLSI technique was 74.9% and 94.6%, respectively. By the CDS method, 60 (20.3%)

Table 2 Comparison of discrepancies and agreement between the CDS and Etest method for 295 *N. gonorrhoeae* isolates

Antimicrobial agent	No. of discrepancies			% Agreement		Correlation coefficient (r)
	Minor	Major	Very major	Complete	Essential	
Penicillin	25	0	0	91.5	100	0.99
Ciprofloxacin	21	0	0	92.9	100	0.99
Spectinomycin	0	0	0	100	100	1.0
Ceftriaxone	5	0	0	98.3	100	1.0
Tetracycline	0	2	0	99.3	99.3	1.0
All	51	2	0	82.0	99.3	1.0

Minor, 'S' and 'R' as 'LS' or 'LS' as 'S' or 'R'; Major, 'S' as 'R'; Very major, 'R' as 'S'.

isolates were observed to be TRNG and 235 (79.7%) N-TRNG (table 1). Only two (0.7%) isolates were found under major discrepancy on comparison of the CDS method with Etest method. Both complete and essential per cent agreement between CDS and Etest method was 99.3% (table 2). Tetracycline demonstrated perfect correlation coefficient ($r=1$) for CDS and Etest method on the basis of TRNG and N-TRNG category comparison (table 2). However, for comparison of 80.3% N-TRNG isolates with Etest method, it was observed to be very poor ($r=0.61$) by the CLSI disc diffusion technique (table 3).

DISCUSSION

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 *N. gonorrhoeae*. The recommended procedure for AST of gonococci is determination of the MICs by an agar dilution technique.¹⁷ However, this method is performed only in research laboratories, mainly in industrialised countries. Moreover, agar dilution technique requiring a number of isolates at one time is not a feasible technique for determining MIC for this organism because the numbers of isolates even in a central-level laboratory are very few and *N. gonorrhoeae* being a fastidious organism is difficult to store for long periods. Etest in a number of studies has already been shown to be an effective alternative and a more appropriate method than the agar dilution technique for MIC determination for *N. gonorrhoeae* by laboratories in developing countries, where resistance of

gonococci to antimicrobial agents is a major public health problem.^{9–11 20} Therefore, the Etest method was considered as the reference method in this study for comparison of MIC results with CDS and CLSI disc diffusion techniques. An excellent essential agreement (92%–99%) was demonstrated between Etest and standard agar dilution technique for the antibiotics commonly used for susceptibility testing of *N. gonorrhoeae*,²⁰ and these percentages were above the recommended limitations ($\geq 90\%$) set by the Food and Drug Administration's *Review Criteria for Assessment of Antimicrobial Susceptibility Devices*, confirming that the Etest satisfactorily approximates the agar reference method.²⁰ Yeung *et al*¹¹ also reported 98% overall agreement between Etest and the agar dilution method.

The original purpose of this study was to assess reliability and comparability of the CDS and CLSI disc diffusion method to predict the interpretative categories of susceptibility as compared with standard Etest method. However, because all test procedures were done from the same inoculum and were carefully controlled, we had a unique opportunity to compare the two methods with Etest method that was used as reference standard and the Etest MIC values were supported by satisfactory Quality Control measures. Protocols in both the disc diffusion techniques differ in their choices of test medium, antibiotic disc content and interpretative breakpoint criteria. Zones of inhibition delineating susceptible, less susceptible and resistant isolates from disc diffusion procedures must correlate with corresponding MICs. In the present study, CDS results' correlation was excellent with Etest MICs that had less

Table 3 Comparison of discrepancies and agreement between the CLSI and Etest method for 295 *N. gonorrhoeae* isolates

Antimicrobial agent	No. of discrepancies			% Agreement		Correlation coefficient (r)
	Minor	Major	Very major	Complete	Essential	
Penicillin	37	0	0	87.5	100	0.99
Ciprofloxacin	34	0	0	88.5	100	0.99
Spectinomycin	0	0	0	100	100	1.0
Ceftriaxone	4	0	0	98.6	100	1.0
Tetracycline	58	16	0	74.9	94.6	0.61
All	133	16	0	49.5	94.6	0.92

CLSI, Clinical and Laboratory Standards Institute; Minor, 'S' and 'R' as 'LS' or 'LS' as 'S' or 'R'; Major, 'S' as 'R'; Very major, 'R' as 'S'.

