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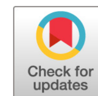

Antimicrobial Susceptibility Profiles To Predict the Presence of Carbapenemase Genes among Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates

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ABSTRACT Detection of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) with carbapenemase-producing (CP) genes is critical for preventing transmission. Our objective was to assess whether certain antimicrobial susceptibility testing (AST) profiles can efficiently identify CP-CRPA. We defined CRPA as *P. aeruginosa* with imipenem or meropenem MICs of ≥8 μg/ml; CP-CRPA was CRPA with CP genes (bla_{KPC}/bla_{IMP}/bla_{NDM}/bla_{OXA-48}/bla_{VIM}). We assessed the sensitivity and specificity of AST profiles to detect CP-CRPA among CRPA isolates collected by CDC's Antibiotic Resistance Laboratory Network (AR Lab Network) and the Emerging Infections Program (EIP) during 2017 to 2019. Three percent (195/6,192) of AR Lab Network CRPA isolates were CP-CRPA. Among CRPA isolates, adding not susceptible (NS) to cefepime or ceftazidime to the definition had 91% sensitivity and 50% specificity for identifying CP-CRPA; adding NS to ceftolozane-tazobactam had 100% sensitivity and 86% specificity. Of 965 EIP CRPA isolates evaluated for CP genes, 7 were identified as CP-CRPA; 6 of the 7 were NS to cefepime and ceftazidime, and all 7 were NS to ceftolozane-tazobactam. Among 4,182 EIP isolates, clinical laboratory AST results were available for 96% of them for cefepime, 80% for ceftazidime, and 4% for ceftolozane-tazobactam. The number of CRPA isolates needed to test (NNT) to identify one CP-CRPA isolate decreased from 138 to 64 if the definition of NS to cefepime or ceftazidime was used and to 7 with NS to ceftolozane-tazobactam. Adding not susceptible to cefepime or ceftazidime to CRPA carbapenemase testing criteria would reduce the NNT by half and can be implemented in most clinical laboratories; adding not susceptible to ceftolozane-tazobactam could be even more predictive once AST for this drug is more widely available.

KEYWORDS AST, CRPA, antibiotic resistance, carbapenemase production

Pseudomonas aeruginosa is a leading cause of health care-associated infections. The substantial mortality and morbidity associated with *P. aeruginosa* infections are in part due to high levels of antimicrobial resistance seen with this organism (1, 2). Multidrug-resistant (MDR) *P. aeruginosa* was first designated a serious threat in CDC's 2013 Antibiotic Resistance Threat Report (3). MDR *P. aeruginosa* caused an estimated 32,600 infections and 2,700 deaths among hospitalized patients in the United States in 2017 (4).

Carbapenem-resistant *P. aeruginosa* (CRPA) isolates are especially concerning because carbapenems are important drugs for clinical management of *P. aeruginosa* infections, given the organism's intrinsic and acquired resistance to many other

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antimicrobial classes. In the United States, approximately 14% of *P. aeruginosa* isolates causing health care-associated infections are not susceptible (NS) (i.e., they test intermediate or resistant) to at least one carbapenem (5), and the incidence of CRPA infections is approximately 20 per 100,000 persons in selected U.S. jurisdictions where population-based surveillance has been conducted (6).

Carbapenem resistance in *P. aeruginosa* frequently occurs because of several chromosomally mediated mechanisms, including porin loss, upregulation of efflux pumps, and expression of intrinsic β-lactamases (7). Carbapenem resistance can also result from carbapenemase production, mediated by the presence and expression of carbapenemase-producing (CP) genes commonly carried on mobile genetic elements (MGE). This form of resistance is highly concerning because of the ability of MGE to transfer resistance within and across different organisms and species, leading to rapid dissemination. Therefore, this form of resistance has been targeted in the United States. for control efforts. Although CP gene-mediated carbapenem resistance is still infrequent in *P. aeruginosa* clinical isolates identified in the United States. (8, 9), it is more common in other countries where it has frequently been associated with extensive spread and substantial increases in the proportion of *P. aeruginosa* isolates that are carbapenem resistant (10). For example, in Russia, there was a 6-fold increase in the proportion of CRPA isolates with a CP gene during 2002 to 2010 (11).

