

Multicenter, Prospective Validation of a Phenotypic Algorithm to Guide Carbapenemase Testing in Carbapenem-Resistant *Pseudomonas aeruginosa* Using the ERACE-PA Global Surveillance Program

Christian M. Gill,¹ Elif Aktaş,² Wadha Alfouzan,^{3,4} Lori Bourassa,⁵ Adrian Brink,⁶ Carey-Ann D. Burnham,⁷ Rafael Canton,⁸ Yehuda Carmeli,⁹ Marco Falcone,¹⁰ Carlos Kiffer,¹¹ Anna Marchese,¹² Octavio Martinez,¹³ Spyros Pournaras,¹⁴ Michael J. Satlin,¹⁵ Harald Seifert,¹⁶ Abrar K. Thabit,¹⁷ Kenneth S. Thomson,¹⁸ Maria Virginia Villegas,¹⁹ and David P. Nicolau^{1,20}, on behalf of the ERACE-PA Global Study Group¹⁰

¹Center for Anti-Infective Research & Development Hartford Hospital, Hartford, Connecticut, USA, ²University of Health Sciences, Sisli Hamidiye Etfal Training and Research Hospital, Clinical Microbiology Laboratory, Istanbul, Turkey, ³Laboratory Medicine, Farwania Hospital, Ministry of Health, Kuwait City, Kuwait, ⁴Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait City, Kuwait, ⁵Department of Laboratory Medicine and Pathology, University of Washington, Seattle, Washington, USA, ⁶Division of Medical Microbiology, Department of Pathology, Faculty of Health Sciences, National Health Laboratory Services, University of Cape Town, Cape Town, South Africa, ⁷Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA, ⁸Servicio de Microbiología. Hospital Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain, ⁹National Institute for Infection Control and Antibiotic Resistance, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel, ¹⁰Infectious Diseases Division, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy, ¹¹Internal Medicine Department and LEMC-Alerta Lab, Escola Paulista de Medicina, UNIFESP, São Paulo, Brazil, ¹²Department of Surgical Sciences and Integrated Diagnostics (DISC), University of Genoa, and Clinical Microbiology Unit, San Martino Policlinico Hospital—IRCCS for Oncology and Neuroscience, Genoa, Italy, ¹³Department of Pathology and Microbiology, University of Miami Miller School of Medicine, Miami, Florida, USA, ¹⁴Laboratory of Clinical Microbiology, Attikon University Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, ¹⁵Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine, New York, New York, USA, ¹⁶Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Köln, Germany, ¹⁷Pharmacy Practice Department, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, ¹⁸University of Louisville School of Medicine, Louisville, Kentucky, USA, ¹⁹Grupo de Resistencia Antimicrobiana y Epidemiología Hospitalaria (RAEH), Universidad El Bosque, Bogotá, Colombia, and ²⁰Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut, USA

Background. Carbapenemase-producing, carbapenem-resistant *Pseudomonas aeruginosa* (CP-CRPA) is a global challenge. However, detection efforts can be laborious because numerous mechanisms produce carbapenem resistance. A minimum inhibitory concentration–based algorithm (imipenem- or meropenem-resistant plus ceftazidime-nonsusceptible plus cefepime-nonsusceptible) was proposed to identify the isolates most likely to harbor a carbapenemase; however, prospective validation in geographies displaying genotypic diversity and varied carbapenemase prevalence is warranted.

Methods. CRPA isolates were collected during the Enhancing Rational Antimicrobials for *P. aeruginosa* (ERACE-PA) global surveillance program from 17 sites in 12 countries. Isolates underwent susceptibility testing following local standards to ceftazidime, cefepime, and ceftolozane/tazobactam. Isolates underwent initial phenotypic carbapenemase screening followed by molecular testing if positive. The primary algorithm criteria were applied, and results were compared with phenotypic carbapenemase results to assess the performance of the algorithm. A secondary criterion, the algorithm criterion or imipenem- or meropenem-resistant plus ceftolozane/tazobactam-nonsusceptible, was assessed.

