AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

SUSCEPTIBILITY

Core Antibiotic-Induced Transcriptional Signatures Reflect Susceptibility to All Members of an Antibiotic Class

Melanie A. Martinsen,[a] Alexis Jaramillo Cartagena,[a] Roby P. Bhattacharyya[a,b]

[a]Infectious Disease and Microbiome Program, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA
[b]Infectious Diseases Division, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA

ABSTRACT Current growth-based antibiotic susceptibility testing (AST) is too slow to guide early therapy. We previously developed a diagnostic approach that quantifies antibiotic-induced transcriptional signatures to distinguish susceptible from resistant isolates, providing phenotypic AST 24 to 36 h faster than current methods. Here, we show that 10 transcripts optimized for AST of one fluoroquinolone, aminoglycoside, or beta-lactam reflect susceptibility when the organism is exposed to other members of that class. This finding will streamline development and implementation of this strategy, facilitating efficient antibiotic deployment.

KEYWORDS Gram-negative infections, antibiotic resistance, mechanism of action, molecular diagnostic, transcription

Worldwide, more than 700,000 people die annually from antibiotic-resistant infections (1), and gaps in global antibiotic resistance tracking suggest that this burden is severely underestimated (2, 3). Antibiotic-resistant infections lead to higher medical costs, longer hospital stays, and increased mortality (4, 5). Current growth-based antibiotic susceptibility testing (AST) is too slow to guide therapy in real time (6–8). This diagnostic delay causes overreliance on empiric broad-spectrum antimicrobials, contributing to the emergence of resistance and poor patient outcomes (9, 10).

Antibiotic-induced transcriptional signatures predict susceptibility. We recently developed a novel microbial diagnostic assay called GoPhAST-R (combined genotypic and phenotypic AST through RNA detection) that can provide AST in <4 h directly from a positive blood culture bottle, 24 to 36 h faster than standard growth-based methods (11). GoPhAST-R measures specific antibiotic-responsive mRNA expression signatures using a commercially available hybridization-based RNA detection platform, NanoString, which is quantitative and multiplexed and works directly from crude lysate with minimal hands-on time (11, 12). After brief antibiotic exposure at the clinical breakpoint concentration, susceptible cells become stressed and exhibit rapid transcriptional changes that distinguish them from unharmed resistant cells. By targeting markers of susceptibility, GoPhAST-R allows accurate and robust phenotypic AST classification, agnostic to resistance mechanism. In addition to detecting these antibiotic-induced transcripts for phenotypic AST, GoPhAST-R can simultaneously target known genetic resistance markers to improve accuracy of resistance detection (13) and facilitate molecular epidemiology by tracking the emergence and spread of specific resistance mechanisms (14), with no need for additional testing. This simultaneous integration of genotype and phenotype sets GoPhAST-R apart from purely phenotypic rapid systems, either commercially available (15) or under development (16), that provide growth-based AST within <7 h from positive blood culture bottles.

We previously demonstrated GoPhAST-R for three antibiotics representing different classes, across five pathogens. We first performed transcriptome sequencing (RNA-Seq) to identify candidate genes whose antibiotic-induced expression best distinguishes

Citation Martinsen MA, Jaramillo Cartagena A, Bhattacharyya RP. 2021. Core antibiotic-induced transcriptional signatures reflect susceptibility to all members of an antibiotic class. Antimicrob Agents Chemother 65:e02296-20. https://doi.org/10.1128/AAC.02296-20.

Address correspondence to Roby P. Bhattacharyya, rbhatt@broadinstitute.org.

Received 30 October 2020
Returned for modification 5 December 2020
Accepted 29 March 2021
Accepted manuscript posted online 12 April 2021
Published 18 May 2021

susceptible from resistant isolates and then refined these candidate transcripts on the NanoString assay platform, using machine-learning algorithms to select the top 10 antibiotic-responsive genes, and validated their AST prediction accuracy for each pathogen-antibiotic pair (11). We further validated the simultaneous detection of selected genotypic carbapenem resistance determinants (11).

