



Evaluation of the Accuracy of Various Disks and Strips for Rapid Culture-Based Gonococcal Antimicrobial Susceptibility Screening Tests in China


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Purpose: *Neisseria gonorrhoeae*, resistant to the first-line treatment option ceftriaxone, is widespread in China from 2016. Nowadays, diverse reagents of disks and strips for rapid gonococcal antimicrobial susceptibility tests used in clinics are culture-based disks diffusion and gradient strips methods. This study aimed to evaluate the accuracy, quality, and availability of almost all disks and strips acquired in the Chinese market and serve as a reference for clinical selection.

Methods: We tested the performance of 15 commercial disks and 9 commercial gradient strips acquired in China, compared with traditional agar dilution method. The overall performance was evaluated by the categorical agreement. The reagent accuracy of gradient strips was assessed by the essential agreement.

Results: A total of 167 gonococcal isolates were used to evaluate antimicrobial disks from three brands. The overall categorical agreements were 71.7% to 81.8% for ceftriaxone, less than 58% for cefixime, 100% for spectinomycin, over 98% for ciprofloxacin, below 70.5% for penicillin, and 73.3% to 81.8% for tetracycline. A total of 81 isolates were tested for different gradient strips. Categorical agreements were over 96% for ceftriaxone, 86.2% for azithromycin, 62.3% to 67.1% for penicillin, 41.9% to 67.5% for tetracycline, and 95% for ciprofloxacin. Essential agreements were 57.7% to 87.3% for ceftriaxone, 70% for azithromycin, 64.9% to 68.4% for penicillin, 51.8% to 71.2% for tetracycline, and 91.3% for ciprofloxacin.

Conclusion: Rapid test reagents of disks and strips based on gonococcal culture have sub-optimal performance. Disk diffusion for spectinomycin or ciprofloxacin can be recommended for clinical individualized prescription. The gradient strips are of great value to identify ceftriaxone-resistant gonococcal strains. Furthermore, abundant improvements are required for many reagents to further optimize their accuracy till the fulfillment of molecular detection.

Keywords: *Neisseria gonorrhoeae*, disk diffusion test, gradient strip test, antimicrobial susceptibility

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Introduction

Gonorrhea, caused by *Neisseria gonorrhoeae* (*N. gonorrhoeae*), is one of the most prevalent sexually transmitted infections in the world. If left untreated, it can cause adverse fertility outcomes, and facilitate the transmission of HIV.¹ *N. gonorrhoeae* has become a public health concern globally due to the antimicrobial resistance. *N. gonorrhoeae*, resistant to the first-line treatment option ceftriaxone, has spread in many countries, including China.²⁻⁴ In China, gonorrhea was the fourth most commonly reported notifiable communicable disease, with 117,938 cases in 2019.⁵

However, the isolates collected for antimicrobial susceptibility testing (AST) by the China Gonorrhoeae Resistance Surveillance Program (GRSP) accounted for less than 1.8% (2344/133,156) in 2018,⁵⁻⁷ and these tests were totally based on the traditional agar dilution method. Although the agar dilution method is the gold standard method for AST, it is a time- and labor-consuming method and not suitable for rapid clinical tests. Ideally, the China-GRSP should strengthen the monitoring of the AMR trends and inform the adjustment of treatment guidelines, yet an alternative and comparative AST method remains necessary.

ASTs in *N. gonorrhoeae* include qualitative tests (disk diffusion assay and molecular tests) and quantitative tests (agar dilution method and E-test method). The agar dilution method is the gold standard method to detect minimum inhibitory concentrations (MICs) and is recommended mainly for reference laboratories. The MIC of gonococcal isolates was defined as the lowest concentration of the antibiotic that inhibited its growth, with which clinicians can prescribe antibiotics precisely and individually. The disk diffusion test is relatively simple and also well accepted. Yet the observed diameter of inhibition-zone is relatively imprecise and cannot be converted into a concrete MIC value. In addition, a recent study reported that disks made of Oxoid show suboptimal accuracy for ceftriaxone and cefixime in clinics compared with values determined by the agar dilution method.⁸ E-test (bioMérieux, France), one of the labeled gradient strip methods, is suitable as an alternative test to the agar dilution method, although it has not been approved by the China Food and Drug Administration. Moreover, the molecular tests have a restricted detection range for predicting specific antimicrobial resistance in gonococcal isolates, such as cephalosporin, ciprofloxacin, and azithromycin resistance.^{9,10} Nonetheless, disk diffusion and gradient strip methods are nowadays the first choice in microbiological laboratories and hospitals world-wide. Improvements of these two methods benefit clinics for their timely and precise prescription.

