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Authors

Evans, Scott R
Hujer, Andrea M
Jiang, Hongyu
et al.

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Informing Antibiotic Treatment Decisions: Evaluating Rapid Molecular Diagnostics To Identify Susceptibility and Resistance to Carbapenems against *Acinetobacter* spp. in PRIMERS III

Scott R. Evans,[a] Andrea M. Hujer,[b,c] Hongyu Jiang,[a] Carol B. Hill,[d] Kristine M. Hujer,[b,c] Jose R. Mediavilla,[e] Claudia Manca,[e] Thuy Tien T. Tran,[a] T. Nicholas Domitrovic,[b,c] Paul G. Higgins,[f,g] Harald Seifert,[f,g] Barry N. Kreiswirth,[e] Robin Patel,[h] Michael R. Jacobs,[i] Liang Chen,[e] Rangarajan Sampath,[j] Thomas Hall,[j] Christine Marzan,[j] Vance G. Fowler, Jr.,[d,k] Henry F. Chambers,[l] Robert A. Bonomo,[b,c,m] for the Antibacterial Resistance Leadership Group (ARLG)

Center for Biostatistics in AIDS Research and the Department of Biostatistics, Harvard University, Boston, Massachusetts, USA[a]; Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA[b]; Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA[c]; Duke Clinical Research Institute, Duke University Medical Center, Durham, North Carolina, USA[d]; Public Health Research Institute Center, New Jersey Medical School-Rutgers University, Newark, New Jersey, USA[e]; Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany[f]; German Center for Infection Research (DZIF), Partner Site Bonn, Cologne, Germany[g]; Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA[h]; Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA[i]; IBIS Biosciences Inc., an Abbott Company, Carlsbad, California, USA[j]; Division of Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA[k]; University of California, San Francisco General Hospital, San Francisco, California, USA[l]; Departments of Pharmacology, Molecular Biology and Microbiology, Biochemistry, and Proteomics and Bioinformatics, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA[m]

ABSTRACT The widespread dissemination of carbapenem-resistant *Acinetobacter* spp. has created significant therapeutic challenges. At present, rapid molecular diagnostics (RMDs) that can identify this phenotype are not commercially available. Two RMD platforms, PCR combined with electrospray ionization mass spectrometry (PCR/ESI-MS) and molecular beacons (MB), for detecting genes conferring resistance/susceptibility to carbapenems in *Acinetobacter* spp. were evaluated. An archived collection of 200 clinical *Acinetobacter* sp. isolates was tested. Predictive values for susceptibility and resistance were estimated as a function of susceptibility prevalence and were based on the absence or presence of beta-lactamase (*bla*) NDM, VIM, IMP, KPC, and OXA carbapenemase genes (e.g., $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40}}$, and $bla_{\text{OXA-58}}$ found in this study) against the reference standard of MIC determinations. According to the interpretation of MICs, 49% ($n = 98$) of the isolates were carbapenem resistant (as defined by either resistance or intermediate resistance to imipenem). The susceptibility sensitivities (95% confidence interval [CI]) for imipenem were 82% (74%, 89%) and 92% (85%, 97%) for PCR/ESI-MS and MB, respectively. Resistance sensitivities (95% CI) for imipenem were 95% (88%, 98%) and 88% (80%, 94%) for PCR/ESI-MS and MB, respectively. PRIMERS III establishes that RMDs can discriminate between carbapenem resistance and susceptibility in *Acinetobacter* spp. In the context of a known prevalence of resistance, SPVs and RPVs can inform clinicians regarding the best choice for empiric antimicrobial therapy against this multidrug-resistant pathogen.

KEYWORDS *Acinetobacter*, beta-lactams, carbapenemases

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Address correspondence to Scott R. Evans, evans@sdac.harvard.edu, or Robert A. Bonomo, robert.bonomo@va.gov.

Resistance to antibiotics is a major public health threat, and rapid diagnostic platforms are needed to assist clinicians in choosing effective empiric therapy. In the platforms for rapid identification of multidrug-resistant gram-negative bacteria and evaluation of resistance studies (PRIMERS) I and II, analytical strategies were developed and tested to evaluate whether genotypic results obtained by nucleic acid amplification technologies could identify susceptibility and resistance to beta-lactam antibiotics using a carefully chosen panel of susceptible and highly beta-lactam-resistant *Enterobacteriaceae* (1). The rapid molecular diagnostic (RMD) platforms that formed the testing basis for that investigation were (i) PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS); (ii) molecular beacons (MB); (iii) a DNA microarray kit; and (iv) a next-generation sequencing platform.