To enhance flexibility of development and implementation of this diagnostic approach, we aimed here to test the generalizability of these transcriptional signatures of susceptibility across an antibiotic class. Since antibiotics elicit transcriptional responses related to their mechanism of action (17–20), we hypothesized that antibiotic-responsive genes optimized for one antibiotic could reflect susceptibility when an isolate is exposed to other members of the same drug class. Here, we confirm this hypothesis for two common pathogens with a propensity for multidrug resistance—*Escherichia coli* and *Klebsiella pneumoniae*—treated with multiple members of three major antibiotic classes in regular clinical use: fluoroquinolones, aminoglycosides, and beta-lactams.

Experimental design. We used NanoString probe sets targeting the top 10 genes we previously identified (11) for each of three individual antibiotics—ciprofloxacin (a fluoroquinolone [FQ]), gentamicin (an aminoglycoside [AG]), and meropenem (a beta-lactam [BL] in the carbapenem subclass)—and assessed whether they reflect susceptibility when exposed to other members of their respective class (Table 1). All strains were obtained from clinical or reference microbiological laboratories, representing diverse geographic locations and resistance mechanisms when possible, and all MICs were verified by broth microdilution (6) (see Data Set S1 in the supplemental material). For the FQ and AG classes, strains were grown in Mueller-Hinton broth (MHB) and treated at early log phase (optical density at 600 nm [OD_{600}], ~0.2). For BLs, we previously found that strains with prominent inoculum effects (21–23) may exhibit decreased induction of transcriptional susceptibility signatures at high cell density (11). To account for this effect for the BL class, we treated isolates at the Clinical and Laboratory Standards Institute (CLSI)-recommended MIC inoculum range of 2×10^5 to 8×10^5 CFU ml^{-1} by growing them in MHB to early log phase, back-diluting to 2×10^5 CFU ml^{-1} in fresh MHB, and incubating for 60 min prior to treatment.

After brief antibiotic exposure (60 min for FQs and AGs, 120 min for BLs) at their respective clinical susceptibility breakpoint concentrations (6, 11, 24), or an equivalent control incubation without antibiotics, samples were mechanically lysed and used as input for NanoString assays as previously described (11). Using the NanoString platform (12), we quantified expression of the top 10 responsive and 10 control genes we previously identified for individual pathogen-antibiotic pairs in multiplexed fashion (11). Control genes, whose expression is unaffected by antibiotics, were used to scale

TABLE 1 Classification of antibiotics used in this study with respective treatment conditions

			Antibiotic treatment	
Antibiotic	**Class**	**Subclass**	**Concn[a] (mg/liter)**	**Duration (min)**
Ciprofloxacin	FQ	2nd-generation FQ	0.25	60
Levofloxacin	FQ	3rd-generation FQ	0.5	60
Moxifloxacin	FQ	3rd-generation FQ	0.25	60
Gentamicin	AG		4	60
Tobramycin	AG		4	60
Amikacin	AG		16	60
Ampicillin	BL	Aminopenicillin	8	120
Cefazolin	BL	1st-generation cephalosporin	2	120
Ceftriaxone	BL	3rd-generation cephalosporin	1	120
Aztreonam	BL	Monobactam	4	120
Piperacillin-tazobactam	BL	Ureidopenicillin/beta-lactamase inhibitor	16/4	120
Ertapenem	BL	Carbapenem	0.5	120
Meropenem	BL	Carbapenem	1	120

[a]The concentration for antibiotic treatment was chosen as the clinical susceptibility breakpoint established by the CLSI for all antibiotics whenever available (24) or as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint for moxifloxacin (29).

for cell number at lysis, resulting in a normalized fold induction of each responsive gene in antibiotic-treated versus untreated samples for each strain, calculated as previously described (11). To assess assay performance, these normalized fold inductions were compared with the known AST classification of each isolate. For all analyses, we used the code we previously wrote and executed, as described elsewhere (11). Code and NanoString raw data files can be made available upon request.