Results. A total of 807 CRPA were assessed, and 464 isolates met the algorithm criteria described above. Overall, testing was reduced by 43% compared with testing all CRPA. Carbapenemase-positive isolates missed by the algorithm were largely driven by Guiana extended spectrum (GES). Addition of the criterion of imipenem- or meropenem-resistant plus ceftolozane/tazobactam-nonsusceptible decreased the number of CP-CRPA missed by the algorithm (21 vs 40 isolates, respectively), reducing number of isolates tested by 39%.

Conclusions. Application of the initial algorithm (imipenem- or meropenem-resistant plus ceftazidime-nonsusceptible plus cefepime-nonsusceptible) performed well in a global cohort, with 33% phenotypically carbapenemase-positive isolates. The addition of imipenem- or meropenem-resistant plus ceftolozane/tazobactam-nonsusceptible reduced the number of phenotypically carbapenemase-positive isolates missed and may be useful in areas with a prominence of GES.

Keywords. algorithm; carbapenemase; genotypic; molecular diagnostics; *Pseudomonas aeruginosa*.

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Correspondence: David P. Nicolau, PharmD, FCCP, FIDSA, Center for Anti-Infective Research and Development, Hartford Hospital, 80 Seymour Street, Hartford, CT 06102 (david.nicolau@hhchealth.org).

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Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is a well-described nosocomial pathogen that is associated with limited treatment options and substantial morbidity and mortality. Numerous mechanisms of resistance can cause carbapenem resistance in *P. aeruginosa* [1]. In the United States, the most common mechanisms of carbapenem resistance are alterations in porins coupled with overexpression of cephalosporinases and/or efflux pumps [2]. However, globally carbapenemase-producing CRPA (CP-CRPA) are common, and the underappreciation of

their contribution in the United States may be due to limited testing [3]. Detection of CP-CRPA is important because these carbapenemases are typically encoded by genes on plasmids that can be transferred among bacteria and often confer resistance to new β -lactam/ β -lactamase inhibitors [4].

A number of phenotypic and genotypic tests are available for carbapenemase detection in *P. aeruginosa* [5–7]. However, carbapenem resistance alone may be indiscriminate for CP-CRPA vs CRPA due to noncarbapenemase mechanisms [8]. Additionally, carbapenemase testing may have a low yield relative to the resources needed to test all carbapenem-resistant *P. aeruginosa* isolates [4]. Strategies to identify the isolates that are most likely to harbor carbapenemases may streamline detection workflows as well as improve the cost-effectiveness of additional screening procedures for *P. aeruginosa*.

We previously described the derivation of a pragmatic, minimum inhibitory concentration (MIC)-based algorithm to guide definitive carbapenemase testing in carbapenem-resistant *P. aeruginosa* [8]. This algorithm was further optimized in a separate cohort from German medical centers and modified to the following: imipenem- or meropenem-resistant (-R) plus ceftazidime-nonsusceptible (-NS) plus cefepime-NS [9]. This algorithm represents a starting point to guide carbapenemase testing while reducing overall workload that would encompass testing all CRPA. The evaluation of these criteria in a global cohort that represents regions with varying prevalence and diversity of carbapenemase genes would further define the utility

of this algorithmic approach. Herein, we describe the application of the algorithm in the previously described Enhancing Rational Antimicrobials for *P. aeruginosa* (ERACE-PA) Global Surveillance Program, which included 33% of isolates testing phenotypically positive for carbapenemase production, of which 86% were genotypically confirmed for carbapenemases [10].

METHODS

Bacterial Isolates

Isolates were compiled as part of the ERACE-PA Global Surveillance Program, which enrolled 17 sites from 12 countries spanning Europe, the Middle East, the United States, South America, and Africa [10]. Isolates were sent to a central laboratory (Center for Anti-Infective Research and Development, Hartford, CT, USA) for storage and frozen at -80°C in skim milk until assessment.

Isolates were eligible for inclusion if they were identified as *P. aeruginosa* by local standards of practice and determined to be carbapenem-resistant at the submitting site. Nonduplicate isolates were included from any age patient or any anatomical source over 2019–2021 [10]. Figure 1, A and B outlines the application of the primary and primary or secondary algorithm criteria, respectively.

