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Implementation and Evaluation of Gradient Strip Antimicrobial Susceptibility Testing in US Public Health Laboratories to Respond to Resistant Gonorrhea

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Abstract

Background: Gradient strip antimicrobial susceptibility testing using Etest is conducted by local public health jurisdictions participating in the Strengthening the US Response to Resistant Gonorrhea (SURRG) program to inform public health responses to resistant gonorrhea. Proficiency testing results across the participating laboratories were analyzed and a comparison of Etest with the agar dilution method was conducted.

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Methods: Laboratories participating in SURRG performed Etest for azithromycin (AZM), cefixime (CFX), and ceftriaxone (CRO). Concurrence between minimum inhibitory concentrations (MICs) obtained with Etest versus the agar dilution method using corresponding isolates was defined as ±1 double dilution. Specific levels of reduced susceptibility were termed “alerts” and included isolates with the following MICs: ≥2.0 μg/mL (AZM), ≥0.25 μg/mL (CFX), and ≥0.125 μg/mL (CRO). Categorical (alert/nonalert) agreement was calculated for MICs determined using Etest and agar dilution methods.

Results: Strengthening the US Response to Resistant Gonorrhea laboratories had high proficiency testing scores (≥98%) and low levels of interlaboratory variations in MICs. The overall concurrence of MICs (essential agreement) determined using agar dilution, and Etest was 96% (CRO), 96% (CFX), and 95% (AZM). Depending on the antibiotic tested, between 27% and 66% of isolates with alert MICs determined by Etest also had alert MICs using the reference agar dilution methodology; however, most of these alert MICs were detected at threshold levels.

Conclusions: This study demonstrates that MICs produced by SURRG laboratories using Etest have a high level of concurrence with agar dilution. Although confirmation of specific alert MICs varied, Etest facilities rapid detection and response to emerging resistant gonorrhea.

In 2017, the Centers for Disease Control and Prevention (CDC) initiated a partnership with local public health departments through the Strengthening the US Response to Resistant Gonorrhea (SURRG) project. One of the objectives of SURRG is to enhance laboratory capacity for culturing and performing antimicrobial susceptibility testing (AST) of *Neisseria gonorrhoeae* (NG) using gradient strips. Local public health laboratories (PHLs) isolate NG from patient specimens and conduct AST for azithromycin (AZM), ceftriaxone (CRO), and cefixime (CFX) using Etest (bioMérieux, Durham, NC) gradient strips. After performing ASTon site, isolates are sent to 1 of 4 Antibiotic Resistance Laboratory Network (AR Lab Network) regional laboratories to confirm AST results by the standard agar dilution methodology for 7 antibiotics including AZM, CRO, and CFX.[1]

A total of 8 PHLs agreeing to participate in SURRG had different initial levels of experience in NG culture and AST. Antimicrobial susceptibility testing training was offered to all laboratories; 6 of the laboratories accepted. On-site training was provided to the 6 PHLs and included activities ranging from performing Etest AST to minimum inhibitory concentration (MIC) reading and interpretation. Strengthening the US Response to Resistant Gonorrhea requires that 3 QC strains are run simultaneously with all test isolates. Further support was provided to help conduct validations required to ensure testing was compliant with Clinical Laboratory Improvement Amendments (CLIA) regulations. Prior to commencing Etest AST for SURRG, all laboratories were required to pass AST using a blind panel comprising 5 NG isolates in triplicate.

In this study, we explored how implementation of rapid AST conducted at the SURRG laboratories can enhance detection of NG isolates with elevated MICs to select antibiotics (including those recommended for treatment of gonorrhea). Etest is less expensive and faster than the reference agar dilution AST method. The purpose of this study was to determine how Etest results obtained in local PHLs align with agar dilution AST results. The accuracy

of local rapid detection of antibiotic resistant NG is a crucial part of the public health response aimed at stopping the spread of resistant gonorrhea.

METHODS

Data Sources

Data (including isolate source, date of collection, Etest MIC, etc.) for 13,182 isolates from 2017 through 2019 were analyzed. Data were collected from the 8 participating SURRG grantees: California (San Francisco County), Colorado (Denver County/Denver), Indiana (Marion County/Indianapolis), Hawaii (Honolulu County/Honolulu), New York City, North Carolina (Guilford County/Greensboro), Washington (King County/Seattle), and Wisconsin (Milwaukee City). Data also included corresponding agar dilution MICs generated at the AR Lab Network regional laboratories. The CDC's institutional review board reviewed the SURRG protocol and determined the project to be a public health activity and not human subject research.

