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COMMENTARY

Lowering the Barriers to Routine Whole-Genome Sequencing of Bacteria in the Clinical Microbiology Laboratory

Daniel D. Rhoads[a,b]

[a]University Hospitals Cleveland Medical Center, Cleveland, Ohio, USA
[b]Case Western Reserve University, Department of Pathology, Cleveland, Ohio, USA

ABSTRACT Whole-genome sequencing of bacterial isolates is increasingly being used to predict antibacterial susceptibility and resistance. Mason and coauthors describe the phenotypic susceptibility interpretations of more than 1,300 *Staphylococcus aureus* isolates tested against a dozen antistaphylococcal agents, and they compared these findings to susceptibility predictions made by analyzing whole-genome sequence data (J Clin Microbiol 56:e01815-17, 2018, https://doi.org/10.1128/JCM.01815-17). The genotype-phenotype susceptibility interpretations correlated in 96.3% (2,720/2,825) of resistant findings and 98.8% (11,504/11,639) of susceptible findings. This work by Mason and colleagues is helping to lower the barriers to using whole-genome sequencing of *S. aureus* in clinical microbiology practice.

High-throughput sequencing is not routinely performed in most hospital-based clinical microbiology laboratories. However, when high-throughput sequencing methods are employed in clinical microbiology, the data are typically used in one of four ways: (i) to characterize an isolate using whole-genome sequencing (WGS) (1–3), (ii) to characterize an organism using targeted sequencing (4, 5), (iii) to detect a pathogen using metagenomics (6–9), or (iv) to characterize a microbiome using metagenomics (10–12). All of these applications (and others such as transcriptome profiling of the host or microbe) have potential utility in clinical microbiology practice, but barriers remain that prevent rapid widespread adoption. Mason and coauthors' study specifically focuses on WGS applications in *Staphylococcus aureus* isolates, and their study has helped to lower the barriers to bringing WGS of *S. aureus* to clinical microbiology practice (3).

CHARACTERIZING *STAPHYLOCOCCUS AUREUS* CLINICAL ISOLATES USING WHOLE-GENOME SEQUENCING

Mason and colleagues add to their published work, which correlates *S. aureus* genome data with phenotypic susceptibility interpretations (3, 13, 14). In their current article, they compare three data analysis software tools to determine their adequacy in detecting virulence genes and resistance genes in more than 1,300 *S. aureus* isolates from WGS data, which were generated as short reads (150 bp) using Illumina HiSeq (3). Also, phenotypic susceptibility testing was performed on the isolates, and phenotypic susceptibility interpretations were compared to the susceptibility predictions based on the WGS data for 12 antimicrobial agents: ciprofloxacin, clindamycin, erythromycin, fusidic acid, gentamicin, methicillin, mupirocin, penicillin, rifampin, tetracycline, trimethoprim, and vancomycin. Agreements between phenotypic testing and all three analysis pipelines were identified in 96.3% (2,720/2,825) of resistant findings and 98.8% (11,504/11,639) of susceptible findings. The authors concluded that all three analysis pipelines performed similarly in their abilities to detect virulence and resistance genes.

Mason et al.'s study is interesting to clinical microbiologists for at least three reasons. First, it reinforces previous studies' findings that correlated *S. aureus* WGS data with

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Address correspondence to ddr2@case.edu.

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antimicrobial susceptibility and resistance (13–15). This study builds on the evidence that WGS data can be used to accurately predict phenotypic susceptibility and resistance in *S. aureus*. Second, the study highlights the need to compare and validate laboratory-developed analysis pipelines when using sequencing data in clinical microbiology. The three pipelines described in Mason et al.'s study performed similarly, but the authors describe subtle differences in interpretation tendencies between the software tools. Third, the authors selectively reported known virulence genes that were identified in the WGS analyses. Currently, clinical microbiology laboratories do not routinely interrogate *S. aureus* for virulence genes, but if WGS becomes standard practice, then detecting and reporting virulence genes may become routine. However, the clinical utility of identifying and reporting *S. aureus* virulence genes would need empirical support, which has not yet been established. The work by Mason and colleagues reinforces the accuracy, feasibility, and potential value-added features of using WGS in routine clinical microbiology, and their work forces clinical microbiologists to consider not "if" but "when" WGS will become part of the repertoire of tools used in routine practice.