Although detection of CP genes is critical to preventing further spread, clinical laboratories historically have focused their efforts on phenotypic testing. To enhance detection and characterization of CP-CRPA, CDC's Antibiotic Resistance Laboratory Network (AR Lab Network) (https://www.cdc.gov/drugresistance/solutions-initiative/ar-lab-network.html), since its inception in 2016, has tested CRPA for the presence of CP genes. Among nearly 15,000 isolates tested during 2017 and 2018, 2% had CP genes detected (5), demonstrating a need for more efficient testing strategies.

We evaluated whether certain antimicrobial susceptibility profiles of CRPA isolates are associated with the presence of a CP gene and whether more stringent selection criteria based on antimicrobial susceptibility profiles from clinical laboratories could improve the specificity of CP gene detection among CRPA isolates with minimal loss of sensitivity. To conduct this analysis, we used data from CRPA isolates tested through (i) the AR Lab Network, including CDC's laboratory, and (ii) CDC's population-based surveillance for CRPA through the Emerging Infection Program (EIP).

MATERIALS AND METHODS

Data sources. (i) Antibiotic Resistance Laboratory Network. CDC's AR Lab Network was established in 2016 to support nationwide laboratory capacity to rapidly detect and contain the spread of antibiotic-resistant organisms (https://www.cdc.gov/drugresistance/solutions-initiative/ar-lab-network.html). Through this program, CDC funds 55 public health laboratories (PHLs) to conduct specialized testing to detect CP genes among pathogens of high concern, including CRPA (5). The AR Lab Network PHLs obtain isolates from clinical laboratories to conduct this testing.

AR Lab Network PHLs are asked to test *P. aeruginosa* isolates that are found to be resistant to doripenem, imipenem, or meropenem (MICs of $\geq$8 μg/ml) at participating clinical laboratories. In states where CRPA is reportable and isolate submission is required, the PHLs may receive all CRPA isolates. Where CRPA is not reportable or isolate submission is not required, state PHLs partner with clinical laboratories for submission of isolates, and some clinical laboratories, especially those with a high volume of CRPA isolates, may send a subset of isolates for characterization. States may require CRPA isolates submitted for carbapenemase testing to be resistant to other classes of drugs in addition to carbapenems.

PHLs characterized isolates submitted by clinical laboratories as CRPA; methods and workflows varied by PHL but included performing species confirmation by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) (Bruker, Billerica, MA), Vitek 2 (bioMérieux, Marcy-l'Étoile, France), MicroScan (Beckman Coulter, Brea, CA), or biochemical methods and conducting antimicrobial susceptibility testing (AST) of confirmed *P. aeruginosa* by broth microdilution (Thermo Fisher's Sensititre system), Kirby Bauer/disk diffusion, or Etests (bioMérieux, Marcy-l'Étoile, France). Drugs tested varied by PHL and may include doripenem, imipenem, meropenem, cefepime, ceftazidime, aztreonam, piperacillin-tazobactam, ciprofloxacin, levofloxacin, amikacin, tobramycin, and gentamicin. CRPA isolates also underwent phenotypic carbapenemase production testing using the modified carbapenem inactivation method (mCIM) or Rapidec Carba NP (bioMérieux, Marcy-l'Étoile, France) and genotypic detection of targeted CP genes (bla_{KPC}, bla_{IMP}, bla_{NDM}, $bla_{oxa\text{-}48}$, or bla_{VIM}) by real-time PCR protocols, Cepheid GeneXpert Carba-R (Cepheid, Sunnyvale, CA), or the Verigene Gram-negative blood culture test (BC-GN) (Nanosphere, Northbrook, IL). Isolates that demonstrated carbapenemase production and did not have

carbapenemase genes detected on initial Cepheid GeneXpert Carba-R testing underwent additional testing for targeted CP genes by real-time PCR. Some PHLs conducted molecular testing on all CRPA isolates, while others conducted molecular testing only on isolates that demonstrated phenotypic carbapenemase production.

