Development and Application of a Pragmatic Algorithm for the Detection of Carbapenemase-Producing *Pseudomonas aeruginosa* (CP-PA)

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ABSTRACT

Background: Historically, carbapenem-resistance in P. aeruginosa (PA) has been mediated by inducible AmpC, drug efflux, and porin loss; however, carbapenemase production is an increasingly recognized entity. Of these mechanisms, carbapenemases can drastically reduce treatment options and rapidly disseminate. Since broad applications of phenotypic (mCIM/eCIM) and PCR-based detection can be labor intensive and costly, we developed an MIC derived algorithm to streamline use of these definitive carbapenemase detection methodologies.

Methods: To develop the testing criteria, a challenge set of PA (n=92), NDM, IMP, VIM, KPC, SPM, GES, cephalosporinase or efflux/porin mutation and wild-type isolates were utilized. Broth microdilution MICs were determined for: ceftazidime (CAZ), cefepime (FEP), piperacillin/tazobactam (TZP), meropenem (MEM), imipenem (IPM), ceftolozane/tazobactam (C/T), and ceftazidime/avibactam (CZA). To assess the utility of CAZ, FEP, TZP, and C/T screening criteria from the challenge set, 1,209 clinical PA isolates from a US surveillance program were tested. Confirmatory genotypic and phenotypic testing for evidence of carbapenemases was conducted on all criteria-derived isolates using the Xpert Carba-R assay and the modified carbapenem inactivation method (mCIM)/EDTA-modified carbapenem inactivation method (eCIM), respectively.

Results: Test performance and characteristics of the challenge set are displayed in Table 1. Of the 1,209 clinical isolates, 230 (19%) were IPM and MEM resistant. 116 isolates met the defined criteria (using most common anti-pseudomonal β-lactams) of: IPM and MEM resistance; non-susceptibility to CAZ, FEP, and TZP. Carba-R identified 5 carbapenemase-producing isolates (all blaVIM-positive), while the mCIM/eCIM detected 7 carbapenemase-producing isolates (including the 5 blaVIM-positive isolates).

Conclusion: In the presence of carbapenem resistance, non-susceptibility to FEP, CAZ, and TZP (or C/T when available) is a useful starting point to delineate CP-PA versus non-CP-PA. This MIC criterion combined with either mCIM/eCIM or PCR-based testing is a pragmatic and streamlined approach to identify CP-PA, while providing vital information to guide therapeutic and infection control measures.

INTRODUCTION

- Carbapenem-resistant *P. aeruginosa* are a notable clinical challenge both in the US and abroad.[1]
- Carbapenem resistance in *P. aeruginosa* in the US is typically mediated by efflux/porin loss and over-expression of cephalosporinases although carbapenemases have been detected including in outbreaks.[2]
- Detection of carbapenemase-producing *P. aeruginosa* is imperative as it is associated with nosocomial spread and limited treatment options.[3]
- Broad applications of phenotypic (mCIM/eCIM) and PCR-based detection for carbapenem resistant *P. aeruginosa* can be labor intensive and costly.
- A pragmatic algorithm using accessible phenotypic profiles (i.e., MICs) may help guide directed carbapenemase testing.

OBJECTIVE

1) To develop a phenotypic algorithm to guide definitive carbapenemase testing using a challenge set of *P. aeruginosa* including: carbapenemase-producing, carbapenemase-negative, carbapenem-resistant *P. aeruginosa,* and wild-type *P. aeruginosa.*
2) Apply the derived testing criteria to a US, multicenter surveillance program to identify carbapenemase-producing *P. aeruginosa*.

METHODS

P. aeruginosa Challenge Set

- The challenge set comprised of 92 *P. aeruginosa* isolates.
- Isolates included NDM-, IMP-, VIM-, KPC-, SPM-, and GES-positive strains.
- Twenty isolates were carbapenemase negative with documented cephalosporinase/efflux mutations to serve as negative controls.
- Seven wild-type strains were included in the challenge set as additional negative controls.