To further characterize the isolates used in this study, we also sequenced those not previously deposited in the NCBI Sequence Read Archive (SRA). Genomic DNA was extracted using a Qiagen DNeasy blood and tissue kit, prepared using an Illumina Nextera XT DNA library prep kit, sequenced on an Illumina MiSeq (or NextSeq, for strain 16) platform, and analyzed for known resistance determinants using ResFinder (25) (Data Set S1).

Generalizability of 10-transcript signatures across FQs and AGs. We tested the ciprofloxacin 10-transcript signatures across the FQs ciprofloxacin, levofloxacin, and moxifloxacin and the gentamicin 10-transcript signatures across the AGs gentamicin, tobramycin, and amikacin. For each class, we selected six isolates of each species: three susceptible and three resistant to all class members (Data Set S1). For both species, heat maps illustrate that the top 10 genes identified for AST of ciprofloxacin and gentamicin showed similar normalized fold induction upon treatment with three FQs and three AGs, respectively (Fig. 1a and b). One-dimensional projections summarizing these transcriptional data (11, 26) show robust distinction of susceptible and resistant isolates across each class (Fig. S1a and b).

Generalizability of a 10-transcript signature across BLs. We next tested BLs, a large class of diverse compounds comprising multiple subclasses that span a wide spectrum of antibacterial activity. The diversity of the BLs challenges the generalizability of transcriptional signatures of susceptibility within a drug class and offers the most clinical benefit from rapid and efficient antibiotic deployment if successful. We tested the 10-transcript signatures identified for meropenem across treatments of seven BL antibiotics spanning multiple subclasses and ranging in spectrum of activity: ampicillin, cefazolin, ceftriaxone, aztreonam, piperacillin-tazobactam, ertapenem, and meropenem. We selected eight clinical isolates for each species that vary in susceptibility across the different BLs, from pan-susceptible to pan-resistant (Data Set S1). Note that although *K. pneumoniae* is intrinsically resistant to ampicillin, strain 23 has an MIC that falls in the susceptible range; whole-genome sequencing (WGS) revealed a premature stop codon (E88*) in the chromosomal $bla_{\text{SHV-89}}$ that may explain this unusual phenotype. Normalized fold induction of the meropenem-responsive genes tested across the seven BLs is shown as heat maps (Fig. 1c) and summarized as one-dimensional projections (Fig. S1c). In both species, each BL induced the top 10 meropenem-responsive transcripts in only the susceptible isolates, allowing susceptibility distinction despite variability in expression levels of certain genes across the class. Strains with MICs closer to the breakpoint exhibited partial induction, consistent with our previous finding that the magnitude of transcriptional response to antibiotic exposure at the breakpoint correlates with MIC (11). Specific carbapenemases from our panel of common carbapenemases and extended-spectrum beta-lactamases were found in the same assay, explaining resistance phenotypes in most carbapenem-resistant isolates (Fig. S2). Notably, strain 14 is correctly distinguishable from susceptible isolates upon ertapenem exposure, despite lacking genotypic markers from our panel. WGS of this isolate revealed three beta-lactamases, including $bla_{\text{CMY-2}}$ (27, 28), as well as polymorphisms in the OmpC and OmpF porins (Data Set S1), suggesting a possible atypical basis for ertapenem resistance that would have been difficult to predict by genotype alone. This exemplifies GoPhAST-R's ability to determine phenotypic resistance to each antibiotic, independent of resistance mechanism.