In PRIMERS I and II, we showed that RMD platforms could help inform empiric beta-lactam therapy against *Escherichia coli* and *Klebsiella pneumoniae*. Moreover, our efforts demonstrated that it was possible to transform beta-lactam resistance genotypic data into a practical decision-making tool, which may be useful to clinicians when the prevalence of resistance for a given population is applied.

The next challenge is whether RMD platforms and analytical strategies can be employed against other Gram-negative multidrug-resistant (MDR) pathogens. *Acinetobacter* spp. are proving to be among the most problematic pathogens facing contemporary clinicians (2). This nefarious status is attributed to difficulties in identifying *Acinetobacter* spp. to the species level, the increasing panoply of resistance phenotypes that confound treatment decisions, and an emerging understanding of their virulence properties (3, 4). Despite the insights obtained using whole-genome sequencing, vexing questions remain regarding the nosology of syndromes caused by *Acinetobacter* spp., including the choice of optimal initial and definitive therapies.

Regarding the correct identification of *Acinetobacter* spp., multiple commercially available microbiological and RMD platforms exist that try to identify species within the genus. Proper species identification is important, as relevant differences exist between the species with regard to treatment decisions, epidemiology, immunogenicity, and most importantly, resistance profiles (e.g., carbapenem resistant [CR] or susceptible [CS]; *Acinetobacter baumannii* versus *Acinetobacter pittii*). Currently, clinicians may place more weight on the CR and CS designation than correct species identification, but this should change.

Due to rising antimicrobial resistance, carbapenems are the cornerstone of therapy for the treatment of serious infections due to *Acinetobacter* spp. Unfortunately, the widespread dissemination of metallo-beta-lactamases (MBLs) and particularly the OXA carbapenemases has created significant therapeutic challenges. In the United States each year, ~12,000 cases of MDR *Acinetobacter* infections occur and are associated with at least 500 deaths (http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf#page=59). More than half of these isolates are CR, and the number is growing in other parts of the world. Knowing when to use, or not to use, alternative therapies (colistin, polymyxin B, tigecycline, etc.) is critical to patient care and can be lifesaving.

In an attempt to address this dilemma, we employed two RMDs (PCR/ESI-MS and MB) to determine whether the identification of specific genotypes can accurately predict antimicrobial susceptibility (i.e., presence of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24/40}}$, $bla_{\text{OXA-58}}$, bla_{NDM}, bla_{KPC}, bla_{VIM}, and bla_{IMP}, predicting carbapenem resistance). The unique challenge in choosing to study *Acinetobacter* spp. with these two platforms rests upon the observation that multiple resistance determinants can result in a CR phenotype (e.g., OXA carbapenemases, metallo-beta-lactamases, KPCs, etc.). Previously, we found that results from PCR/ESI-MS and MB are very informative, and RMDs can contribute significantly to the decision to use empiric carbapenem therapy among *Enterobacteriaceae*. The current study, PRIMERS III, significantly adds to the knowledge obtained from PRIMERS I and II and further establishes the interpretative power of RMDs coupled with the application of unique analytical methods (1). By detecting specific *bla* genes

TABLE 1 Susceptibility of CS and CR to antimicrobial agents

Antimicrobial agent (susceptible breakpoint, mg/liter)	% Susceptibility[a] (no. susceptible/total no.)	
	CR (n = 98)	CS (n = 102)
Imipenem (≤4)	0 (0/98)	100 (102/102)
Doripenem (≤2)	0 (0/98)	97.1 (99/102)
Meropenem (≤4)	0 (0/98)	98.0 (100/102)
Ciprofloxacin (≤1)	0 (0/98)	81.4 (83/102)
Piperacillin-tazobactam (≤16/4)	1.0 (1/98)	85.3 (87/102)
Gentamicin (≤4)	2.0 (2/98)	83.3 (85/102)
Cefepime (≤8)	5.1 (5/98)	86.3 (88/102)
Ceftazidime (≤8)	6.1 (6/98)	88.2 (90/102)
Tetracycline (≤4)	10.2 (10/98)	82.4 (84/102)
Ampicillin-sulbactam (≤8/4)	19.4 (19/98)	94.1 (96/102)
Amikacin (≤16)	19.4 (19/98)	94.1 (96/102)
Minocycline (≤4)	37.8 (37/98)	96.1 (98/102)
Colistin (≤2)	89.8 (88/98)	96.1 (98/102)
Polymyxin B (≤2)	89.8 (88/98)	98.0 (100/102)
Tigecycline (≤2)	97.0 (95/98)	100 (102/102)