In this study, we tested the performance of different commercial disks (Oxoid, Binhe, Kangtai) and gradient strips (Oxoid, Antu, Kangtai) acquired in China. By comparing the performance with the traditional agar dilution method for the evaluation of suitability and reliability, we recommend which reagents for clinical use in some resource-limited areas where the agar dilution method cannot be used, based on the price, operational complexity, and result reliability. In the meantime, it bridges the gap before the realization of molecular detection.

Materials and Methods

Ethics Approval and Consent to Participate

The gonococcal isolates were collected through the China Gonococcal Resistance Surveillance Programme (China-GRSP), and had been saved for use in the present study. The ethics approval for the study was obtained from the Medical Ethics Committee at the Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College (2014-LS-026). Participants no less than 18 years of age who signed an informed consent to provide urine and vaginal and rectal swabs were enrolled in the study. Samples were inoculated, identified, preserved, and transferred as previously described.⁷

Bacterial Strains

A total of 167 clinical samples from urethral swabs were collected and subsequently cultured on selective Thayer-Martin (TM) media. Microscopy, rapid oxidase reaction, and carbohydrate utilization test were used for verification. All gonococcal isolates were preserved in skimmed milk and stored at -70°C as part of routine diagnostics. Strains for further tests were randomly selected, then cultured onto selective TM media and sub-cultured on GC agar base media (Oxide, Hampshire, England) supplemented with 10% defibrinated sheep blood (Bianzhen Biotechnology, Nanjing, China) and 1% Iso VitaleX Enrichment (BD Diagnostics, New Jersey, USA) at 36°C in a moist 5% CO_2 -enrichment atmosphere for 18 to 20 hours.

Agar Dilution Test

The MICs were determined according to the agar dilution method recommended by WHO as previously described.⁷ The 2016 WHO *N. gonorrhoeae* reference strains panels (G, K, L, and P) were included as outer quality control in every batch of experiments.⁷

Disk Diffusion and Gradient Strip Test

Commercialized disks (Oxoid for ceftriaxone, cefixime, spectinomycin, penicillin, tetracycline, and ciprofloxacin; Binhe for ceftriaxone, cefixime, spectinomycin, penicillin, tetracycline, and ciprofloxacin; Kangtai for ceftriaxone, cefixime, spectinomycin, penicillin, tetracycline, and ciprofloxacin) and commercialized MIC gradient strips (Oxoid for penicillin, tetracycline, and ciprofloxacin; Antu for ceftriaxone, azithromycin, and penicillin; Kangtai for ceftriaxone, penicillin, and tetracycline) were evaluated for their

antimicrobial susceptibility accuracy. Three manufacturers' antimicrobial disks were assessed for ceftriaxone, azithromycin, cefixime, spectinomycin, penicillin, tetracycline, and ciprofloxacin, in addition to disks from Oxoid for azithromycin. All disks and strips were purchased for routine diagnostic use, and none of the companies had any influence on the design or performance of our study.

All tests were performed in accordance with the manufacturer's instruction and WHO recommendations.¹¹ The same GC agar base media supplemented with sheep blood and enrichment were used for strain culture. The identical reference strains used in agar dilution test were included in every batch of testing. The results were interpreted by reading the diameter of the inhibition zone measured with a ruler (disk diffusion) or the lowest growth inhibition concentration (gradient strip). Nearest diameter breakpoints and MIC breakpoints from the Clinical and Laboratory Standards Institute (CLSI; em100.edaptivedocs.net/dashboard.aspx) were used for the categorization of susceptibility (S), intermediate (I), and resistance (R). For azithromycin, where CLSI states no SIR breakpoints, MIC breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org/clinical_breakpoints/) were applied.

Statistical Analyses

The overall performance of the assays was evaluated by determining the categorical agreement (CA). Results were considered CA when isolates had the same susceptible, intermediate, susceptible dose-dependent, and resistant category as the agar dilution method category result. Very major errors were R strains being misclassified as S. Major errors were S strains being misclassified as R. Minor errors occurred when one method categorized an isolate as I while the other method defined it as S or R, as previously described.¹² With no breakpoints for azithromycin, we determined the Pearson's correlation tests between diameters and MIC values.

The reagent accuracy of gradient strips was assessed by calculating the essential agreement (EA), which was defined as the percentage of strains with predicted MICs that did not deviate by more than ± 1 doubling dilution from agar dilution MICs. Ideally, an EA between different tests should be $>90\%$.