Algorithm Criteria

The primary phenotypic algorithm of imipenem or meropenem resistance plus ceftazidime-NS plus cefepime-NS to guide

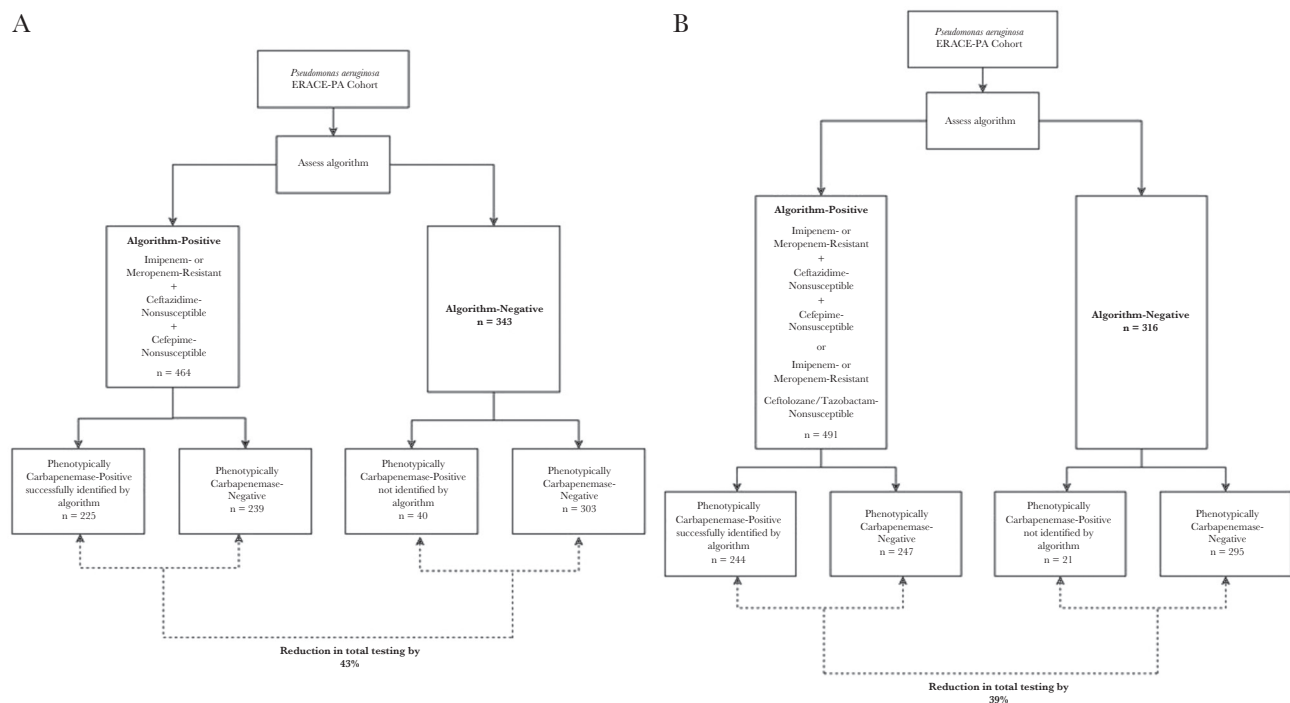


Figure 1. Phenotypic carbapenemase designation of algorithm-positive and -negative isolates using (A) imipenem- or meropenem-R plus ceftazidime-NS plus cefepime-NS and (B) imipenem- or meropenem-R plus ceftazidime-NS plus cefepime-NS OR imipenem- or meropenem-R plus ceftazidime-NS plus cefepime-NS OR imipenem- or meropenem-R plus ceftazidime-NS plus cefepime-NS plus ceftolozane/tazobactam-NS. Abbreviations: ERACE-PA, Enhancing Rational Antimicrobials for *P. aeruginosa*; NS, nonsusceptible; R, resistant.

carbapenemase testing in carbapenem-resistant *P. aeruginosa* was assessed [8, 9]. To limit the number of carbapenemase producers missed by the algorithm, a secondary criterion was evaluated to assess the performance of testing isolates that meet the algorithm criterion or that meet a second criterion: imipenem- or meropenem-resistant plus ceftolozane/tazobactam-nonsusceptible. Ceftazidime/avibactam was not assessed as many serine carbapenemase-producing *P. aeruginosa* may test susceptible, and thus this would have poor sensitivity [8].