Antimicrobial Susceptibility Testing

Specimens for NG culture were collected from urethral, vaginal, endocervical, rectal, and pharyngeal sites of infection. Various culture transport systems and primary NG culture media were used by the STD clinics and other community clinics participating in SURRG. NG isolate confirmation included biochemical assays (eg, API NH, bioMérieux) and/or MALDI-ToF. Gradient strip AST for AZM, CRO, and CFX was conducted using Etest (bioMérieux) strips, and agar dilution was conducted as previously described.[2]

Laboratories also ensure that QC strains have MIC ranges within established acceptable ranges. Between 2017 and 2019, 3 QC strains were used: F-18, SPJ-15, and SPL-4. The expected MIC ranges for these strains were as follows: F-18 (0.25–1.0 μg/mL [AZM], 0.004–0.03 μg/mL [CFX], 0.004–0.016 [CRO]), SPJ-15 (1.0–8.0 μg/mL [AZM], 0.008–0.06 μg/mL [CRX], 0.004–0.016 μg/mL [CRO]), and SPL-4 (0.125–1.0 μg/mL [AZM], 0.25–0.5 μg/mL [CFX], 0.03–0.25 μg/mL [CRO]). While the Clinical and Laboratory Standards Institute (CLSI) does not define resistance breakpoints for AZM, CRO, and CFX, SURRG defines alert MICs, which trigger programmatic action because of the reduced susceptibility for these antibiotics. Alert values are defined as follows: AZM MIC of 2.0 μg/mL or greater, CRO MIC of 0.125 μg/mL or greater, and CFX MIC of 0.25 μg/mL or greater.

NG Etest AST Proficiency Testing

To ensure accuracy of AST data, all SURRG laboratories are required to enroll in a proficiency testing (PT) program and partake in 2 PT challenges per year. In 2017, when SURRG began the implementation of Etest AST for NG, there were no PT providers for this test. US Centers for Disease Control and Prevention partnered with the Wisconsin State Laboratory of Hygiene (WSLH) to develop a PT program for gonococcal Etest AST. Wisconsin State Laboratory of Hygiene is a Center for Medicaid Services approved agency for providing PT. Ever since, all laboratories participating in the SURRG project are required to enroll in the Etest AST PT administered by the WSLH. Two times each year, the WSLH sends to each laboratory a challenge panel consisting of 5 gonococcal isolates which

are tested in triplicate. All laboratories are required to perform Etest AST against CRO, CFX, and AZM on the PT panel. The WSLH also analyzes and provides a PT report to each laboratory. The PT passing score is 80% and is calculated based on ±1 doubling dilution of the modal MIC reported by the laboratories performing the PT. Interlaboratory Etest MIC variation was calculated based on the exact MICs reported by each laboratory.

Data Analysis

Concurrence of Etest results was defined as MICs within ±1 doubling dilution. Although the lower limit of agar dilution AST was below 0.016 μg/mL, the Etest strips used by most of the participating laboratories during the study period had a lower limit of 0.016 μg/mL, and MIC concurrence could only be assessed at or above this level. Because alert MICs are associated with a specific threshold, we also calculated the percentage of isolates with an Etest alert MIC for each antibiotic that also had an agar dilution MIC at or above the alert threshold. All statistical analyses were conducted using R.[3]

RESULTS

PT of Etest Conducted in Local PHLs

From 2017 to 2019, the average PT scores across the 8 current laboratories participating in SURRG were 99.7%, 98.7%, and 98.0% for AZM, CFX, and CRO, respectively. In addition, the 3-year average interlaboratory MIC variations were 26.5% (AZM), 18.2% (CFM), and 21.7% (CRO).

Comparison of Agar Dilution MICs to Etest

The modal agar dilution MIC for CRO was 0.008 μg/mL (Table 1). The overall MIC concurrence between Etest and agar dilution CRO MICs was 96%. At the agar dilution alert MIC (0.125 μg/mL), the alert category concurrence using Etest results was only 83%. For Etest, most of the discordant MICs were 0.032 μg/mL or greater.

The modal agar dilution MIC for CFX was 0.016 μg/mL (Table 1). The overall MIC concurrence between Etest and agar dilution CFX MICs was 96%. At the agar dilution alert MIC (0.25 μg/mL), the alert category concurrence using Etest results was 86%. For Etest, most of the discordant MICs were ≤0.064 μg/mL.

The modal agar dilution MIC for AZM was 0.25 μg/mL (Table 1). The overall MIC concurrence between Etest and agar dilution AZM MICs was 95%. At the agar dilution alert MIC (2.0 μg/mL), concurrence with Etest results was 99%. For Etest, more than half of the discordant MICs were 8 μg/mL or greater. Notably, the Etest concordance for isolates with an AZM agar dilution MIC of 0.016 μg/mL was only 63%; all of the discordant Etest MICs were 0.064 μg/mL or greater.