AR Lab Network isolates were often forwarded to CDC when they met one or more of the following criteria: confirmed to harbor ≥1 CP gene, suspected to have a novel carbapenemase (phenotypic test positive and PCR negative for bla_{IMP}, bla_{KPC}, bla_{NDM}, bla_{oxa-48}, or bla_{VIM} genes), and not susceptible to all antibiotics tested at the clinical laboratory and PHL. At CDC, isolates underwent testing by mCIM for carbapenemase activity and real-time PCR for carbapenemase genes, as well as by reference broth microdilution panels (e.g., frozen panels) to assess susceptibility to newer antibiotics, including ceftazidime-avibactam and ceftolozane-tazobactam.

(ii) Emerging Infections Program. From 1 August 2016 through 31 July 2018, CDC conducted laboratory- and population-based surveillance for CRPA through the Emerging Infections Program (EIP) in selected metropolitan areas in eight states, involving over 80 clinical laboratories (12). EIP defined CRPA as *P. aeruginosa* that tested resistant to imipenem, meropenem, or doripenem at the clinical laboratory. An incident CRPA case was defined as the first case where CRPA was isolated from the lower respiratory tract, urine, wound, or normally sterile site of a resident of the EIP catchment area in a 30-day period. Clinical laboratory AST data were abstracted from the medical record for each case; clinical laboratories generally did not perform carbapenemase testing. A sample of isolates was submitted to CDC for further characterization. At CDC, isolates underwent confirmation of species by MALDI-TOF, AST by reference broth microdilution panels (e.g., frozen panels) for 15 antibiotics (which is more antibiotics tested than what is typically conducted in clinical laboratories and includes ceftazidime-avibactam and ceftolozane-tazobactam), and real-time PCR to detect CP genes. For the purposes of this analysis, we excluded cases and isolates obtained from cystic fibrosis patients.

AST phenotypic definitions and performance criteria. We included all *P. aeruginosa* isolates tested at the AR Lab Network laboratories that met the following criteria: (i) being isolated from specimens collected in 2018, (ii) being resistant to imipenem and/or meropenem at the PHL, (iii) having AST results available for the drug(s) being evaluated for a given definition, (iv) having a phenotypic carbapenemase test or having CP gene PCR results available, and (v) being from a state that submitted at least 10 CRPA isolates during 2018. Doripenem was not included in the CRPA definition for this analysis because most PHLs used broth microdilution panels that did not distinguish between intermediate and resistant isolates.

We defined CP-CRPA as a CRPA isolate with molecular identification of ≥1 CP gene (among bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, and bla_{VIM}) at the PHL. Isolates that demonstrated phenotypic carbapenemase production without detection of a CP gene were categorized as non-CP-CRPA for the purpose of this analysis but were included in a secondary analysis to evaluate how the AST-based definitions performed in detecting these isolates. We applied Clinical and Laboratory Standards Institute (CLSI) interpretative criteria (13) to categorize isolates as resistant (R), intermediate (I), or susceptible (S) to a given antibiotic. Isolates that were I or R were considered NS.

We evaluated the sensitivity and specificity of the following beta-lactam and monobactam AST phenotype-based definitions for detecting CP-CRPA: (i) R to imipenem and meropenem, (ii) R to cefepime, (iii) NS to cefepime, (iv) R to ceftazidime, (v) NS to ceftazidime, (vi) NS to cefepime or ceftazidime, (vii) R to aztreonam, and (vii) NS to aztreonam. We focused on these beta-lactam and monobactam drugs because the spectrum of activity against these agents is expected to be broader for carbapenemases than other mechanisms of carbapenem resistance.

