5. Grundmeier M, Tuchscherr L, Bruck M et al. Staphylococcal strains vary greatly in their ability to induce an inflammatory response in endothelial cells. J Infect Dis **2010**; 201:871–80.
6. Lam TT, Giese B, Chikkaballi D et al. Phagolysosomal integrity is generally maintained after *Staphylococcus aureus* invasion of nonprofessional phagocytes but is modulated by strain 6850. Infect Immun **2010**; 78:3392–403.

Received 10 October 2016; accepted 10 October 2016; published online 14 December 2016.

Correspondence: A. S. Zinkernagel, Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Rämistrasse 100, 8091 Zürich, Switzerland

The Journal of Infectious Diseases® **2017;215:490–1**
© The Author 2016. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.
DOI: 10.1093/infdis/jiw504

Codon 91 Gyrase A Testing Is Necessary and Sufficient to Predict Ciprofloxacin Susceptibility in *Neisseria gonorrhoeae*

To the Editor—We read with great interest the article by Grad et al [1]. We agree with their conclusion that gyrase A (*gyr*A) genotype testing of *Neisseria gonorrhoeae* is a valuable means of resistance testing; however, we believe that *gyr*A testing, specifically of codon 91, is both necessary and sufficient for predicting susceptibility to ciprofloxacin. There have been 11 studies (N=4777 specimens) comparing real-time polymerase chain reaction (RT-PCR) genotype results with conventional antimicrobial susceptibility testing methods, all of which have demonstrated high sensitivity and specificity (93.8%–100% and 93.2%–100%, respectively). Positive and negative predictive values were similarly impressive (94.4%–100% and 87.5%–100%, respectively). Furthermore, 4 studies found that mutation at codon 91 of the *gyr*A gene as determined by RT-PCR was 100% specific for *N. gonorrhoeae* compared with other *Neisseria* species [2–5].

Other mutations have been shown to contribute to ciprofloxacin resistance, but previous studies have shown that other mutations in general occur in conjunction with a mutation in the *gyr*A gene [6, 7]. In addition, it is estimated that approximately 80% of *N. gonorrhoeae* infections in the United States are susceptible to ciprofloxacin [8]. Those 2 facts support the implementation of *gyr*A genotype testing to promote the use of targeted ciprofloxacin therapy. That may in turn reduce overuse of ceftriaxone. A recent article showed that treatment may be a major driver of ceftriaxone resistance in *Neisseria gonorrhoeae* [9], which has been called one of the top 3 urgent threats to public health by the Centers for Disease Control and Prevention [10].

We developed a rapid codon 91 *gyr*A genotypic assay using RT-PCR techniques [6], and we verified the assay in accordance with Clinical Laboratory Improvement Amendments [2]. UCLA Health introduced that assay into routine clinical practice for all *N. gonorrhoeae*–positive specimens in November 2015. Further studies are underway to characterize the impact of that implementation.

Notes

Financial support. This research was supported by the National Institutes of Health (grants R21AI117256 and R21AI109005).

Potential conflicts of interest. Both authors: No reported conflicts.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Lao-Tzu Allan-Blitz[1] and Jeffrey D. Klausner[2]

[1]David Geffen School of Medicine, Department of Epidemiology and [2]Division of Infectious Diseases, Department of Medicine and Fielding School of Public Health, University of California, Los Angeles