GoPhAST-R as a robust diagnostic platform. This work demonstrates that the same antibiotic-induced 10-transcript signatures reflect antibiotic susceptibility for all drugs within a class, consistent with a conserved core transcriptional response to each of three major antibiotic classes. Despite their diversity, even BL compounds share a

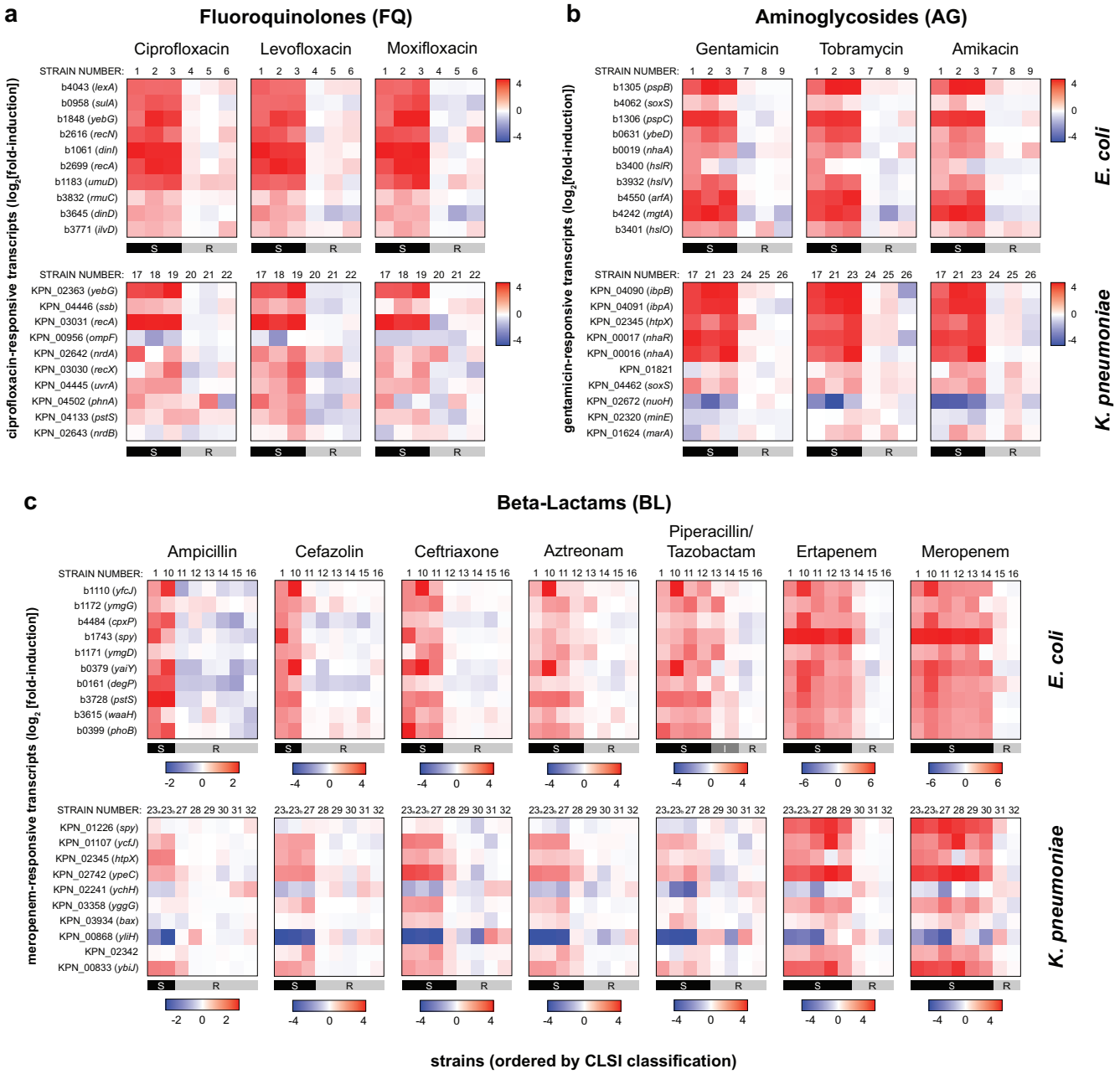

FIG 1 Differential expression of the same antibiotic-induced 10-transcript signatures reflect susceptibility for all drugs within a class. The figure shows heat maps of normalized, log-transformed fold induction (treated/untreated) of the top 10 antibiotic-responsive transcripts we previously identified for *E. coli* (top panels) and *K. pneumoniae* (bottom panels) treated with (a) ciprofloxacin, (b) gentamicin, and (c) meropenem upon exposure at CLSI breakpoint concentrations to other fluoroquinolones, aminoglycosides, and beta-lactams, respectively. Color scales indicate range of $\log_2$(fold induction) for transcripts in the respective heat map(s), symmetrically scaled to each plot. Strains are indicated by numbers over the heat map columns (Data Set S1), with CLSI classifications of each strain based on broth microdilution shown below (S, susceptible; I, intermediate; R, resistant). Gene identifiers for antibiotic-responsive transcripts are listed on the left, as defined for NCBI reference sequences NC_000913 (*E. coli*; top panels) and NC_009648 (*K. pneumoniae*; bottom panels).

susceptibility signature across all subclasses, implying a common stress response to their similar cellular targets. The 10-transcript signatures used in this study, previously designed for individual antibiotics, may not represent the top 10 genes for the whole class or for each individual member, but they clearly report on susceptibility for each compound in a given class. Moreover, WGS of isolates used in this study revealed diverse genotypic resistance mechanisms (target site mutations, efflux pumps, porin mutations, or antibiotic-modifying enzymes [see Data Set S1]), further illustrating how