number of major and minor category discrepancies in comparison to CLSI disc diffusion technique, that is, 2 and 52 versus 16 and 133. Complete per cent agreement of CDS with Etest was 82.0%, while for CLSI disc diffusion method, it was 49.5% that was very poor agreement. CDS method showed excellent correlation coefficient ($r=1$) with Etest for all five antimicrobial agents tested in comparison to CLSI ($r=0.92$). Previously, study of our centre had shown 96.9% agreement for ceftriaxone for comparison of CDS with Etest technique.¹³ Interpretation of disc inhibition zones in CDS method was easier than in the CLSI technique especially when the zone size was near the breakpoint. Double zone of inhibition was observed many times in CLSI technique leading to difficulty in interpretation.

In another study from WHO Collaborating Centre for STD in Australia and Sweden, the correlation between results of CDS disc diffusion testing (cefpodoxime) and MIC determinations with agar dilution and Etest using cefpodoxime, cefixime and ceftriaxone for the detection of decreased ESC susceptibility in *N. gonorrhoeae* was investigated.¹⁴ CDS technique using cefpodoxime 10 µg disc was shown to provide high sensitivity for detection of gonococcal isolates with decreased ESC susceptibility, and it was suggested that this disc test will make it possible to provide AMR surveillance data also from less-developed and/or less-resourced settings, where disc testing is the only practical and affordable means of AMR testing.¹⁴

Our findings reveal poor correlation between CLSI disc diffusion results and Etest MICs, most notably for tetracycline, penicillin and ciprofloxacin. Furthermore, if tetracycline was removed from the CLSI analysis, then the correlation coefficients between the two methods and the Etest were identical. It is the reported in the literature that there are problems on the reproducibility of results with tetracycline, especially when different media are used. Excellent correlation was observed for ceftriaxone and spectinomycin, reason being that resistance was not reported for these antimicrobial agents in the present study. Our results compared well with that from China,²¹ where poor per cent agreement was reported between the CLSI and agar dilution technique for MIC testing. It was 73.6%, 72.3% and 71.4% for ciprofloxacin, penicillin and ceftriaxone, respectively. Given the disparity among susceptibility test results presented here, errors associated with susceptibility testing may result in the unwarranted utilisation or elimination of these antibiotics as part of possible treatment regimens.

The results of our study suggest that CDS disc diffusion technique could be reliably used in resistance surveillance programmes for public health purposes and it can be recommended for use by all the focal point laboratories in WHO GASP network in SEAR because of its excellent agreement with Etest results and also it was simple cost-effective and results are easier to interpret. In many developing countries in SEAR and other

regions, most afflicted by gonorrhoea and AMR, MIC determination using agar dilution method or Etest is not readily accessible or affordable. Especially in those countries, the availability of a sensitive, rapid, inexpensive, easily performed and effective disc test can be highly valuable. Use of a standardised agar diffusion method is practical in these situations and allows fast and reproducible results for clinical microbiology laboratories if standards are observed.²²

To conclude, this is the first study to compare CDS and CLSI disk diffusion method with Etest and the CDS technique yielded excellent category agreement results when compared with the Etest. The data obtained in the present study suggest that the CDS technique is an accurate alternative method for susceptibility testing of *N. gonorrhoeae* for various antimicrobial agents. It is much less cumbersome than the current reference method because of its simplicity, less consumption of media and glassware and is a more appropriate technique in settings with minimal microbiological resources. CDS offers the advantage for those laboratories that process small numbers of specimens, and these laboratories could determine the susceptibilities of gonococcal isolates reasonably accurately. This could facilitate direct and meaningful comparison of resistance data generated within different national and international laboratories.

Acknowledgements The authors are indebted to A/Professor John W. Tapsall AM, Ms. Athena E. Limnios and A/Professor Monica M. Lahra, WHO collaborating centre for STD, Department of Microbiology, The Prince of Wales Hospital, Sydney, Australia, for supplying WHO Reference strains and low concentration antibiotic discs. The support of WHO and NACO is gratefully acknowledged. We are grateful to Mrs Leelamma Peter and Mr Naveen Chandra Joshi for their excellent technical assistance.

Contributors MB designed the study and coordinated the work. VS and MB carried out analysis of data and preparation of manuscript. MK participated in data analysis and VR collaborated in the writing of the manuscript.

Funding This research received no specific grant from any funding agency in public, commercial or not-for-profit sectors.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement There is no additional data available.

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