Confirmation of Isolates With Alert MICs

A total of 1449 isolates had an Etest alert to at least 1 antibiotic (CRO, CFX, or AZM), most commonly for AZM (94%). Isolates with alert MICs to CRO or CFX accounted for 3% and

4% of all alerts, respectively. Isolates with alert MICs to both CRO and CFX accounted for just 1% of all Etest alerts.

A total of 52 isolates with CRO Etest alerts were detected (51 of which had an MIC of 0.125 μg/mL), but only 14 (27%) of these isolates also had an alert MIC by agar dilution (Table 2). There were 42 isolates with CFX Etest alert MICs, and 25 (60%) of these isolates also had alert MICs by agar dilution. Of the isolates with CFX Etest alert MICs, 36 (86%) had an MIC = 0.25 μg/mL. For AZM, there were 1358 isolates with Etest alert MICs, and 903 (66%) of these had corresponding agar dilution alert MICs. Of the isolates with AZM Etest alert MICs, 1047 (77%) had an MIC of 2.0 μg/mL.

DISCUSSION

Antimicrobial susceptibility testing using gradient strips (such as Etest) is faster and less labor-intensive than agar dilution. Extensive media preparation time is required for agar dilution, which uses multiple plates of media containing different concentrations of antibiotics. Moreover, accurate methods to replicate inoculum on each plate are needed. Although PHLs participating in SURRG had differences in previous capacity for performing GC culture and AST, all sites were able to conduct method validations to support CLIA regulations and pass initial blinded competency assessments. Notably, at the inception of SURRG, only nonsusceptible breakpoints had been adopted by CLSI, and AZM had no defined breakpoints. As a result, programs needed to conduct performance evaluations of Etest as a laboratory-developed test.

To support ongoing PT for the SURRG laboratories, CDC collaborated with an external laboratory (WSLH) to provide 2 PT panels annually. The high PT scores (≥98%) and low level of interlaboratory variations in MICs suggest that AST data generated through SURRG are reliable and valid. These data lend confidence to the use of Etest AST results for programmatic and clinical applications.

Concurrence between Etest and agar dilution was defined as MICs within ±1 doubling dilution. There was a high concurrence between the AST methods for the 3 antibiotics tested in SURRG; 96% (CRO), 96% (CFX), and 95% (AZM). At the agar dilution alert MIC for CRO and CFX, most discordant Etest MICs were lower by 2 or more doubling dilutions. Although the concurrence of Etest MICs at the AZM alert MIC was high (99%), concurrence at the Etest lower limit MIC (0.016 μg/mL) was only 63%. Notably, the discordant Etest MICs were 2 or more doubling dilutions greater.

Although Etest concurrence with agar dilution (ie, essential agreement) was very high, this analysis allowed some variation in MICs (±1 doubling dilution). Categorical agreement requires that MICs produced by the 2 methods fall into the same category (eg, resistant, susceptible, etc.). Clinical and Laboratory Standards Institute defines only susceptibility breakpoints for AZM (≤1 μg/mL), CFX (≤0.25 μg/mL), and CRO (≤0.25 μg/mL).[4] The susceptible categorical agreement between the Etest and agar dilution methods were as follows: 100% (CRO), >99.9% (CRX), and >99.9% AZM. For programmatic action (eg, partner services), SURRG defines alert MICs at or above a specific threshold.[5] Confirmation

of alert MICs determined by Etest using agar dilution was successful for only 27% to 66% of isolates (depending on the antibiotic tested). This reduction in the number of confirmed Etest alert MICs using agar dilution methodology is likely due to a combination of factors including expected variation in MICs generated using a different method (ie, agar dilution) and most isolates with Etest alerts (77–98%) had MICs at the alert threshold. As a result, a single lower doubling dilution MIC using agar dilution obviates an isolate's alert category concurrence. Although isolates with Etest alert MICs have variable levels of alert category confirmation using agar dilution methodology, Etest can be used to efficiently detect and respond to resistant gonorrhea in local jurisdictions.

Other studies have demonstrated high concordance between Etest and agar dilution using smaller datasets. In a previous study,[6] AZM MIC agreement (±1 doubling dilution) across replicate isolates was 100% using Etest and agreement with agar dilution was 91%. In a separate study,[7] MICs for CRO, CFX, and cefpodoxime using Etest and agar dilution methods were within 1 doubling dilution for 80% or greater of isolates and within 2 doubling dilutions for greater than 90% of isolates tested.

There are several limitations to this study. Because participating laboratories implemented local procedures for conducting Etest, differences in media used by SURRG laboratories for Etest could have effects on concordance of MICs with agar dilution. Indeed, Liao et al[8] found differences in Etest MICs using corresponding isolates tested on GC agar or chocolate agar. Nonetheless, the low level of interlaboratory variation suggests differences in media use among SURRG laboratories is unlikely to be a significant confounding factor. Another potential limitation to the study is that confirmatory agar dilution AST was conducted at different AR Lab Network regional laboratories creating an additional source of variation. Further analysis of this dataset could help elucidate possible interlaboratory variation, as well as identify potential differences in AST method concurrence associated variables, such as with Etest media usage or specimen type from which the isolate was recovered.

