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COMMENTARY

Precision Medicine and Mysteries in Clinical Microbiology: Rationalizing Epidemiology, Genotype, and Phenotype To Guide Therapeutics

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ABSTRACT Whole-genome sequencing (WGS) using MinION was used to characterize high-risk clones of *Escherichia coli* and *Klebsiella pneumoniae* harboring bla_{NDM-5}, $bla_{OXA-181}$, and $bla_{CTX-M-15}$, as well as *Pseudomonas aeruginosa* harboring bla_{NDM}, in a patient who received health care in India. Synergy testing demonstrated the activity of aztreonam and ceftazime-avibactam in combination. This case illustrates a "precision medicine" approach where deeper understanding of the genotype through WGS and of the phenotype through synergy testing formed the basis for rational combination therapy.

Khan et al. describe the fascinating case of a woman who, after receiving medical care in India, developed infection with Gram-negative pathogens resistant to carbapenems (1). Additionally, she was colonized with *Candida auris*, an emerging candidal species characterized by resistance to azoles. Cultures of the urine grew *Escherichia coli*, demonstrated by PCR to harbor bla_{NDM-5}, $bla_{OXA-181}$, and $bla_{CTX-M-15}$, as well as *Klebsiella pneumoniae* containing bla_{NDM-5} and $_{blaCTX-M-15}$; culture of a sputum sample grew *Pseudomonas aeruginosa* harboring bla_{NDM-1}. Guided by this knowledge, clinicians treated her with aztreonam, which is stable in the presence of the metallo-β-lactamase NDM, in conjunction with avibactam (administered as ceftazidime-avibactam), which protects aztreonam from inactivation by CTX-M-15 and OXA-181 β-lactamases. Demonstration of *in vitro* synergy of this combination of antibiotics affirmed their choice of therapy. The patient, whose infection did not respond to colistin despite the *in vitro* activity of this agent, appeared to have a favorable clinical response to aztreonam and ceftazidime-avibactam, although she eventually died after a stroke.

This case features the use of whole-genome sequencing (WGS) to determine bacterial and plasmid sequence types, as well as the resistome of *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Specifically, long-read genomic data were procured with the MinION nanopore sequencer, with additional complementary short-read sequences obtained using Illumina. MinION is a portable and affordable device that permits, for instance, real-time genomic analysis for the rapid diagnosis, detection of antibiotic resistance, and surveillance of *Mycobacterium tuberculosis* (2). The use of MinION provided insights that, in conjunction with other microbiological and pharmacological information, were

Citation Perez F, Bonomo RA. 2020. Precision medicine and mysteries in clinical microbiology: rationalizing epidemiology, genotype, and phenotype to guide therapeutics. Antimicrob Agents Chemother 64:e02264-19. https://doi.org/10.1128/AAC.02264-19.

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For the case discussed, see https://doi.org/10.1128/AAC.01466-19.

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Accepted manuscript posted online 18 November 2019
Published 27 January 2020

clinically useful and herald a "precision medicine" approach for the treatment of Gram-negative bacterial infections (3).

The strain of *E. coli* identified in this case, sequence type 167 (ST167), usually harbors bla_{NDM-5} or $bla_{OXA-181}$ within a plasmid of the IncX3 type, which can also be hosted by other sequence types of *E. coli* and other bacterial species (4). The IncX3 plasmid found in this isolate did not contain carbapenemase genes but contained the cephalosporinase $bla_{CTX-M-15}$. Rather, bla_{NDM} was in an IncF plasmid surrounded by a genetic environment like that of other bla_{NDM} plasmids in *E. coli* from Asia and Europe (5). Similarly, *K. pneumoniae* ST16, also isolated in this case, has been reported harboring bla_{NDM-5} in Asia and Europe. Variability in the plasmid and resistance content of *K. pneumoniae* ST16 suggests that different variants of that clone circulate worldwide (6). *P. aeruginosa* harboring bla_{NDM}, found in this case, is not uncommon and was reported recently in association with ST773 (7).