We also wanted to evaluate definitions that included R and NS to piperacillin-tazobactam and newer β-lactam/β-lactamase inhibitor combination antibiotics, i.e., ceftazidime-avibactam and ceftolozane-tazobactam, but AST for these drugs was not available or performed by most AR Lab Network PHLs in 2018. To evaluate definitions that included R or NS to piperacillin-tazobactam, we used CRPA isolates tested in the Wadsworth Center, New York State Department of Health laboratory, and Texas Department of State Health Services Laboratory during 2017 to 2019 because these laboratories conducted AST for a large number of isolates and antimicrobial drugs and were able to share data with CDC. To evaluate the performance of definitions that included R and NS to ceftazidime-avibactam and ceftolozane-tazobactam, we used AST data from CRPA isolates received at CDC through the AR Lab Network or EIP after 1 January 2018 through October 2019.

Whole-genome sequencing (WGS) data were used to inform associations of definition performance and different sequence types (ST) and presence of CP genes. Briefly, genomic DNA from CRPA isolates was sequenced on the Illumina MiSeq system at the Wadsworth Center Applied Genomic Technologies Core. Illumina reads were *de novo* assembled with SPAdes v3.12.0 0 (14), prior to multilocus sequence typing analysis (MLST) with mlst v2.16.2 (https://github.com/tseemann/mlst) and AR gene identification with ABRicate v0.8.13 (https://github.com/tseemann/abricate).

Applying AST definitions of interest to clinical laboratory data. Many PHLs only receive a subset of CRPA isolates from clinical laboratories for testing to identify CP organisms (e.g., some may choose to send the first 10 isolates each month, some may send the most resistant isolates, and some may send a random sample), and AST methods may vary between PHLs and clinical laboratories. Therefore, to assess how AST-based definitions might perform in clinical laboratories, we used clinical laboratory AST data obtained through a systematically collected set of CRPA isolates submitted through EIP laboratory- and population-based surveillance in eight metropolitan areas to (i) identify the proportion of clinical laboratories that test *P. aeruginosa* isolates for certain antimicrobials to assess the feasibility of implementing different AST definitions and (ii) determine what proportion of isolates, based on their AST profile in the

clinical laboratory, could be eliminated from needing further testing at a PHL for the presence of CP genes. We then used the subset of EIP isolates tested for CP genes at CDC to compare the number of isolates needed to test (NNT) to identify one CP-CRPA isolate for all CRPA isolates versus those meeting definitions that performed well in analysis of the AR Lab network isolates.

Data analysis and ethics review. A schematic figure of the source of isolates, years of inclusion, the analysis aspects they were used for, and overlap between data sources are shown in Fig. S1 in the supplemental material. We analyzed data using SAS version 9.4 (SAS Institute, Cary, NC). The human subject advisors at the National Center for Emerging and Zoonotic Infectious Diseases reviewed this activity and determined that the activity constituted public health surveillance. At EIP sites, CRPA surveillance activities were either approved with waiver of informed consent or deemed a nonresearch activity.

RESULTS

AR Lab Network isolate evaluation. A total of 6,192 AR Lab Network isolates obtained in 2018 from 36 states and Puerto Rico met inclusion criteria for the main analyses; 195 (3%) met the CP-CRPA definition and harbored bla_{VIM} ($n = 120$; 62%), bla_{KPC} ($n = 51$; 26%), bla_{IMP} ($n = 13$; 7%), or bla_{NDM} ($n = 12$; 6%) genes. There was one isolate with two CP genes detected (bla_{KPC} and bla_{VIM}); no isolates had bla_{OXA-48}. Of the 195 with CP gene, 163 also had a positive phenotypic test, 6 were negative, and 2 were indeterminate; 24 were missing results for phenotypic test. An additional 77 (1%) isolates did not have a CP gene detected but demonstrated phenotypic carbapenemase activity.