BARRIERS TO HIGH-THROUGHPUT SEQUENCING

Cost was previously seen as a significant barrier to high-throughput sequencing. In 2001, the National Institutes of Health (NIH) estimated the cost of sequencing a megabase of DNA to be more than $5,000, but the NIH's most recent estimate puts the cost of sequencing a megabase of DNA at a little more than a penny ($0.012) (https://www.genome.gov/27541954/dna-sequencing-costs-data/). Although cost is still a concern, it is no longer seen as an insurmountable barrier (16). Now, barriers other than costs are the most concerning: turnaround time (17, 18), empirical knowledge enabling genotype-phenotype correlation (2, 19), and data analysis workflow (2, 16).

Barrier 1: turnaround time. The barrier of a long turnaround time is being overcome by technical improvements in sequencing and data analysis, which are moving toward real-time DNA sequencing analysis (18, 20, 21). Turnaround time needs to be rapid enough to significantly impact clinical decision making, and it should be at least as rapid as established methods. When the etiology of infection is unclear and no good alternatives to high-throughput sequencing exist for achieving an accurate diagnosis, then the tolerance for a long turnaround time metagenomics approach is more acceptable (7). However, when sequencing is being used to produce an interpretation that could be achieved using more traditional methodology, then there is little tolerance for the WGS turnaround time to be longer than the traditional method's turnaround time. For example, to adopt the use of WGS as an adjunct or replacement to phenotypic susceptibility testing, the turnaround time to produce susceptibility and resistance interpretations using WGS needs to be at least as rapid as routine phenotypic testing.

Mason and colleagues recognize the need for a short turnaround time. The WGS data used in their analyses were short reads (150 bp), which can be produced relatively quickly. They also point out that two of the analysis tools, Genefinder and Mykrobe, only take a couple of minutes of computer processing time. The turnaround time for identifying a susceptibility genotype in *S. aureus* using Mason et al.'s approach is near to the time required to identify its susceptibility phenotype (13). As technology improves and sequencing turnaround time is decreased, it is likely that identifying susceptibility using WGS will be more rapid than identifying susceptibility by traditional phenotypic methods.

Barrier 2: genotype-phenotype correlation. The correlation of high-throughput sequence data and the associated phenotype is a barrier that can only be overcome by empirically building the knowledge base. For WGS data of bacteria, the clinical impact of each virulence gene needs to be studied in order for clinicians to be able to meaningfully interpret the relevance of the gene, because reporting a virulence gene without clearly linking that finding to a diagnosis, prognosis, or management choice would invoke clinical confusion rather than clarity.

Correlating the phenotypic susceptibility and resistance of a bacterial isolate to a WGS genotype also needs to be performed on an organism-by-organism basis. Recent studies have empirically demonstrated that predicting susceptibility and resistance using WGS data is feasible or potentially feasible for *S. aureus* (13–15), *Enterobacteriaceae* (22–25), *Pseudomonas aeruginosa* (26, 27), *Campylobacter* (28), *Helicobacter pylori* (29), *Neisseria gonorrhoeae* (30, 31), and *Mycobacterium tuberculosis* (13, 18, 32–35). In one of these studies, WGS data from more than 1,700 isolates of *Salmonella enterica* were used to predict susceptibility and resistance to 13 antimicrobial agents (22). In another study, machine learning was used to analyze WGS data and phenotypic susceptibility interpretations from more than 1,800 *M. tuberculosis* isolates (34). The machine learning approach improved upon the accuracy of resistance prediction in *M. tuberculosis* compared to a rules-based analysis of WGS data (34, 35), and machine learning approaches may also help to improve the accuracy of genotype-phenotype correlations in other taxa.

Mason and colleagues have worked to improve the genotype-phenotype antimicrobial susceptibility correlation for *S. aureus* by characterizing over 1,300 isolates, including 16,000 phenotypic susceptibility results—the largest published set to date (3). Studies like this will provide the prerequisite knowledge foundation that will foster the eventual implementation of WGS in clinical microbiology. The article builds upon the authors' previous work and reinforces the findings that the presence of resistance genes or mutations within housekeeping genes can be used to predict the susceptibility phenotype in *S. aureus* (13, 14). However, challenges will remain in identifying uncommon phenotypes that are not clearly understood genetically (e.g., staphylococci not susceptible to vancomycin) and recognizing novel resistance mechanisms.