References

1. Grad YH, Harris SR, Kirkcaldy RD, et al. Genomic epidemiology of gonococcal resistance to extended spectrum cephalosporins, macrolides, and fluoroquinolones in the United States, 2000–2013. J Infect Dis **2016**; 214:1579–87.
2. Hemarajata P, Yang S, Soge OO, Humphries RM, Klausner JD. Performance and verification of a real-time PCR assay targeting the gyrA gene for prediction of ciprofloxacin resistance in *Neisseria gonorrhoeae*. J Clin Microbiol **2016**; 54:805–8.
3. Donà V, Kasraian S, Lupo A, et al. Multiplex real-time PCR assay with high-resolution melting analysis for characterization of antimicrobial resistance in *Neisseria gonorrhoeae*. J Clin Microbiol **2016**; 54:2074–81.
4. Peterson SW, Martin I, Demczuk W, et al. Molecular assay for detection of ciprofloxacin resistance in *Neisseria gonorrhoeae* isolates from cultures and clinical nucleic acid amplification test specimens. J Clin Microbiol **2015**; 53:3606–8.
5. Zhao L, Zhao S. TaqMan real-time quantitative PCR assay for detection of fluoroquinolone-resistant *Neisseria gonorrhoeae*. Curr Microbiol **2012**; 65:692–5.
6. Siedner MJ, Pandori M, Castro L, et al. Real-time PCR assay for detection of quinolone-resistant *Neisseria gonorrhoeae* in urine samples. J Clin Microbiol **2007**; 45:1250–4.
7. Kulkarni S, Bala M, Sane S, Pandey S, Bhattacharya J, Risbud A. Mutations in the gyrA and parC genes of quinolone-resistant *Neisseria gonorrhoeae* isolates in India. Int J Antimicrob Agents **2012**; 40:549–53.
8. Kirkcaldy RD, Harvey A, Papp JR, et al., *Neisseria gonorrhoeae* antimicrobial susceptibility surveillance—the Gonococcal Isolate Surveillance Project, 27 sites, United States, 2014. MMWR Surveill Summ **2016**. 65:1–19.
9. Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev **2014**; 27:587–613.
10. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. Atlanta, Ga: Centers for Disease Control and Prevention, **2013**.

Received 27 October 2016; editorial decision 4 November 2016; accepted 9 November 2016; published online 11 January 2017.

Correspondence: L.-T. Allan-Blitz, David Geffen School of Medicine, University of California Los Angeles, 10833 Le Conte Ave, Los Angeles, CA 90095 (lallanblitz@mednet.ucla.edu)

The Journal of Infectious Diseases® **2017;215:491**
© The Author 2017. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.
DOI: 10.1093/infdis/jiw551

Reply to Allan-Blitz and Klausner

To the Editor—We thank Allan-Blitz and Klausner [1] for the citations to their group's work in this area and to the efforts underway to test diagnostics for quinolone resistance in *Neisseria gonorrhoeae*. Although our study investigated the genetic basis of resistance and assessed the positive and negative predictive values of specific mutations for resistance in the set of samples we analyzed [2], we take no position on the suitability of particular diagnostics. We note, however, that the US Food and Drug Administration has published guidance for antimicrobial susceptibility test systems [3]. The lower end of the range in negative predictive value cited by Allan-Blitz and Klausner (87.5%) is considerably lower than the 99% we observed,

possibly owing to sampling from a different gonococcal population, in which alternative mechanisms of resistance may exist. This emphasizes the importance of establishing rates of major and very major discrepancy [3], as well as regularly monitoring the circulating gonococcal lineages to ensure the diagnostic test characteristics accurately reflect the distribution of resistance mechanisms in gonococci, which may vary over time and by geographic and demographic groups.

Notes

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health.

Financial support. This work YHG was supported by the National Institutes of Health (grant K08-AI104767 to Y. H. G.). ML was supported by from the National Institute of General Medical Sciences (cooperative agreement U54GM088558 to M. L.).

***Potential conflicts of interest*.** Both authors: no reported conflicts.

Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Yonatan H. Grad[1,3] and Marc Lipsitch[1,2]

[1]Department of Immunology and Infectious Diseases, and [2]Center for Communicable Disease Dynamics, Department of Epidemiology, Harvard T.H. Chan School of Public Health, and [3]Division of Infectious Diseases, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

References

1. Allan-Blitz LT, Klausner JD. Codon 91 gyrase A testing is necessary and sufficient to predict ciprofloxacin susceptibility in *Neisseria gonorrhoeae*. J Infect Dis **2016**.
2. Grad YH, Harris SR, Kirkcaldy RD, et al. Genomic epidemiology of gonococcal resistance to extended-spectrum cephalosporins, macrolides, and fluoroquinolones in the United States, 2000–2013. J Infect Dis **2016**; 214:1579–87.
3. US Food and Drug Administration. class II special controls guidance document: antimicrobial susceptibility test (AST) systems. Guidance for industry and FDA. Silver Spring, MD: US Food and Drug Administration, **2009**. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080564.htm. Accessed 2 November 2016.