Overall, the implementation of Etest AST at local PHLs demonstrates high concurrence with agar dilution AST. The ability of these results to be generated locally helps drive more rapid responses to antibiotic resistant gonorrhea. Moreover, lessons learned from implementation of the laboratory aspects of SURRG can help guide further expansion of rapid AST in other jurisdictions.

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REFERENCES

1. Kersh EN, Pham CD, Papp JR, et al. Expanding U.S. Laboratory Capacity for *Neisseria gonorrhoeae* Antimicrobial Susceptibility Testing and Whole-Genome Sequencing through the CDC's Antibiotic Resistance Laboratory Network. J Clin Microbiol 2020; 58:e01461–e01419. [PubMed: 32024723]
2. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 11th ed. Wayne, PA: CLSI standard M07. Clinical and Laboratory Standards Institute, 2018.
3. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2020. Available at: https://www.R-project.org/.
4. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 31st ed. Wayne, PA: CLSI supplement M100. Clinical and Laboratory Standards Institute, 2021.
5. Schlanger K, Kirkcaldy RD. Rising to meet the programmatic public health challenges of emerging Neisseria gonorrhoeae antimicrobial resistance: Strengthening the United States Response to Resistant Gonorrhea. Sex Transm Dis 2021; 48(12S):S91–S92. [PubMed: 34310529]
6. McAuliffe GN, Smith M, Cooper G, et al. Variability in azithromycin susceptibility results for *Neisseria gonorrhoeae* obtained using gradient MIC strip and agar dilution techniques. J Clin Microbiol 2019; 57: e01353–e01319. [PubMed: 31578264]
7. Liu H, Taylor TH Jr., Pettus K, et al. Assessment of Etest as an alternative to agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. J Clin Microbiol 2014; 52:1435–1440. [PubMed: 24554750]
8. Liao CH, Lai CC, Hsu MS, et al. Antimicrobial susceptibility of Neisseria gonorrhoeae isolates determined by the agar dilution, disk diffusion and Etest® methods: comparison of results using GC agar and chocolate agar. Int J Antimicrob Agents 2010; 35:457–460. [PubMed: 20188518]

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Table 1.

Percentage of NG Isolates That Have Etest AST Results Within 1 Doubling Dilution of Agar Dilution Susceptibility Testing Results, by Antimicrobial and MIC, SURRG, 2017–2019

	CRO*		CFX*		AZM†	
Agar Dilution MIC (µg/mL)	No. Isolates‡	% Agreement§	No. Isolates	% Agreement	No. Isolates	% Agreement
0.001	250	ND	0	ND	0	ND
0.002	435	ND	150	ND	0	ND
0.004	2323	ND	483	ND	0	ND
0.008	5418	ND	2405	ND	14	ND
0.016	3006	97	5700	96	60	63
0.032	1283	93	3080	99	416	81
0.064	279	89	874	78	1054	88
0.125	30	83	285	87	3397	94
0.25	1	0	44	86	4601	98
0.5	0	ND	4	75	1419	95
1.0	0	ND	0	ND	1075	95
2.0	0	ND	0	ND	798	99
4.0	0	ND	0	ND	65	94
8.0	0	ND	0	ND	44	93
16.0	0	ND	0	ND	85	94

*Lower limit of CRO and CFX Etest MIC = 0.016 µg/mL.

†Upper limit of AZM Etest MIC = 250 µg/mL. Concurrence with agar dilution result defined as MIC ≥16 µg/mL.

‡Restricted to isolates with agar dilution MIC and corresponding Etest MIC.

§Percentage of isolates with concurrent Etest MIC (±1 doubling dilution of agar dilution MIC). ND, not determined.

TABLE 2.

Etest Alert MIC and Confirmation Using Agar Dilution, SURRG, 2017–2019

		Etest Alerts* (No. Isolates)		Comparison of Etest to Agar Dilution† (No. Isolates, %)		
Antibiotic	**Alert MIC, μg/mL**	**≥ Alert MIC**	**Exactly Alert MIC (%)**	**< Alert MIC**	**Exactly Alert MIC**	**> Alert MIC**
CRO	0.125	52	51 (98%)	38 (73%)	14 (27%)	0 (0%)
CFX	0.25	42	36 (86%)	17 (40%)	22 (52%)	3 (7%)
AZM	2	1358	1047 (77%)	455 (34%)	712 (52%)	191 (14%)

*Restricted to isolates with Etest MIC and corresponding agar dilution MIC.

†Comparison of Etest and agar dilution based MICs for corresponding isolates. Percentages based on number of isolates with ≥ Etest alert MIC.