Among the 1,989 CRPA isolates tested in New York and Texas PHLs during 2017 to 2019 and used to evaluate how definitions, including resistant or not susceptible to piperacillin-tazobactam, performed, 159 (8%) were CP-CRPA and harbored bla_{VIM} ($n = 100$), bla_{KPC} ($n = 42$), bla_{IMP} ($n = 12$), or bla_{NDM} ($n = 5$).

Among 903 CRPA isolates sent to CDC during 2018 to 2019 and tested against ceftazidime-avibactam and ceftolozane-tazobactam, there were 223 (25%) CP-CRPA isolates with the following distribution of CP genes: bla_{VIM} ($n = 76$), bla_{KPC} ($n = 74$), bla_{NDM} ($n = 29$), and bla_{IMP} ($n = 34$). Ten had more than one carbapenemase gene detected: bla_{NDM} and bla_{VIM} ($n = 4$), bla_{NDM} and bla_{IMP} ($n = 3$), bla_{VIM} and bla_{IMP} ($n = 2$), and bla_{KPC} and bla_{VIM} ($n = 1$). An additional 94 isolates did not have a CP gene detected but demonstrated phenotypic carbapenemase activity.

Table 1 shows, for each AST phenotype definition we evaluated, the total number of isolates included in that analysis and the number of true positives (i.e., those that met the definition and had a CP gene present), false positives (those that met the definition but had no CP gene), false negatives (those that did not meet the definition but had a CP gene present), and true negatives (those that did not meet the definition and had no CP gene) and the sensitivity and specificity of the given definition to detect CP-CRPA isolates among CRPA isolates. The definitions with at least 50% specificity that maintained sensitivity higher than 80% were definition 3 (NS to cefepime [sensitivity, 83%; specificity, 53%]) and definition 5 (NS to ceftazidime [sensitivity, 94%; specificity, 61%]); definition 6, which combined NS to cefepime or ceftazidime, had a sensitivity of 91% and a specificity of 50%. Definition 13 (NS to ceftolozane-tazobactam) had 100% sensitivity and 86% specificity. Other antimicrobials evaluated, including aztreonam, piperacillin-tazobactam, and ceftazidime-avibactam, did not have favorable sensitivity and specificity profiles.

In further examining the false negatives (the CP-CRPA isolates that would be missed by definitions 3 [NS to cefepime] and 5 [NS to ceftazidime]), we found that among the 34 CP-CRPA isolates missed by definition 3, 2 harbored bla_{KPC} and 32 harbored bla_{VIM}; 22 bla_{VIM}-containing isolates were from a single-state CP-CRPA outbreak of ST 111. The 9 CP-CRPA missed by definition 5 included 8 with bla_{VIM}, of which 7 were part of the aforementioned ST111 bla_{VIM} CP-CRPA outbreak, and 1 bla_{KPC} CP-CRPA isolate also missed by definition 3.

Definition 6 (NS to cefepime or ceftazidime) had sensitivity (83%) for detecting the 77 AR Lab Network isolates that were phenotypically positive for carbapenemase production but did not have one of the five CP genes detected. Of 903 isolates evaluated at CDC against definition 13 (NS to ceftolozane-tazobactam), 94 were phenotypically

TABLE 1 Sensitivity and specificity of AST-based definitions for identifying carbapenemase-producing carbapenem-resistant *P. aeruginosa* isolates among CRPA isolates, United States, 2017 to 2019