Phenotypic MIC Determination

- Broth microdilution MICs were determined for ceftazidime, cefepime, piperacillin/tazobactam, meropenem, imipenem, ceftolozane/tazobactam, and ceftazidime/avibactam per CLSI standards.[4]
- The phenotypic profile was used to compare different algorithm testing criteria to capture the highest number of carbapenemase-producing strains while minimizing the number non-carbapenemase strains selected.

Data Analysis

- Sensitivity and specificity was calculated for various testing criteria to compare how well each criteria performed within the challenge set.

Application of Algorithm to a US Surveillance Study

- The derived algorithm was applied to clinical *P. aeruginosa* isolates from a multicenter, US surveillance study, n = 1,209[5]
- MICs were determined using broth microdilution per CLSI methodology[4]
- Isolates meeting the algorithm testing criteria were selected to undergo phenotypic (mCIM/eCIM) and genotypic (Xpert® Carba-R) carbapenemase testing.

Modified Carbapenem Inactivation Method/ EDTA-Modified Carbapenem inactivation method (mCIM/eCIM)

- mCIM testing was conducted as outlined in CLSI M100.[4]
- eCIM testing was conducted simultaneous to mCIM as previously described.[6]
- Four quality control strains were included with each mCIM/eCIM run: two negative controls (*K. pneumonia* ATCC 1706 and *P. aeruginosa* ATCC 27853), one serine-carbapenemase control (*K. pneumoniae* ATCC 1705, KPC-positive), and a Metallo-β-lactamase positive control (*K. pneumoniae* CDC Bank #505, NDM-positive).

Cepheid Carba-R Testing

- Testing using the Xpert® Carba-R assay was conducted at the Center for Anti-Infective Research and Development per the package insert.[7]
- Quality control was conducted weekly using the Xpert® Carba-R QC Panel (Maine Molecular).

Whole Genome Sequencing

- Any isolate with discordant results between mCIM/eCIM and Carba-R assay underwent whole-genome sequencing (WGS).

Antibiotic abbreviations
IPM = imipenem; MEM = meropenem; CAZ = ceftazidime; FEP = cefepime; TZP = piperacillin/tazobactam; C/T = ceftolozane/tazobactam; CZA = ceftazidime/avibactam; R = resistant; NS = non-susceptible

RESULTS

Table 1. Characteristics of the Challenge Set of 92 *P. aeruginosa* isolates utilized in algorithm development.

Susceptibility Criteria	Carbapenemase Producers, n = 57	Non-Carbapenemase Producers,		Test Performance	
		Cephalosporinase or Efflux/Porin Mutation, n = 20	Wild Type, n = 15	Sensitivity % (95% CI)	Specificity % (95% CI)
IPM + MEM- R	57 (100%)	15 (75%)	1 (7%)	100% (94–100%)	54% (37–71%)
IPM + MEM- R AND FEP + CAZ + TZP- NS	57 (100%)	12 (60%)	0 (0%)	100% (94–100%)	66% (48–81%)
IPM + MEM- R AND FEP + CAZ + TZP- R	47 (82%)	6 (30%)	0 (0%)	83% (70–91%)	83% (66–93%)
IPM + MEM- R AND FEP + CAZ + TZP- NS + CZA- R	49 (86%)	8 (40%)	0 (0%)	86% (74–94%)	77% (60–90%)
IPM + MEM- R AND FEP + CAZ + TZP- NS + C/T- R	57 (100%)	4 (20%)	0 (0%)	100% (94–100%)	89% (73–97%)
IPM + MEM- R AND FEP + CAZ + TZP- NS + C/T- R+ CZA- R	49 (86%)	3 (15%)	0 (0%)	86% (74–94%)	91% (77–98%)

Derivation of Phenotypic Screening Criteria in the Challenge Set

- Piperacillin/tazobactam-resistance decreased the test performance because 10 carbapenemase producers resulted with intermediate MICs.
- The test performance of ceftazidime/avibactam-resistance was contingent on carbapenemase epidemiology as some classes (i.e., KPC- and some GES-) may be susceptible.
- Since ceftolozane/tazobactam and ceftazidime/avibactam MICs may be delayed or unavailable, the algorithm base used imipenem and meropenem-resistant AND ceftazidime, cefepime, and piperacillin/tazobactam- non-susceptible as the starting criteria (**Figure 1**).