Received 3 November 2016; editorial decision 4 November 2016; accepted 10 November 2016; published online 11 January 2017.

Correspondence: Y. H. Grad, Harvard T.H. Chan School of Public Health, 665 Huntington Ave, Bldg 1, Room 715, Boston MA 02115 (ygrad@hsph.harvard.edu).

The Journal of Infectious Diseases® 2017;215:491–2
© The Author 2017. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.
DOI: 10.1093/infdis/jiw552

Antiviral Activity of the Combination of Interferon and Ribavirin Against Chikungunya Virus: Are the Results Conclusive?

To the Editor—We agree with Gallegos and colleagues [1] that chikungunya virus (CHIKV) is a significant public health problem today, and that effective antiviral agents are urgently needed to treat severe cases of CHIKV fever. On the basis of their results, the authors suggest that ribavirin (RBV) and interferon (IFN) α2a are effective, when used in combination, against CHIKV replication in Vero cells, and that such combination represents a promising therapeutic strategy against the infection [1]. Although these findings are interesting and meaningful, we are concerned about some shortcomings in the experimental design, which might lead to misleading conclusions.

First, the evaluation of the antiviral activity performed to support the development of an "investigational" product should include an assay directed against a broad range of clinical and laboratory viral isolates, including different clades, subtypes, or genotypes. In their study, Gallegos et al. [1] did not consider the possibility that the level of sensitivity of the CHIKV to the antiviral action of IFN could be virus strain–dependent. Indeed, they used the CHIKV vaccine strain 181/clone 25, which is a live-attenuated derivative of Southeast Asian human isolate strain AF15561, not necessarily mirroring the real level of natural IFN resistance potentially exploited by clinical CHIKV strains [2–6]. To this regard, we found that the envelope surface glycoprotein E1 (A226V) adaptive mutation, which improved the fitness of CHIKV for a secondary vector, *Aedes albopictus* (facilitating its spread during the outbreaks in the Indian Ocean area, in India and in north-eastern Italy [7–8]), can significantly affect the sensitivity of CHIKV to the antiviral action of different type I IFN preparations. In particular, CHIKV strain (East Central South African [ECSA]) with the A226V mutation [8] was more sensitive to IFNs in the Vero cell line compared to the viral strain without A226V (Table 1).

Second, the assessment of antiviral activity to support the development of an "investigational" product requires to test at least the effect of an increasing multiplicity of infection (MOI) and, whenever possible, the antiviral activity in different cellular lines, giving priority to human cells (which are more likely to reflect *in vivo* condition). Gallegos et al. [1] have analyzed the potency of the IFNα against CHIKV by using a very low MOI (eg, 0.0001 plaque-forming units/cell), we found that the antiviral activity of IFNs against CHIKV strains significantly decreased by increasing the MOI (Table 1). Furthermore, the authors estimated the antiviral activity of IFNα2a against CHIKV in Vero cells only. This is a nonhuman primate cell line that lacks an intact type I IFN signaling [9]. In relation to that, we found that the antiviral activity of IFNα, IFNβ, and IFNω against both strains of CHIKV is different in Hep-2, a human cell line, compared to that recorded in Vero cells (Table 1). Perhaps more importantly, the antiviral activity of RBV seems to be naturally limited in many cell types, including the Vero cell line (personal observations, [10]), thus indicating the importance of using multiple cell lines of different origin when antiviral activity and potency are studied for new and/or established drugs *in vitro*.

Lastly, Gallegos et al. evaluated the anti-CHIKV activity of only one type I IFN preparation (eg, IFNα2a), not addressing/discussing at all the issue of the different commercially available