No.	Definition	No. of isolates with AST data[a]	No. (%) of confirmed CP-CRPA isolates	No. of[b]:				%	
				True positives	False positives	False negatives	True negatives	Sensitivity	Specificity
1	R to imipenem and meropenem	5,394	177 (3)	169	3,338	8	1,879	96	36
	R to imipenem or meropenem AND:								
2	R to cefepime	6,159	195 (3)	128	1,459	67	4,505	66	76
3	NS to cefepime	6,159	195 (3)	161	2,778	34	3,186	83	53
4	R to ceftazidime	5,299	147 (3)	106	1,528	41	3,624	72	70
5	NS to ceftazidime	5,299	147 (3)	139	2,021	9	3,131	94	61
6	NS to cefepime or ceftazidime	6,192	195 (3)	178	2,979	17	3,018	91	50
7	R to aztreonam	6,095	184 (3)	97	2,926	87	2,985	53	51
8	NS to aztreonam	6,095	184 (3)	120	4,098	64	1,813	65	31
9	R to piperacillin-tazobactam	1,989[c]	159 (8)	75	684	84	1,146	47	57
10	NS to piperacillin-tazobactam	1,989[c]	159 (8)	129	1,013	30	817	81	41
11	NS to ceftazidime-avibactam	903[d]	223 (25)	169	123	54	557	76	82
12	R to ceftolozane-tazobactam	903[d]	223 (25)	211	56	12	624	95	92
13	NS to ceftolozane-tazobactam	903[d]	223 (25)	223	94	0	586	100	86

[a]Number of isolates with AST data available for antimicrobial drugs evaluated in the definition. CLSI interpretative criteria were applied to designate isolates as susceptible (S), intermediate (I), or resistant (R); isolates classified as not susceptible (NS) include isolates designated I and R.
[b]True positives met the definition and had a CP gene present; false positives met the definition but had no CP gene; false negatives did not meet the definition but had a CP gene present; true negatives did not meet the definition and had no CP gene.
[c]Analysis was limited to CRPA isolates tested at New York and Texas public health laboratories (PHLs) during 2017 to 2019; these were the only state PHLs in which a majority of isolates had susceptibility data for piperacillin-tazobactam. Isolates tested during 2018 and 2019 are also included in the analysis of definitions 1 to 8.
[d]Analysis was limited to CRPA isolates submitted to CDC during 2018 and 2019 through the AR Lab Network and EIP. AST for ceftazidime-avibactam and ceftolozane-tazobactam was routinely performed at CDC but not at PHLs.

positive for carbapenemase production but did not have one of the five CP genes detected; of these, 35% were NS to ceftolozane-tazobactam.

Evaluation of definitions against clinical laboratory data from EIP. During the 2-year EIP surveillance period, 4,182 CRPA were identified by 87 clinical laboratories across the 8 sites. All of the 87 participating clinical laboratories tested at least one CRPA isolate for cefepime susceptibility during the reporting period and 91% tested for ceftazidime susceptibility. Among the 4,182 CRPA isolates, 96% had AST results for cefepime, of which 63% were susceptible, and 80% had clinical laboratory AST data for ceftazidime, of which 65% were susceptible. Of the 3,268 isolates tested against both cefepime and ceftazidime, 1,733 (53%) were susceptible to both drugs and therefore would not meet carbapenemase testing criteria when a definition that includes NS to cefepime or ceftazidime in addition to resistance to carbapenems was used. Only 148 (4%) had ceftolozane-tazobactam AST results from the clinical laboratory, of which 127 (86%) were susceptible.

Of 965 CRPA isolates from EIP which underwent genotypic testing at CDC, 21 showed carbapenemase activity by mCIM assay, of which 7 ($<$1%) were identified as having a CP gene (*bla*$_{VIM}$ [three isolates], *bla*$_{KPC}$ [three isolates], and *bla*$_{IMP}$ [one isolate]). Six of seven CP-CRPA isolates were NS to cefepime and ceftazidime; the sensitivity of definition 6 for these isolates was 86%, and the specificity was 54%. All seven were NS to ceftolozane-tazobactam. Testing only CRPA isolates from EIP that were NS to cefepime or ceftazidime would decrease the NNT to identify one CP-CRPA isolate from 138 isolates to 64. Limiting testing to CRPA isolates that were NS to ceftolozane-

tazobactam would be expected to decrease the NNT to identify one CP-CRPA isolate from 138 isolates to 7.