The empirical approach used by Mason and colleagues on *S. aureus* will be necessary for each taxon in which WGS is to be used, and so susceptibility and resistance can be confidently and accurately predicted in each taxon. It is likely that not all taxa will be as amenable as *S. aureus* for using WGS to predict phenotypic susceptibility, but these challenges need to be identified and shared in published studies. Identifying these challenges will lead to more study and subsequent understanding of antimicrobial resistance mechanisms. Once these challenging resistance mechanisms are characterized, they could potentially be detected using ancillary nucleic acid methods, such as transcriptomic or epigenetic analyses.

Barrier 3: the pipeline. The software used in data analysis workflow (also known as the "pipeline") interprets high-throughput sequence data and translates the findings into clinically useful reporting. The main goal of a pipeline is to extract as much clinically applicable and accurate information from sequencing data as possible in as little time as possible and to present this information in a manner that is easily interpreted by humans. Parallel pipelines can be built and used to analyze the same data set to address different needs (e.g., virulence detection, clonality detection [outbreak surveillance], and antimicrobial resistance detection) (36). These pipelines are often designed and maintained by each laboratory performing the analyses, and this practice will likely remain commonplace until an FDA-cleared solution is created.

Mason and colleagues' study points to the need for head-to-head comparisons of different pipelines. The authors identified minor trends in interpretations between the pipelines studied and some significant differences in processing time (i.e., minutes versus hours) between the pipelines. For example, Mykrobe had more false positives for *blaZ* than the other two pipelines, Genefinder had a better ability to detect mutations in genes invoking linezolid resistance, and Typewriter was more prone to fail to detect a few virulence genes that the other pipelines detected. The differences between pipelines may go unnoticed unless formal comparisons are undertaken.

A well-curated, dynamic reference database is a key component of the analysis process, and variants of unknown significance in key genes need to be studied as they are encountered in order to determine whether or not they confer resistance.

FDA-ARGOS (https://www.fda.gov/MedicalDevices/ScienceandResearch/DatabaseforReferenceGradeMicrobialSequences/default.htm) is being developed as a reference-grade microbial sequence database and may become a viable resource and standard in the development and validation of clinical WGS. A database like FDA-ARGOS could serve as the truth source for novel and emerging resistance mechanisms and for outbreak strain genotypes. Maintaining a well-curated database in the cloud or locally will be a challenging but necessary component of clinical WGS.

Mason and colleagues point out that using WGS has better interlaboratory reproducibility than phenotypic susceptibility testing, but they also recognize the need for proficiency testing material for WGS. As this study demonstrates, different pipelines can produce minor differences in WGS data interpretations. Because each laboratory potentially uses a unique pipeline, WGS proficiency testing material would be a valuable addition to laboratories' quality plans.

SUMMARY

The barriers to implementing WGS of *S. aureus* in clinical microbiology practice are being lowered by the work of Mason and colleagues (3, 13, 14). In their most recent work, they have again demonstrated that *S. aureus* WGS data can be used to predict the phenotypic susceptibility interpretations of common antistaphylococcal agents. Their approach should serve as a model for researchers and clinical microbiologists who work to investigate the genotype-phenotype correlations of other microbial taxa. The barriers to implementing WGS in clinical microbiology will continue to be lowered as sequencing technologies become more rapid and as scientific investigation unravels the correlation between genotype and phenotype.