All these sequence types are deemed "international high-risk clones" and appear to be stable platforms for the maintenance of carbapenemase genes. High-risk clones seem to have a propensity to acquire plasmids and undergo genetic rearrangements that facilitate horizontal transmission of antibiotic resistance genes and, as in this case, disseminate across continents (8). Although the exact interplay between high-risk clones, plasmids, and other mobile genetic elements is not clear, it must serve the process of evolution and permit bacterial adaptation to conditions in the environment, including antibiotic selective pressure.

This case is extraordinary because of the simultaneous infection with *E. coli*, *K. pneumoniae*, and *P. aeruginosa* harboring multiple carbapenemases, as well as colonization with *C. auris*. Despite genetic details obtained through WGS, it remains impossible to reconstruct how this occurred. Whether she became colonized inside or outside the hospital in India where she was initially treated, or even plausibly in the rehabilitation facility in the United States where she was recovering, remains unknown. Also, recapitulating the type, duration, and sequence of antibiotics she received is not feasible, and therefore the evolutionary path by which resistance emerged cannot be retraced (9). Clarifying those details could recast this patient's unlikely misfortune as the logical culmination of acquisition, rearrangement, and dissemination of mobile genetic elements and high-risk clones in the context of environmental exposure, antibiotic selective pressure, and international travel. Furthermore, it would point toward strategies for the prevention and control of such events.

The promise of precision medicine is that a deeper understanding of the mechanisms of disease can be a compass pointing toward better therapies. The rationale for the use of aztreonam and ceftazidime-avibactam was grounded on the hydrolytic profiles of each of the enzymes against aztreonam and the ability of avibactam to inhibit key β-lactamases (10). This knowledge supported the use of a combination containing aztreonam and avibactam but was insufficient in the absence of information about the bacterial phenotype garnered through more conventional microbiologic methods. Therefore, demonstration through antimicrobial susceptibility testing of *in vitro* activity of aztreonam against *P. aeruginosa* and synergy between ceftazidime-avibactam and aztreonam against *E. coli* and *K. pneumoniae* was essential.

Similarly, colistin appeared active *in vitro* against all the pathogens, yet this polymyxin failed in achieving a clinical response. This underscores the notion that colistin has serious shortcomings for the treatment of infections caused by Gram-negative bacteria. The evidence documenting how and why colistin fails is robust: antibiotic concentrations are insufficient at the site of infection, the therapeutic window is narrow with frequent nephrotoxicity, and resistance can emerge during treatment (11). Conversely, there are unexplored aspects of how and why the combination of aztreonam and ceftazidime-avibactam is effective against metallo-β-lactamase-harboring bacteria, such as optimal timing and dosing, antibiotic exposures in different compartments, and the adjuvant role of ceftazidime targeting penicillin-binding proteins. Answering these and other questions, and proving the efficacy and safety of this combination in well-designed clinical trials, should be the aim of a conscientious research program.

Ultimately, the use of genotypic and phenotypic data in this case mattered because it led to the selection of an effective antibiotic combination against carbapenemase-producing bacteria where the usual therapy failed. This was possible because of progress in the basic science of resistance mechanisms and investments in the development of new antibiotics. Even though this case is of interest to the community of scientists and clinicians who have access to these tools, it remains to be seen whether precision medicine as illustrated here increases quality, safety, and value and if it is affordable and universally applicable. The latter aspect is crucial, considering that the burden of carbapenemase-mediated resistance is highest in regions of the world with low incomes. By improving access to WGS, platforms such as the MinION sequencer may help upend diagnostic and treatment paradigms, which could be transformative in the global fight against antibiotic resistance.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (NIH) to R.A.B. under award numbers R01AI100560, R01AI063517, and R01AI072219 and by funds and/or facilities provided by the Cleveland Department of Veterans Affairs, award number 1I01BX001974 to R.A.B. from the Biomedical Laboratory Research & Development Service of the VA Office of Research and Development, and the Geriatric Research Education and Clinical Center VISN 10. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the Department of Veterans Affairs.

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