DISCUSSION

Based on analysis of over 6,000 CRPA isolates submitted to PHLs from clinical laboratories across the United States in 2018, we found that adding not susceptible to cefepime or ceftazidime to the CRPA definition has a sensitivity of >90% for detecting CRPA with targeted CP genes (bla_{KPC}, bla_{IMP}, bla_{NDM}, and bla_{VIM}) while reducing the number of isolates requiring carbapenemase testing by half. A definition that incorporates NS to ceftolozane-tazobactam was even more sensitive and specific; susceptibility testing for this antibiotic is not yet widespread among clinical laboratories, but this could be a promising strategy for identifying likely CP gene carriers as testing availability increases. Using AST-based profiles of CRPA to eliminate further testing of isolates that are unlikely to have carbapenemases has the potential to make carbapenemase testing of *P. aeruginosa* more feasible and efficient and ultimately improve CP-CRPA detection efforts in clinical and PHL laboratories. Maintaining the same level of testing as is currently being performed in PHLs but being selective about which isolates to work up for the presence of CP genes would result in improved detection of CP-CRPA and enhance the impact of public health responses to these organisms.

Our objective was to identify a definition with good performance that is feasible for clinical laboratories and PHLs to implement. Although definition 5 (NS to ceftazidime) was more sensitive than definition 3 (NS to cefepime), EIP data indicate that fewer laboratories include ceftazidime in their panel of antibiotics tested against *P. aeruginosa*, making this definition less broadly applicable than one that includes NS to cefepime. In the interest of developing a definition that is broadly adoptable, we chose to include NS to cefepime or ceftazidime in our final definition. This definition could be used by clinical laboratories to inform testing protocols, including which isolates should be selected for phenotypic or genotypic testing to identify carbapenemase production and CP gene-carrying organisms. As a result of the work described herein, this definition has already been adopted by AR Lab Network laboratories and their submitters.

The poor sensitivity of definition 11 (NS to ceftazidime-avibactam) compared to definitions 12 and 13 (R and NS to ceftolozane-tazobactam, respectively) was unsurprising given that bla_{KPC}-carrying organisms are susceptible to this drug and bla_{KPC} was the second most common CP gene identified in our analysis. These isolates accounted for the majority of false negatives for definition 11. Clinical laboratories and PHLs that conduct ceftolozane-tazobactam susceptibility testing can employ the additional criterion of NS to ceftolozane-tazobactam to identify isolates that should be tested for the presence of CP genes.

Our findings are corroborated by two recent publications which also identified NS to cefepime, ceftazidime, and ceftolozane-tazobactam as key to increasing specificity while preserving sensitivity (15, 16). Our data add to these previous two publications in two ways: (i) we derived the definitions by analyzing a very large sample of >6,000 CRPA isolates collected from across the country, and (ii) we accounted for availability of AST from clinical laboratories to ensure that the definitions would be widely implementable (for example, clinical laboratories might test against only the one antipseudomonal carbapenem on their clinical formulary, and not all laboratories test for ceftazidime susceptibility; therefore, we chose to include resistance to either imipenem or meropenem in our definition and NS to either cefepime or ceftazidime).