REFERENCES

1. Jackson BR, Tarr C, Strain E, Jackson KA, Conrad A, Carleton H, Katz LS, Stroika S, Gould LH, Mody RK, Silk BJ, Beal J, Chen Y, Timme R, Doyle M, Fields A, Wise M, Tillman G, Defibaugh-Chavez S, Kucerova Z, Sabol A, Roache K, Trees E, Simmons M, Wasilenko J, Kubota K, Pouseele H, Klimke W, Besser J, Brown E, Allard M, Gerner-Smidt P. 2016. Implementation of nationwide real-time whole-genome sequencing to enhance listeriosis outbreak detection and investigation. Clin Infect Dis 63:380–386. https://doi.org/10.1093/cid/ciw242.
2. Long SW, Williams D, Valson C, Cantu CC, Cernoch P, Musser JM, Olsen RJ. 2013. A genomic day in the life of a clinical microbiology laboratory. J Clin Microbiol 51:1272–1277. https://doi.org/10.1128/JCM.03237-12.
3. Mason A, Foster D, Bradley P, Golubchik T, Doumith M, Gordon NC, Pichon B, Iqbal Z, Staves P, Crook D, Walker AS, Kearns A, Peto T. 2018. Accuracy of different bioinformatics methods in detecting antibiotic resistance and virulence factors from *Staphylococcus aureus* whole-genome sequences. J Clin Microbiol 56:e01815-17. https://doi.org/10.1128/JCM.01815-17.
4. Berg MG, Yamaguchi J, Alessandri-Gradt E, Tell RW, Plantier JC, Brennan CA. 2016. A pan-HIV strategy for complete genome sequencing. J Clin Microbiol 54:868–882. https://doi.org/10.1128/JCM.02479-15.
5. Talundzic E, Ravishankar S, Kelley J, Patel D, Plucinski M, Schmedes S, Ljolje D, Clemons B, Madison-Antenucci S, Arguin PM, Lucchi NW, Vannberg F, Udhayakumar V. 2018. Next-generation sequencing and bioinformatics protocol for malaria drug resistance marker surveillance. Antimicrob Agents Chemother 62:e02474-17. https://doi.org/10.1128/AAC.02474-17.
6. Piantadosi A, Kanjilal S, Ganesh V, Khanna A, Hyle EP, Rosand J, Bold T, Metsky HC, Lemieux J, Leone MJ, Freimark L, Matranga CB, Adams G, McGrath G, Zamirpour S, Telford S, III, Rosenberg E, Cho T, Frosch MP, Goldberg MB, Mukerji SS, Sabeti PC. 2018. Rapid detection of Powassan virus in a patient with encephalitis by metagenomic sequencing. Clin Infect Dis 66:789–792. https://doi.org/10.1093/cid/cix792.
7. Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, Salamat SM, Somasekar S, Federman S, Miller S, Sokolic R, Garabedian E, Candotti F, Buckley RH, Reed KD, Meyer TL, Seroogy CM, Galloway R, Henderson SL, Gern JE, DeRisi JL, Chiu CY. 2014. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med 370:2408–2417. https://doi.org/10.1056/NEJMoa1401268.
8. Chiu CY, Coffey LL, Murkey J, Symmes K, Sample HA, Wilson MR, Naccache SN, Arevalo S, Somasekar S, Federman S, Stryke D, Vespa P, Schiller G, Messenger S, Humphries R, Miller S, Klausner JD. 2017. Diagnosis of fatal human case of St. Louis encephalitis virus infection by metagenomic sequencing, California, 2016. Emerg Infect Dis 23:1964–1968. https://doi.org/10.3201/eid2310.161986.
9. Allcock RJN, Jennison AV, Warrilow D. 2017. Towards a universal molecular microbiological test. J Clin Microbiol 55:3175–3182. https://doi.org/10.1128/JCM.01155-17.
10. Fuentes S, Rossen NG, van der Spek MJ, Hartman JH, Huuskonen L, Korpela K, Salojarvi J, Aalvink S, de Vos WM, D'Haens GR, Zoetendal EG, Ponsioen CY. 2017. Microbial shifts and signatures of long-term remission in ulcerative colitis after faecal microbiota transplantation. ISME J 11:1877–1889. https://doi.org/10.1038/ismej.2017.44.
11. Staley C, Kelly CR, Brandt LJ, Khoruts A, Sadowsky MJ. 2016. Complete microbiota engraftment is not essential for recovery from recurrent *Clostridium difficile* infection following fecal microbiota transplantation. mBio 7:e01965-16. https://doi.org/10.1128/mBio.01965-16.
12. Wolcott RD, Hanson JD, Rees EJ, Koenig LD, Phillips CD, Wolcott RA, Cox SB, White JS. 2016. Analysis of the chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing. Wound Repair Regen 24:163–174. https://doi.org/10.1111/wrr.12370.
13. Bradley P, Gordon NC, Walker TM, Dunn L, Heys S, Huang B, Earle S, Pankhurst LJ, Anson L, de Cesare M, Piazza P, Votintseva AA, Golubchik T, Wilson DJ, Wyllie DH, Diel R, Niemann S, Feuerriegel S, Kohl TA, Ismail N, Omar SV, Smith EG, Buck D, McVean G, Walker AS, Peto TE, Crook DW, Iqbal Z. 2015. Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*. Nat Commun 6:10063. https://doi.org/10.1038/ncomms10063.
14. Gordon NC, Price JR, Cole K, Everitt R, Morgan M, Finney J, Kearns AM, Pichon B, Young B, Wilson DJ, Llewelyn MJ, Paul J, Peto TE, Crook DW, Walker AS, Golubchik T. 2014. Prediction of *Staphylococcus aureus* antimicrobial resistance by whole-genome sequencing. J Clin Microbiol 52:1182–1191. https://doi.org/10.1128/JCM.03117-13.
15. Anson LW, Chau K, Sanderson N, Hoosdally S, Bradley P, Iqbal Z, Phan H, Foster D, Oakley S, Morgan M, Peto TEA, Modernizing Medical Microbiology Informatics Group Mmmig, Crook DW, Pankhurst LJ. 2018. DNA extraction from primary liquid blood cultures for bloodstream infection