our phenotypic AST approach is fundamentally agnostic to resistance mechanism. Thus, this study provides further evidence for transcriptional profiling as a robust phenotypic measure of antimicrobial stress, underscoring the flexibility and breadth of GoPhAST-R: the same minimal gene set derived for one specific antibiotic can assess susceptibility not only across diverse strains but also across drugs with a shared mechanism of action. This finding will streamline GoPhAST-R implementation, contributing to the critical effort to employ rapid AST diagnostics to guide upfront selection of the narrowest effective agent against a given pathogen. Efficient, informed deployment of antibiotics will improve patient outcomes while minimizing selection for resistance.

Data availability. Raw sequencing files of strains not previously deposited in the NCBI Sequence Read Archive (SRA) were deposited in the SRA under project no. PRJNA707347.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.03 MB.

ACKNOWLEDGMENTS

This work was supported in part by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under award 1K08AI119157-04 (R.P.B.) and in part by the Massachusetts General Hospital's Pilot Translational Research Grant. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

We thank Gowtham Thakku, James Gomez, Jonathan Livny, and Peijun Ma for helpful discussion, Lorrie He for laboratory support during data collection, Alejandro Pironti for porin mutation analysis of strain 14, and Ashlee Earl, the CDC ARBank, and the New York State Department of Health for strains.

M.A.M. contributed to the project conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, and writing (original draft, review, and editing). A.J.C. contributed to a portion of the investigation as well as writing (review and editing). R.P.B. contributed to project conceptualization, formal analysis, funding acquisition, methodology, supervision, and writing (review and editing). All authors read and approved the manuscript.

R.P.B. is a coinventor on subject matter in U.S. provisional application no. 62/723,417, now pending in PCT/US2019/048114, filed by the Broad Institute directed to RNA signatures for AST, as described in this paper. NanoString, Inc., has licensed the intellectual property for RNA-based AST from the Broad Institute. No payments or services from a third party were received.