Restricting genotypic testing to a subset of CRPA isolates based on their AST profile has the potential to miss some CP-CRPA isolates. We found that applying a definition that requires NS to cefepime or ceftazidime in addition to R to carbapenems would have missed an outbreak of ST 111 CRPA harboring bla_{VIM} in one state. Sequencing analyses conducted at CDC and previously published studies on ST 111

indicate that susceptibility to cefepime or ceftazidime does not appear to be a common feature of ST 111 CRPA harboring *bla*$_{VIM}$ (17); therefore, we do not anticipate that this definition will systematically exclude carbapenemase-producing ST111 isolates from carbapenemase testing. However, this finding highlights the importance of laboratories, clinicians, and public health officials using discretion when applying this definition. If there is a cluster of carbapenem-resistant *P. aeruginosa* cases, it is important to consider mechanism testing, even if the isolates are susceptible to cefepime and ceftazidime. Similarly, CRPA from patients recently hospitalized outside the United States, who are at higher risk of acquiring carbapenemase-producing organisms, should be prioritized for testing even if these isolates are susceptible to extended-spectrum cephalosporins.

We also found that using not susceptible to cefepime or ceftazidime as a criterion for further testing of CRPA isolates for CP gene detection captured approximately 80% of isolates that phenotypically demonstrated carbapenemase production but did not have any of the targeted CP genes (*bla*$_{KPC}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{oxa-48}$, or *bla*$_{VIM}$). Notably, published data describing isolates with novel CP genes, such as *bla*$_{GES}$ with carbapenemase activity and *bla*$_{HMB}$, indicate that isolates identified with these genes hydrolyze cefepime, ceftazidime, and ceftolozane and exhibit phenotypic resistance to them (12, 18–20), and they would therefore be identified for further testing using definition 6. However, we cannot rule out the possibility that some novel carbapenemases could be missed through application of this definition.

There were multiple limitations to our analysis. We focused this analysis on detecting CP-CRPA among CRPA isolates, not among all *P. aeruginosa* isolates, including isolates that have intermediate MICs of carbapenems. Therefore, we do not know the true sensitivity of our definitions for carbapenemase detection among all *P. aeruginosa* isolates, only their performance among CRPA isolates. Conclusions based on AR Lab Network isolates are limited in several ways. (i) The AR Lab Network system is designed for early detection of concerning resistance rather than systematic disease surveillance; thus, laboratories that submit a subset of CRPA isolates may pick those that are most resistant or have other systematic biases. (ii) AST methods used by PHLs may differ from those used by clinical laboratories; automated AST methods might result in classifying isolates as NS differently than with MICs and might affect the sensitivity and specificity of the AST definitions we identified in this analysis. (iii) We did not use doripenem susceptibility to assess CRPA isolates submitted to PHLs because the broth microdilution plates used at most AR Lab Network labs did not distinguish between intermediate and resistant MICs for doripenem. However, we do not have reason to believe that these definitions would perform differently for isolates defined as CRPA because of their resistance to doripenem.

To overcome the limitations presented by AR Lab Network data, we also assessed performance of the definitions among a more representative set of isolates using AST results collected from clinical laboratories through population-based EIP surveillance. However, EIP surveillance data also have distinct limitations: (i) only 8 metropolitan areas are included, and findings may not be nationally generalizable; (ii) only a sample of isolates underwent carbapenemase testing; and (iii) CP genes were rarely identified. Finally, isolates tested at CDC tend to be more resistant than isolates tested at clinical or PHL laboratories. Definition 13 (NS to ceftolozane-tazobactam) may be even more selective for CP-CRPA when used against a more representative set of isolates; 95% of 42 non-carbapenemase-producing CRPA isolates in one study were susceptible to ceftolozane-tazobactam (21).

Prompt identification of CP genes in CRPA is critical to prevent further emergence of these highly antibiotic-resistant strains. As it is currently not feasible to test all CRPA isolates for the presence of CP genes, applying definitions that narrow carbapenemase testing to isolates most likely to harbor targeted CP genes may increase overall detection of CP-CRPA. Laboratorians and clinicians should have high suspicion for carbapenemases in *P. aeruginosa* isolates that are resistant to carbapenems and also not susceptible to ceftazidime or cefepime and very high suspicion for those that are NS to

ceftolozane-tazobactam; these isolates should be targeted for carbapenemase testing and infection control interventions.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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