Figure 1. Algorithm for carbapenemase detection in *Pseudomonas aeruginosa*.

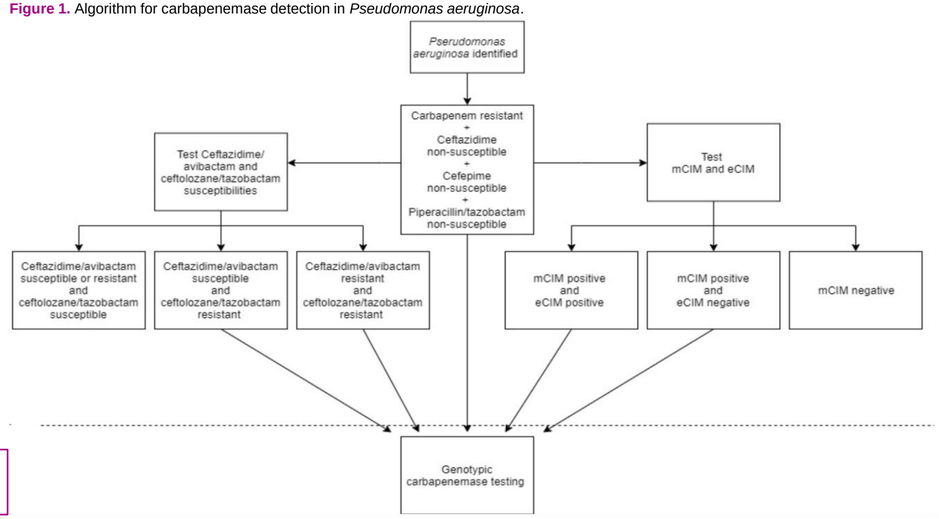

Table 2. Application to 1,209 clinical *P. aeruginosa* isolates from a US surveillance study.

Algorithm-Derived Screening Criteria	Number Meeting Criteria	Carbapenemase Producers Detected	Carbapenemase Producers Missed By Criteria
IPM + MEM- R AND FEP + CAZ + TZP- NS	116	7/116	0
IPM + MEM- R AND FEP + CAZ + TZP- NS + CZA- R	43	7/43	0
IPM + MEM- R AND FEP + CAZ + TZP- NS + C/T- R	21	6/21	1*
IPM + MEM- R AND FEP + CAZ + TZP- NS + C/T- R + CZA-R	19	6/19	1*

*Isolate harbored OXA-2, OXA-50

Carbapenemases Detected from Multicenter US Surveillance Program

- The Xpert Carba-R detected five carbapenemase harboring strains all harboring ***bla*VIM**.
- mCIM/eCIM testing confirmed all **five *bla*VIM-positive** strains and detected two additional isolates that were mCIM-positive and eCIM-negative suggesting a non-metallo-β-lactamases that are outside the current Carba-R targets.
  - Whole genome-sequencing revealed one isolate that was **GES-20-positive,** a known carbapenemase, while the second was **OXA-2, OXA-50** positive but lacking a known carbapenemase.

DISCUSSION/CONCLUSIONS

- Imipenem and meropenem-resistance alone poorly predicted carbapenemase activity in the challenge set (**Table 1**).
- Addition of ceftazidime, cefepime, and piperacillin/tazobactam-non-susceptibility improved the test performance and was used as the base of the phenotypic algorithm (**Table 1** and **Figure 1**).
- Test performance is further enhanced by introducing ceftolozane/tazobactam and ceftazidime/avibactam-resistance (**Table 1** and **Table 2**).
- Application of the testing criteria using the genotypic and phenotypic detected **five VIM positive** strains, **one GES-positive** strain, and one strain without known carbapenemase production.
- Application of this phenotypic algorithm may be a more targeted approach to carbapenemase testing in *P. aeruginosa* but further validation is needed.

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