In Vitro Susceptibility Testing

In vitro susceptibility testing was conducted by the submitting sites per local standards of practice including automated susceptibility systems and disk diffusion. If the susceptibility test result was not available due to the laboratory's standard of care, the susceptibility testing was conducted using broth microdilution, per Clinical and Laboratory Standards Institute (CLSI) standards at the central laboratory. MICs were assessed for ceftazidime, cefepime, and ceftolozane/tazobactam and interpreted per CLSI and European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards as the MIC breakpoints that constitute nonsusceptibility match between both interpretations [5, 11]. For EUCAST, the "I" interpretation was considered susceptible as it implies susceptible increased exposure [11].

Phenotypic and Genotypic Carbapenemase Categorization

Carbapenemase testing as defined previously was used in the present analysis [10]. Briefly, isolates were tested for carbapenemase production by local genotypic testing, if available. All isolates underwent testing with the modified carbapenem inactivation method (mCIM) as previously described [5]. Phenotypically carbapenemase-positive isolates were then tested genotypically using the Carba-R, Carba-R NxG (Cepheid, Sunnyvale, CA, USA) and whole-genome sequencing (if negative on the first 2 platforms) [10]. In total, 265 of the 807 isolates (33%) tested phenotypically positive for carbapenemase activity [10]. Of these, 228 had genotypically confirmed carbapenemase genes including *blaVIM*, *blaIMP*, *blaNDM*, *blaKPC*, *blaGES*, and multiple carbapenemases (*blaVIM* and *blaIMP*, *blaVIM* and *blaKPC*, *blaVIM* and *blaOXA-48*) [10]. The remaining 37 isolates did not have detection of any previously defined carbapenemase genes on polymerase chain reaction (PCR). Whole-genome sequencing was conducted on 21 of these 37 isolates to assess for other carbapenemases, of which none were detected. The other 16 isolates had similar phenotypes and similar geographic origins as isolates that were assessed via whole-genome sequencing.

Analysis

Isolates were categorized as algorithm-positive (meeting the criteria) or algorithm-negative (not meeting algorithm criteria) as they correspond to the primary and secondary criteria.

To analyze the performance of each algorithm criterion, phenotypic carbapenemase testing was utilized as the reference standard to which the algorithm results were compared. This was selected as a conservative assessment as genotypic testing may be limited by the included genotypic targets but phenotypic testing is not target specific and thus represents a global assessment of carbapenemase activity. The mCIM has a test performance for *P. aeruginosa* of sensitivity 93%–98% and specificity 85%–95% [5–7]. The number (%) of isolates that would be tested for carbapenemase genes (meeting algorithm criteria) was compared with all included carbapenem-resistant *P. aeruginosa* isolates. The number of isolates missed by the algorithm (algorithm-negative) but positive by phenotypic carbapenemase testing was categorized. Sensitivity and specificity were calculated for each algorithm criterion. Positive and negative predictive values were calculated using the 33% phenotypically carbapenemase-positive isolates from the cohort to describe prevalence.