REFERENCES

1. O'Neill J. 2014. Antimicrobial resistance. Tackling a crisis for the health and wealth of nations. The Wellcome Trust, London, United Kingdom.
2. WHO. 2014. Antimicrobial resistance: global report on surveillance. World Health Organization, Geneva, Switzerland.
3. WHO. 2020. Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2020. World Health Organization, Geneva, Switzerland.
4. Mauldin PD, Salgado CD, Hansen IS, Durup DT, Bosso JA. 2010. Attributable hospital cost and length of stay associated with health care-associated infections caused by antibiotic-resistant Gram-negative bacteria. Antimicrob Agents Chemother 54:109–115. https://doi.org/10.1128/AAC.01041-09.
5. Kadri SS, Adjemian J, Lai YL, Spaulding AB, Ricotta E, Prevots DR, Palmore TN, Rhee C, Klompas M, Dekker JP, Powers JH, 3rd, Suffredini AF, Hooper DC, Fridkin S, Danner RL, National Institutes of Health Antimicrobial Resistance Outcomes Research Initiative. 2018. Difficult-to-treat resistance in Gram-negative bacteremia at 173 US hospitals: retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. Clin Infect Dis 67:1803–1814. https://doi.org/10.1093/cid/ciy378.
6. Wiegand I, Hilpert K, Hancock REW. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc 3:163–175. https://doi.org/10.1038/nprot.2007.521.
7. Caliendo AM, Gilbert DN, Ginocchio CC, Hanson KE, May L, Quinn TC, Tenover FC, Alland D, Blaschke AJ, Bonomo RA, Carroll KC, Ferraro MJ, Hirschhorn LR, Joseph WP, Karchmer T, MacIntyre AT, Reller LB, Jackson AF. 2013. Better tests, better care: improved diagnostics for infectious diseases. Clin Infect Dis 57(Suppl 3):S139–S170. https://doi.org/10.1093/cid/cit578.
8. Burnham CD, Leeds J, Nordmann P, O'Grady J, Patel J. 2017. Diagnosing antimicrobial resistance. Nat Rev Microbiol 15:697–703. https://doi.org/10.1038/nrmicro.2017.103.
9. Rhee C, Kadri SS, Dekker JP, Danner RL, Chen HC, Fram D, Zhang F, Wang R, Klompas M, CDC Prevention Epicenters Program. 2020. Prevalence of