diagnosis using whole genome sequencing. J Med Microbiol 67:347–357. https://doi.org/10.1099/jmm.0.000664.
16. Kwong JC, McCallum N, Sintchenko V, Howden BP. 2015. Whole genome sequencing in clinical and public health microbiology. Pathology 47:199–210. https://doi.org/10.1097/PAT.0000000000000235.
17. Raven K, Blane B, Churcher C, Parkhill J, Peacock SJ. 5 April 2018. Are commercial providers a viable option for clinical bacterial sequencing? Microb Genom https://doi.org/10.1099/mgen.0.000173.
18. Votintseva AA, Bradley P, Pankhurst L, Del Ojo Elias C, Loose M, Nilgiriwala K, Chatterjee A, Smith EG, Sanderson N, Walker TM, Morgan MR, Wyllie DH, Walker AS, Peto TEA, Crook DW, Iqbal Z. 2017. Same-day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples. J Clin Microbiol 55:1285–1298. https://doi.org/10.1128/JCM.02483-16.
19. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Koser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen O, Woodford N. 2017. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST subcommittee. Clin Microbiol Infect 23:2–22. https://doi.org/10.1016/j.cmi.2016.11.012.
20. Quick J, Ashton P, Calus S, Chatt C, Gossain S, Hawker J, Nair S, Neal K, Nye K, Peters T, De Pinna E, Robinson E, Struthers K, Webber M, Catto A, Dallman TJ, Hawkey P, Loman NJ. 2015. Rapid draft sequencing and real-time nanopore sequencing in a hospital outbreak of *Salmonella*. Genome Biol 16:114. https://doi.org/10.1186/s13059-015-0677-2.
21. Lemon JK, Khil PP, Frank KM, Dekker JP. 2017. Rapid nanopore sequencing of plasmids and resistance gene detection in clinical isolates. J Clin Microbiol 55:3530–3543. https://doi.org/10.1128/JCM.01069-17.
22. Tyson GH, Zhao S, Li C, Ayers S, Sabo JL, Lam C, Miller RA, McDermott PF. 2017. Establishing genotypic cutoff values to measure antimicrobial resistance in *Salmonella*. Antimicrob Agents Chemother 61:e02140-16. https://doi.org/10.1128/AAC.02140-16.
23. Tyson GH, McDermott PF, Li C, Chen Y, Tadesse DA, Mukherjee S, Bodeis-Jones S, Kabera C, Gaines SA, Loneragan GH, Edrington TS, Torrence M, Harhay DM, Zhao S. 2015. WGS accurately predicts antimicrobial resistance in *Escherichia coli*. J Antimicrob Chemother 70:2763–2769. https://doi.org/10.1093/jac/dkv186.
24. Stoesser N, Batty EM, Eyre DW, Morgan M, Wyllie DH, Del Ojo Elias C, Johnson JR, Walker AS, Peto TE, Crook DW. 2013. Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data. J Antimicrob Chemother 68:2234–2244. https://doi.org/10.1093/jac/dkt180.
25. Zankari E, Hasman H, Kaas RS, Seyfarth AM, Agerso Y, Lund O, Larsen MV, Aarestrup FM. 2013. Genotyping using whole-genome sequencing is a realistic alternative to surveillance based on phenotypic antimicrobial susceptibility testing. J Antimicrob Chemother 68:771–777. https://doi.org/10.1093/jac/dks496.
26. Kos VN, Deraspe M, McLaughlin RE, Whiteaker JD, Roy PH, Alm RA, Corbeil J, Gardner H. 2015. The resistome of *Pseudomonas aeruginosa* in relationship to phenotypic susceptibility. Antimicrob Agents Chemother 59:427–436. https://doi.org/10.1128/AAC.03954-14.