RESULTS

Eight hundred seven CRPA were assessed, and 464 isolates met the initial algorithm criterion (imipenem- or meropenem-R plus ceftazidime-NS plus cefepime-NS) and thus would have been selected for carbapenemase testing, resulting in a 43% reduction in testing compared with testing all carbapenem-resistant isolates. When tested against phenotypically carbapenemase-positive isolates, a total of 40 (15% of phenotypically carbapenemase-positive) isolates would have not been tested due to not meeting algorithm criteria. Figure 1A depicts the number of phenotypically carbapenemase-positive isolates that were algorithm-positive and -negative. The summary of test performance and genotypic characteristics of isolates that were algorithm-negative but phenotypically carbapenemase-positive is reported in Table 1. Notably, 20 of the 40 (50%) phenotypically carbapenemase-positive isolates missed by the algorithm lacked a known carbapenemase gene by PCR or whole-genome sequencing. The most commonly missed carbapenemase class was Guiana extended spectrum (GES)-harboring isolates (n = 13 of the 20 isolates), including isolates from 4 different countries (Turkey, Kuwait, Saudi Arabia, and Italy). Table 2 describes the genotypic carbapenemase targets that were true positives and false negatives by each algorithm assessed. The 40 algorithm false negatives were also assessed by broth microdilution, which confirmed algorithm false negativity in 83% of these isolates.

The addition of a secondary carbapenemase testing criterion to the algorithm—imipenem- or meropenem-R plus ceftolozane/tazobactam-NS—resulted in 491 isolates tested and thus a 39% decrease in testing compared with testing all CRPA. Figure 1B depicts the number of phenotypically carbapenemase-positive isolates that were algorithm-positive and -negative with

Table 1. Performance Characteristics of the 2 Evaluated Algorithm Criteria Against a Cohort of 807 Globally Collected Carbapenem-Resistant *P. aeruginosa* That Included 265 Phenotypically Carbapenemase-Positive Isolates

Criteria	No. of Isolates Tested	% Reduction in Carbapenemase Tests	No. of Phenotypically CP-CRPA Successfully Identified (% of All CP-CRPA)	No. of Phenotypically CP-CRPA Missed (% of All CP-CRPA)	Genotype of Phenotypically Carbapenemase-Positive Isolates Missed
Primary algorithm criterion: imipenem- or meropenem-R plus ceftazidime-NS plus cefepime-NS	464	43	225 (85)	40 (15)	Isolates with classically described carbapenemase gene, n = 20 -GES, n = 13 -VIM, n = 6 -KPC, n = 1 Isolates without classically described carbapenemase genes after whole-genome sequencing, n = 20 -OXA-10-like + OXA-50-like + PDC, n = 2 -n = 11, not sequenced but have similar phenotypes and same site -OXA-2-like + OXA-50-like + PDC, n = 3 -OXA-50-like + PDC, n = 2 -n = 2, not sequenced but have similar phenotypes and same site
Primary algorithm criterion OR secondary algorithm criterion: imipenem- or meropenem-R plus ceftazidime-NS plus cefepime-NS (algorithm) OR imipenem- or meropenem-R plus ceftolozane/tazobactam-NS	491	39	244 (92)	21 (8)	Isolates with classically described carbapenemase gene, n = 4 -GES, n = 1 -VIM, n = 2 -KPC, n = 1 Isolates without classically described carbapenemase genes after whole-genome sequencing, n = 17 -OXA-10-like + OXA-50-like + PDC, n = 1 -n = 11, not sequenced but have similar phenotypes and same site -OXA-2-like + OXA-50-like + PDC, n = 1 -OXA-50-like + PDC, n = 2 -n = 2, not sequenced but have similar phenotypes and same site

Abbreviations: CP-CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; GES, Guiana extended spectrum; KPC, *Klebsiella pneumoniae* carbapenemase; NS, nonsusceptible; OXA, oxacillinase; PDC, *Pseudomonas*-derived cephalosporinase; R, resistant; VIM, Verona integron-encoded metallo- β -lactamase.

the addition of the ceftolozane/tazobactam-based criteria. The number of phenotypically carbapenemase-positive isolates that were missed by the addition of this criterion decreased to 21 isolates (8% of all carbapenemase-positive). Similarly, 81% of phenotypically carbapenemase-positive isolates missed lacked classic carbapenemases when tested by PCR or whole-genome sequencing (Table 1).

The test performance of each algorithm is presented in Table 3. Indeed, despite the addition of the secondary algorithm criterion reducing the number of false-negative isolates, the increase in sensitivity and negative predicted value was marginal due to the high performance of the primary algorithm.