Results

During each batch of the tests, MIC values obtained from the reference strains were identical or within 1 doubling dilution of those previously reported.¹³

A total of 167 randomly selected gonococcal isolates were used to evaluate antimicrobial disks from three brands. Specific numbers of categorical accuracy are shown in Table 1. For ceftriaxone and cefixime, breakpoints from the CLSI can only distinguish susceptibility and non-susceptibility, with no very major errors or major errors. The overall categorical agreements of disk diffusion method to ceftriaxone were 71.7% for Oxoid, 73.2% for Binhe, and 81.8% for Kangtai, respectively. Categorical agreements for cefixime were suboptimal, showing less than 58% for all three manufacturers. Categorical agreements for spectinomycin were 100% because no resistant strains were tested in our study. Likewise for ciprofloxacin, categorical agreements of all three disks surpassed 98%. Concordance rates for penicillin went below to 70.5%. For tetracycline, agreements were 73.3% (Oxoid), 76.4% (Binhe), and 81.8% (Kangtai). Without recommended diameter breakpoints being proposed in CLSI or EUCAST, we calculated the Pearson's correlation coefficients between their diameters and MIC values. Disks for azithromycin made in Kangtai (r , 0.59) correlated better than those from Binhe (r , 0.52) with a statistical significance.

Nearly 81 isolates were tested for susceptibility accuracy of different gradient strips. The overall performances for five antimicrobials are provided in Table 2. For ceftriaxone, essential agreements were 87.3% for Antu, and 57.7% for Kangtai, with categorical agreements for both exceeding 96%. For azithromycin, only strips made from Antu were detected, with 70.0% and 86.3%, respectively, for its essential and categorical concordance. Gradient strips of Oxoid, Antu, and Kangtai were evaluated for penicillin, with no major or very major errors found, and the agreements ranged from 62.3% to 68.4%. For tetracycline, accuracy of Oxoid was higher than that of Kangtai. Only strips of Oxoid were purchased for ciprofloxacin, with 91.3% and 95% separately for essential and categorical agreements, respectively.

Discussion

Monitoring the trend of clinical *N. gonorrhoeae* with reduced susceptibility to different antimicrobials is an important task for public health agencies. Yet, currently there is no large-scale comparative study on definite accuracy of different AST agents in China for clinically widely used disks or strips methods. In this study, we evaluated the performance of 27 commercial reagents for disk diffusion and gradient strip methods, using strains randomly

Table 1 Categorical Agreements of Disk Diffusion Reagents for 6 Antimicrobials

	Ceftriaxone			Cefixime			Spectinomycin			Penicillin			Tetracycline			Ciprofloxacin		
	Oxoid	Binhe	Kangtai	Oxoid	Binhe	Kangtai	Oxoid	Binhe	Kangtai	Oxoid	Binhe	Kangtai	Oxoid	Binhe	Kangtai	Oxoid	Binhe	Kangtai
Number tested	166	164	165	157	98	159	166	157	166	166	167	166	165	157	165	165	162	164
Categorical agreement (No.)	119	120	135	90	34	91	166	157	166	116	112	117	121	120	135	163	159	162
Categorical agreement rate (%)	71.7	73.2	81.8	57.3	34.7	57.2	100	100	100	70.0	67.1	70.5	73.3	76.4	81.8	97.8	98.1	98.7
Very major errors (No.)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Major errors (No.)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Minor errors (No.)	47	39	50	67	64	68	0	0	0	50	54	49	44	36	30	2	3	2
Minor error rate (%)	28.3	23.8	30.3	42.7	65.3	42.8	0.0	0.0	0.0	30.1	32.3	29.5	26.7	22.9	18.2	1.2	1.9	1.2

selected from China GRSP, in order to formulate our recommended methods and reagents.