27. Jaillard M, van Belkum A, Cady KC, Creely D, Shortridge D, Blanc B, Barbu EM, Dunne WM, Jr, Zambardi G, Enright M, Mugnier N, Le Priol C, Schicklin S, Guigon G, Veyrieras JB. 2017. Correlation between phenotypic antibiotic susceptibility and the resistome in *Pseudomonas aeruginosa*. Int J Antimicrob Agents 50:210–218. https://doi.org/10.1016/j.ijantimicag.2017.02.026.
28. Zhao S, Tyson GH, Chen Y, Li C, Mukherjee S, Young S, Lam C, Folster JP, Whichard JM, McDermott PF. 2016. Whole-genome sequencing analysis accurately predicts antimicrobial resistance phenotypes in *Campylobacter* spp. Appl Environ Microbiol 82:459–466. https://doi.org/10.1128/AEM.02873-15.
29. van Vliet AH, Kusters JG. 2015. Use of alignment-free phylogenetics for rapid genome sequence-based typing of *Helicobacter pylori* virulence markers and antibiotic susceptibility. J Clin Microbiol 53:2877–2888. https://doi.org/10.1128/JCM.01357-15.
30. Lee RS, Seemann T, Heffernan H, Kwong JC, Goncalves da Silva A, Carter GP, Woodhouse R, Dyet KH, Bulach DM, Stinear TP, Howden BP, Williamson DA. 2018. Genomic epidemiology and antimicrobial resistance of *Neisseria gonorrhoeae* in New Zealand. J Antimicrob Chemother 73:353–364. https://doi.org/10.1093/jac/dkx405.
31. Eyre DW, De Silva D, Cole K, Peters J, Cole MJ, Grad YH, Demczuk W, Martin I, Mulvey MR, Crook DW, Walker AS, Peto TEA, Paul J. 2017. WGS to predict antibiotic MICs for *Neisseria gonorrhoeae*. J Antimicrob Chemother 72:1937–1947. https://doi.org/10.1093/jac/dkx067.
32. Nimmo C, Doyle R, Burgess C, Williams R, Gorton R, McHugh TD, Brown M, Morris-Jones S, Booth H, Breuer J. 2017. Rapid identification of a *Mycobacterium tuberculosis* full genetic drug resistance profile through whole genome sequencing directly from sputum. Int J Infect Dis 62:44–46. https://doi.org/10.1016/j.ijid.2017.07.007.
33. Brown AC, Bryant JM, Einer-Jensen K, Holdstock J, Houniet DT, Chan JZ, Depledge DP, Nikolayevskyy V, Broda A, Stone MJ, Christiansen MT, Williams R, McAndrew MB, Tutill H, Brown J, Melzer M, Rosmarin C, McHugh TD, Shorten RJ, Drobniewski F, Speight G, Breuer J. 2015. Rapid whole-genome sequencing of *Mycobacterium tuberculosis* isolates directly from clinical samples. J Clin Microbiol 53:2230–2237. https://doi.org/10.1128/JCM.00486-15.
34. Yang Y, Niehaus KE, Walker TM, Iqbal Z, Walker AS, Wilson DJ, Peto TEA, Crook DW, Smith EG, Zhu T, Clifton DA. 2018. Machine learning for classifying tuberculosis drug-resistance from DNA sequencing data. Bioinformatics 34:1666–1671. https://doi.org/10.1093/bioinformatics/btx801.
35. Walker TM, Kohl TA, Omar SV, Hedge J, Del Ojo Elias C, Bradley P, Iqbal Z, Feuerriegel S, Niehaus KE, Wilson DJ, Clifton DA, Kapatai G, Ip CLC, Bowden R, Drobniewski FA, Allix-Beguec C, Gaudin C, Parkhill J, Diel R, Supply P, Crook DW, Smith EG, Walker AS, Ismail N, Niemann S, Peto TEA, Modernizing Medical Microbiology Informatics Group. 2015. Whole-genome sequencing for prediction of *Mycobacterium tuberculosis* drug susceptibility and resistance: a retrospective cohort study. Lancet Infect Dis 15:1193–1202. https://doi.org/10.1016/S1473-3099(15)00062-6.
36. Rhoads DD, Sintchenko V, Rauch CA, Pantanowitz L. 2014. Clinical microbiology informatics. Clin Microbiol Rev 27:1025–1047. https://doi.org/10.1128/CMR.00049-14.