DISCUSSION

Application of our previously derived, clinically applicable algorithm to guide definitive carbapenemase testing among

P. aeruginosa performed well to reduce the number of unnecessary carbapenemase tests compared with testing all carbapenem-resistant isolates in a diverse population of CRPA. Importantly, a high percentage of isolates that tested phenotypically positive for carbapenemase were captured by the primary algorithm criterion. The addition of the secondary testing criterion of imipenem- or meropenem-resistant plus ceftolozane/tazobactam-nonsusceptible further increased the number of phenotypically carbapenemase-positive isolates that would be selected for testing by the algorithm criteria, although the improvement in sensitivity was only marginal. Indeed, both approaches presented above have value to both CLSI and EUCAST interpretations as both were considered in the development. Using this algorithmic approach may help streamline carbapenemase detection efforts.

Some isolates were missed by the algorithm that tested phenotypically positive for carbapenemase production. Introduction

Table 2. Genotypic Carbapenemase Targets Identified in Phenotypically Carbapenemase Positive Isolates

Genotypic Target	Primary Algorithm Criterion: Imipenem- or Meropenem-R Plus Ceftazidime-NS Plus Cefepime-NS		Primary Algorithm Criterion OR Secondary Algorithm Criterion: Imipenem- or Meropenem-R Plus Ceftazidime-NS Plus Cefepime-NS (Algorithm) OR Imipenem- or Meropenem-R Plus Ceftolozane/ Tazobactam-NS	
	True Positive	False Negative	True Positive	False Negative
VIM	130	6	134	2
IMP	15	0	15	0
NDM	13	0	13	0
KPC	7	1	7	1
GES	31	13	43	1
VIM and IMP	3	0	3	0
VIM and OXA	1	0	1	0
VIM and KPC	8	0	8	0
No targets identified	17	20	20	17

Abbreviations: GES, Guiana extended spectrum; IMP, imipenemase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; NS, nonsusceptible; OXA, oxacillinase; R, resistant; VIM, Verona integron-encoded metallo-β-lactamase.

of the second step (ie, ceftolozane/tazobactam-nonsusceptible) reduced the number of isolates missed, adding sensitivity to the previously defined criterion. GES-positive isolates accounted for 13 of the isolates missed by the primary algorithm due to cefepime susceptibility. The addition of the secondary criteria reduced the number of GES isolates missed to 1 isolate. This is not unlike our previous findings where the addition of ceftolozane/tazobactam failed to capture 2 GES-harboring isolates and may be due to the variable hydrolytic potentials of this diverse class of enzymes [9, 12]. Importantly, the addition of the ceftolozane/tazobactam criterion represents an alternative, as not all institutions have these MIC data readily available. The base algorithm still performed well to streamline carbapenemase testing while not excluding a high proportion of phenotypically carbapenemase-positive isolates. Interestingly, over half of phenotypically carbapenemase-positive isolates missed by the algorithm criteria lacked classically categorized carbapenemase genes. This was similar to our previous findings, where phenotypic susceptibility to ceftolozane/tazobactam was noted in an OXA-2- and OXA-50-like harboring isolate that tested mCIM-positive [8]. These mismatched results of

carbapenemase detection that were phenotypically susceptible to various β-lactam agents represent a distinct clinical challenge, as the best therapeutic strategy is unclear, although when using phenotypic detection it is plausible that the test is detecting an enzyme(s) that breaks down the test compound (ie, meropenem in mCIM) but lacks viable hydrolytic activity against other agents (ie, antipseudomonal cephalosporins) [13]. Clinical and in vivo data using human-simulated exposures are needed to help categorize the clinical relevance of these findings to guide treatment decisions [14]. While ceftazidime/avibactam plays an important clinical role in the management of CRPA inclusive of GES- and *Klebsiella pneumoniae* carbapenemase (KPC)-producing strains, inclusion of this compound's phenotypic profile in the algorithm criterion was not undertaken as the high proportion testing susceptible would result in a false-negative screen by the criterion [8, 10]. In the context of the algorithmic carbapenemase screening, the limited number of phenotypically carbapenemase-positive isolates missed by the algorithm further supports its utility in the clinical laboratory.