The disk diffusion method is less labor-intensive and easier to carry out than the traditional agar dilution method, which is, rather, suitable for clinical tests to guide individually treatment or find resistant strains for microbial infections. In our comparative study, categorical agreements showed suboptimal results despite being carried out in one laboratory. For ceftriaxone and cefixime, performance rates of six manufacturers were less than accepted percentages (defined as >90% for categorical agreements and <7% for minor errors), and the performance is worse for cefixime. Agreements in our study, resembling the results conducted in China GRSP,⁶ were much lower than other comparative researches (Oxoid) in foreign countries, all of which showed concordant rates over 89%.¹⁴⁻¹⁶ The range of susceptibility values of these two antimicrobials was larger and comprehensive, which may lead to suboptimal agreements in studies conducted in China. All the disks had excellent agreements for ciprofloxacin and spectinomycin, notwithstanding that the clinical strains collected were mostly resistant or susceptible to them, indicating that these two disks may have clinical efficiencies for individual prescription like molecular assay kits, which performed well in the detection of AMR determinants for predicting phenotypic ciprofloxacin and azithromycin resistance.¹⁷ Disk diffusion has inferior accuracy for tetracycline and penicillin, but reagents from Kangtai performed best. Overall, as cheap and easy as the disk diffusion method is, training and experience are required for reading the results. Reading the diameters of different disks can be subjective, which can affect the outcome, especially for the antimicrobials with low MIC values. Similarly, the E-test method, one of the gradient strip methods, is another alternative AST method. The E-test method has results comparable with the standardized agar dilution method, but is less labor-intensive. However, the E-test strips made by bioMerieux are not commercially available in China, with no allowance of passports and no clinical ratification from CFDA. Strips sold in China perform with the same principle as E-test plastic strips, but are expensive (over \$4.50) due to patent protection. Hence, gradient strips for ceftriaxone and azithromycin in our study are both fabricated domestically. Despite its high concordance in ciprofloxacin, the price made it suboptimal than ciprofloxacin disks. Compared with overseas study on variant gonococcal gradient strips, all the strips tested in our research showed acceptable agreements for ceftriaxone categorical potency.¹⁸ Strips made by

Table 2 Categorical and Essential Agreements of Gradient Strip Reagents for 5 Antimicrobials

	Ceftriaxone		Azithromycin	Penicillin			Tetracycline		Ciprofloxacin
	Antu	Kangtai	Antu	Oxoid	Antu	Kangtai	Oxoid	Kangtai	Oxoid
Number tested	79	78	80	77	77	76	80	81	80
Essential agreement (No.)	69	45	56	50	48	52	57	42	73
Essential agreement rate (%)	87.3	57.7	70.0	64.9	62.3	68.4	71.3	51.9	91.3
Categorical agreement (No.)	77	75	69	48	50	51	54	34	76
Categorical agreement rate (%)	97.5	96.2	86.3	62.3	64.9	67.1	67.5	42.0	95.0
Very major errors (No.)	0	0	1	0	0	0	3	3	0
Major errors (No.)	0	0	0	0	0	0	0	0	0
Minor errors (No.)	2	3	10	29	27	25	23	44	4
Minor error rate (%)	2.5	3.8	12.5	37.7	35.1	32.9	28.8	54.3	5.0

Antu got higher categorical agreements than those by Liofilchem and HiMedia,¹⁸ but all the rates were below 86.3%. In short, the gradient strips can be recommended as an alternative AST method in order to quickly find out the strains resistant to the first-line option ceftriaxone and control the infection at an early stage, regardless of the brands.

Considering variant kinds of antibiotics, the susceptibility profiles to first-line drugs (ceftriaxone or spectinomycin) can directly be distinguished by rapid tests. But, for other treatment options except ciprofloxacin, qualities of the antibiotic disks and strips, no matter their brands, need to be further improved.

This is the first study comparing so many disks and gradient strips for gonococcal ASTs in the Chinese market. Nevertheless, there are also limitations in our study. First, the time for gonococcal incubation needs to be shortened by changing to a more nutrient-rich medium, or a more suitable incubation environment. In China, most doctors prescribe antibiotics for gonococcal infection just after a smear microscopy instead of waiting days for the AST results. Second, the reagents compared in our study cannot be fully matched in the number of tests because some items were out of stock during the experiment.

In conclusion, the overall performances of antimicrobial disks and strips are suboptimal to the agar dilution method for susceptibility testing in *N. gonorrhoeae*, but some reagents can be recommended for rapid clinical detection and routine antimicrobial resistance surveillance. In detail, disk diffusion for spectinomycin or ciprofloxacin can be recommended as an initial detection for

individualized prescription, and the gradient strips are of great value to identify ceftriaxone-resistant gonococcal strains. Furthermore, the price of strips needs to be regulated, and a strict quality assurance (both at manufacturing and reference laboratory level) is required, including internal and external quality controls. Ultimately, before the arrival of a rapid PCR-based molecular method, the traditional disks and strips methods will still dominate the market and are in need of further improvements. Last but not least, all the results were detected from our laboratory, and are intended to serve as primary references for clinical usage.

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Disclosure

The authors report no conflicts of interest in this work.

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