antibiotic-resistant pathogens in culture-proven sepsis and outcomes associated with inadequate and broad-spectrum empiric antibiotic use. JAMA Netw Open 3:e202899. https://doi.org/10.1001/jamanetworkopen.2020.2899.
10. Kadri SS, Lai YL, Warner S, Strich JR, Babiker A, Ricotta EE, Demirkale CY, Dekker JP, Palmore TN, Rhee C, Klompas M, Hooper DC, Powers JH, 3rd, Srinivasan A, Danner RL, Adjemian J. 2020. Inappropriate empirical antibiotic therapy for bloodstream infections based on discordant in-vitro susceptibilities: a retrospective cohort analysis of prevalence, predictors, and mortality risk in US hospitals. Lancet Infect Dis 21:241–251. https://doi.org/10.1016/s1473-3099(20)30477-1.
11. Bhattacharyya RP, Bandyopadhyay N, Ma P, Son SS, Liu J, He LL, Wu L, Khafizov R, Boykin R, Cerqueira GC, Pironti A, Rudy RF, Patel MM, Yang R, Skerry J, Nazarian E, Musser KA, Taylor J, Pierce VM, Earl AM, Cosimi LA, Shoresh N, Beechem J, Livny J, Hung DT. 2019. Simultaneous detection of genotype and phenotype enables rapid and accurate antibiotic susceptibility determination. Nat Med 25:1858–1864. https://doi.org/10.1038/s41591-019-0650-9.
12. Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, Fell HP, Ferree S, George RD, Grogan T, James JJ, Maysuria M, Mitton JD, Oliveri P, Osborn JL, Peng T, Ratcliffe AL, Webster PJ, Davidson EH, Hood L, Dimitrov K. 2008. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol 26:317–325. https://doi.org/10.1038/nbt1385.
13. Lutgring JD, Limbago BM. 2016. The problem of carbapenemase-producing-carbapenem-resistant-Enterobacteriaceae detection. J Clin Microbiol 54:529–534. https://doi.org/10.1128/JCM.02771-15.
14. Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, Grundman H, Hasman H, Holden MTG, Hopkins KL, Iredell J, Kahlmeter G, Köser CU, MacGowan A, Mevius D, Mulvey M, Naas T, Peto T, Rolain JM, Samuelsen Ø, 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.
15. Charnot-Katsikas A, Tesic V, Love N, Hill B, Bethel C, Boonlayangoor S, Beavis KG. 2017. Use of the Accelerate Pheno system for identification and antimicrobial susceptibility testing of pathogens in positive blood cultures and impact on time to results and workflow. J Clin Microbiol 56:e01166-17. https://doi.org/10.1128/JCM.01166-17.
16. Flentie K, Spears BR, Chen F, Purmort NB, DaPonte K, Viveiros E, Phelan N, Krebill C, Flyer AN, Hooper DC, Smalley DL, Ferraro MJ, Vacic A, Stern E. 2019. Microplate-based surface area assay for rapid phenotypic antibiotic susceptibility testing. Sci Rep 9:237. https://doi.org/10.1038/s41598-018-35916-0.
17. O'Rourke A, Beyhan S, Choi Y, Morales P, Chan AP, Espinoza JL, Dupont CL, Meyer KJ, Spoering A, Lewis K, Nierman WC, Nelson KE. 2020. Mechanism-of-action classification of antibiotics by global transcriptome profiling. Antimicrob Agents Chemother 64:e01207-19. https://doi.org/10.1128/AAC.01207-19.
18. Boshoff HIM, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE, III. 2004. The Transcriptional responses of Mycobacterium tuberculosis to inhibitors of metabolism: novel insights into drug mechanisms of action. J Biol Chem 279:40174–40184. https://doi.org/10.1074/jbc.M406796200.
19. Brazas MD, Hancock RE. 2005. Using microarray gene signatures to elucidate mechanisms of antibiotic action and resistance. Drug Discov Today 10:1245–1252. https://doi.org/10.1016/S1359-6446(05)03566-X.
20. Hutter B, Schaab C, Albrecht S, Borgmann M, Brunner NA, Freiberg C, Ziegelbauer K, Rock CO, Ivanov I, Loferer H. 2004. Prediction of mechanisms of action of antibacterial compounds by gene expression profiling. Antimicrob Agents Chemother 48:2838–2844. https://doi.org/10.1128/AAC.48.8.2838-2844.2004.
21. Smith KP, Kirby JE. 2018. The inoculum effect in the era of multidrug resistance: minor differences in inoculum have dramatic effect on MIC determination. Antimicrob Agents Chemother 62:e00433-18. https://doi.org/10.1128/AAC.00433-18.
22. Eng RH, Cherubin C, Smith SM, Buccini F. 1985. Inoculum effect of beta-lactam antibiotics on Enterobacteriaceae. Antimicrob Agents Chemother 28:601–606. https://doi.org/10.1128/aac.28.5.601.
23. Brook I. 1989. Inoculum effect. Rev Infect Dis 11:361–368. https://doi.org/10.1093/clinids/11.3.361.
24. CLSI. 2020. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, Wayne, PA.
25. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75:3491–3500. https://doi.org/10.1093/jac/dkaa345.
26. Barczak AK, Gomez JE, Kaufmann BB, Hinson ER, Cosimi L, Borowsky ML, Onderdonk AB, Stanley SA, Kaur D, Bryant KF, Knipe DM, Sloutsky A, Hung DT. 2012. RNA signatures allow rapid identification of pathogens and antibiotic susceptibilities. Proc Natl Acad Sci U S A 109:6217–6222. https://doi.org/10.1073/pnas.1119540109.
27. Mammeri H, Guillon H, Eb F, Nordmann P. 2010. Phenotypic and biochemical comparison of the carbapenem-hydrolyzing activities of five plasmid-borne AmpC β-lactamases. Antimicrob Agents Chemother 54:4556–4560. https://doi.org/10.1128/AAC.01762-09.
28. van Boxtel R, Wattel AA, Arenas J, Goessens WHF, Tommassen J. 2017. Acquisition of carbapenem resistance by plasmid-encoded-AmpC-expressing Escherichia coli. Antimicrob Agents Chemother 61:e01413-16. https://doi.org/10.1128/AAC.01413-16.
29. EUCAST. 2020. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0. The European Committee on Antimicrobial Susceptibility Testing, Vaxjo, Sweden.