Our work builds upon our previous studies as well as those recently reported using a similar approach [8, 9, 15].

Table 3. Test Performance of Each Algorithm Criteria

	Primary Algorithm Criterion OR Secondary Algorithm Criterion: Imipenem- or Meropenem-R Plus Ceftazidime-NS Plus Cefepime-NS (Algorithm) OR Imipenem- or Meropenem-R Plus Ceftolozane/Tazobactam-NS	
	Primary Algorithm Criterion: Imipenem- or Meropenem-R Plus Ceftazidime-NS Plus Cefepime-NS	Primary Algorithm Criterion OR Secondary Algorithm Criterion: Imipenem- or Meropenem-R Plus Ceftazidime-NS Plus Cefepime-NS (Algorithm) OR Imipenem- or Meropenem-R Plus Ceftolozane/Tazobactam-NS
Sensitivity (95% CI), %	85 (80–89)	92 (88–95)
Specificity (95% CI), %	56 (52–60)	54 (50–59)
Positive predictive value, %	49	50
Negative predictive value, %	88	93

Abbreviations: NS, nonsusceptible; R, resistant.

Vallabhaneni and colleagues recently reported data that showed that adding the criteria of cefepime- or ceftazidime-NS reduced the number needed to test for detection of CP-CRPA, which was further decreased if ceftolozane/tazobactam-NS was employed in CRPA isolates from the United States [15]. The differences in our study designs are notable as we used phenotypic detection of carbapenemases as the standard identification, to which we compared algorithm performance rather than genotypic carbapenemases. Both approaches have their own benefits and limitations, although genotypic detection is limited to the targeted enzymology. In our approach, all CRPA were systematically assessed using phenotypic carbapenemase detection, and positive isolates underwent genotypic testing with Carba-R and Carba-R NxG, which enhances the carbapenemase detection profile in our study to include GES and certain IMP subtypes, among others [16]. Similarly, isolates that were phenotypically carbapenemase-positive but genotypically negative by PCR underwent whole-genome sequencing to further evaluate the underlying mechanisms [10]. Although mCIM has been noted to have excellent sensitivity (93%–98% for *P. aeruginosa*), it is liable to false-positive results, and thus the present study represents a conservative assessment of our proposed algorithm's performance [6, 7]. The number of isolates testing mCIM-positive, genotypically carbapenemase-negative further highlights the need for confirmatory genotypic testing of phenotypically carbapenemase-positive isolates in the clinic. Additionally, our data come from a global collection of CRPA that assessed regions with both high and low prevalence of CP-CRPA including an overall prevalence of phenotypically defined carbapenemase activity in 33% of the CRPA assessed. This compares to the ~3%–10% of CRPA testing positive for carbapenemases from our previous and other studies [9, 15]. These data provide important insight into the algorithm's performance when the baseline prevalence of CP-CRPA is high and thus adds translatability of this approach to areas with higher prevalence of CP-CRPA than previously assessed. Another strength of the present study included that MICs were determined by the standard methods of study sites, further adding to the applicability of this study to the clinic. Indeed, the algorithm false negatives were assessed by reference broth microdilution, revealing that 83% were confirmed to be algorithm-negative (ie, susceptible to either cefepime or ceftazidime). This is not unexpected; particularly when isolates' MICs are at or around the susceptibility breakpoint, there is a high potential for categorical disagreement as the acceptable variability of MIC testing can vary by 100% on either side of the MIC result [17]. In the analysis of the algorithm, we utilized the MIC result from the submitting sites as it represents (1) a worst-case assessment of the proposed algorithm and (2) is clinically applicable as it reflects the methods utilized regularly in clinical practice.

In conclusion, the application of our phenotypic algorithm to guide definitive carbapenemase testing among

carbapenem-resistant *P. aeruginosa* decreased the number of tests conducted compared with testing all carbapenem-resistant isolates. Even in the high-prevalence global cohort with diverse genotypic profiles, a high proportion of phenotypically carbapenemase-positive isolates were captured by the primary algorithm criteria. The addition of imipenem- or meropenem-R plus ceftolozane/tazobactam-NS further improved the algorithm's performance and may be most useful in regions with high prevalence of GES enzymes. These data support the utilization of this approach in regions with varying prevalence and diversity of CP-CRPA. Clinical implementation will aid in streamlining of carbapenemase detection workflow.

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Patient consent. The present study was institutional review board approved under exempt status as not human subjects research, and thus informed consent was not required.

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References

- Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev* **2009**; 22:582–610.
- Woodworth KR, Walters MS, Weiner LM, et al. Vital signs: containment of novel multidrug-resistant organisms and resistance mechanisms - United States, 2006–2017. *MMWR Morb Mortal Wkly Rep* **2018**; 67:396–401.
- Hammoudi Halat D, Ayoub Moubareck C. The current burden of carbapenemases: review of significant properties and dissemination among gram-negative bacteria. *Antibiotics (Basel)* **2020**; 9:186.
- Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria. *Clin Infect Dis* **2019**; 69:S521–8.
- Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. 30th ed. Clinical and Laboratory Standards Institute; **2020**.
- Tamma PD, Simner PJ. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *J Clin Microbiol* **2018**; 56:e01140–18.
- Gill CM, Lasko MJ, Asempa TE, et al. Evaluation of the EDTA-modified carbapenem inactivation method for detecting metallo- β -lactamase-producing *Pseudomonas aeruginosa*. *J Clin Microbiol* **2020**; 58:e02015–19.

8. Gill CM, Asempa TE, Nicolau DP. Development and application of a pragmatic algorithm to guide definitive carbapenemase testing to identify carbapenemase-producing *Pseudomonas aeruginosa*. *Antibiotics (Basel)* **2020**; *9*:738.
9. Gill CM, Kresken M, Seifert H, et al. Evaluation of a phenotypic algorithm to direct carbapenemase testing in *Pseudomonas aeruginosa*: validation in a multicenter German cohort. *Microb Drug Resist*. **2021**; *27*:1243–8.
10. Gill CM, Aktaş E, Alfouzan W, et al; on behalf of the ERACE-PA Global Study Group. The ERACE-PA global surveillance program: ceftolozane/tazobactam and ceftazidime/avibactam in vitro activity against a global collection of carbapenem-resistant *Pseudomonas aeruginosa*. *Eur J Clin Microbiol Infect Dis*. **2021**; *40*:2533–41.
11. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0. **2021**. Available at: <http://www.eucast.org>. Accessed November 17, 2021.
12. Naas T, Dortet L, Iorga BI. Structural and functional aspects of class A carbapenemases. *Curr Drug Targets* **2016**; *17*:1006–28.
13. Yee R, Dien Bard J, Simner PJ. The genotype to phenotype dilemma: how should laboratories approach discordant susceptibility results? *J Clin Microbiol* **2021**; *13*:JCM.00138–20.
14. Gill CM, Brink A, Chu CY, et al. Phenotypic/genotypic profile of OXA-10-like-harboring, carbapenem-resistant *Pseudomonas aeruginosa*: using validated pharmacokinetic/pharmacodynamic in vivo models to further evaluate enzyme functionality and clinical implications. *Antimicrob Agents Chemother* **2021**; *65*:e0127421.
15. Vallabhaneni S, Huang JY, Grass JE, et al. Antimicrobial susceptibility profiles to predict the presence of carbapenemase genes among carbapenem-resistant *Pseudomonas aeruginosa*. *J Clin Microbiol*. **2021**; *59*:e02874–20.
16. Gill CM, Asempa TE, Tickler IA, et al. Evaluation of the Xpert Carba-R NxG assay for detection of carbapenemase genes in a global challenge set of *Pseudomonas aeruginosa* isolates. *J Clin Microbiol* **2020**; *58*:e01098–20.
17. Lasko MJ, Huse HK, Nicolau DP, Kuti JL. Contemporary analysis of ETEST for antibiotic susceptibility and minimum inhibitory concentration agreement against *Pseudomonas aeruginosa* from patients with cystic fibrosis. *Ann Clin Microbiol Antimicrob* **2021**; *20*:9.