[a]CR, carbapenem resistant; CS, carbapenem susceptible.

conferring resistance to carbapenems, clinicians can have confidence in choosing alternative empiric therapies (colistin, tigecycline, etc.) in ~90% of cases.

RESULTS

Antimicrobial susceptibility testing (AST) and genetic analysis. In Table 1 we summarized the MICs determined for the 200 *Acinetobacter* sp. isolates. In this collection of isolates, >90% of *Acinetobacter* spp. that were CS were also susceptible to ampicillin-sulbactam, amikacin, minocycline, colistin, polymyxin B, and tigecycline; 80 to 89% were susceptible to ciprofloxacin, piperacillin-tazobactam, gentamicin, cefepime, ceftazidime, and tetracycline. When faced with the CS phenotype, most clinicians would favor the use of ampicillin-sulbactam over that of colistin or polymyxin B due to a lower risk of renal toxicity (5).

In Table 2, the CR patterns of the 200 isolates are detailed. By AST, 98 isolates were resistant to imipenem (MIC of >4 mg/liter), 100 were resistant to meropenem (MIC of >4 mg/liter), and 101 were resistant to doripenem (MIC of >2 mg/liter). *Acinetobacter* spp. are intrinsically resistant to ertapenem, so this carbapenem was not included in the analysis.

Notably, 98 isolates were resistant to all three carbapenems that are employed in treatment (doripenem, imipenem, and meropenem). From this comparison, we chose imipenem as the reference compound; therefore, we included 98 CR isolates in this analysis. We note that differences in the actual number that are susceptible or resistant may be due to (i) the ability of different carbapenems to penetrate the outer membrane of *Acinetobacter* spp. and (ii) the activities/potencies of each carbapenem versus the carbapenemase harbored by the strain.

TABLE 2 Carbapenem phenotypic profile of 200 isolates studied

No. of isolates	Susceptibility to[a]:		
	Doripenem	Imipenem	Meropenem
98	S	S	S
1	S	S	R
2	R	S	S
1	R	S	R
98	R	R	R
Total	99 S, 101 R	102 S, 98 R	100 S, 100 R

[a]S, susceptible; R, resistant.

TABLE 3 Genotypic profile of 200 isolates studied via PCR ESI-MS/MB

No. of isolates[a]	bla_{OXA-23}	$bla_{OXA-24/40}$	bla_{OXA-58}
89/106	−	−	−
79/82	+	−	−
24/9	−	+	−
3/1	−	−	+
5/2	+	+	−

[a]Isolate count for PCR ESI-MS/MB.

In Table 1, we also summarize the phenotypic profile of CR *Acinetobacter* spp.; clearly these isolates are very drug resistant. The only agents with notable activity against CR strains of *Acinetobacter* spp. were colistin (89.8% susceptible), polymyxin B (89.8% susceptible), and tigecycline (97.0% susceptible). Ampicillin-sulbactam (19.4% susceptible), amikacin (19.4% susceptible), and minocycline (37.8% susceptible) had activity against some but not all CR *Acinetobacter* sp. isolates. In particular, the low level of susceptibility of this CR collection to minocycline was different from previous reports suggesting susceptibility rates of ~60% (6). Further investigations are in progress to determine the genetic basis of this observation.

Table 3 summarizes the number and identity of carbapenemase genes that were detected. It is important to keep in mind that all *A. baumannii* isolates possess a naturally occurring oxacillinase ($bla_{OXA-51-like}$) gene, which can affect CS. However, CR is dependent upon its level of expression and the gene variant (7, 8). The predominant genes present in our analysis that are recognized to produce a CR phenotype were bla_{OXA-23} and $bla_{OXA-24/40}$. MBLs (bla_{NDM}, bla_{VIM}, and bla_{IMP}) were absent from this collection, and at this time it is uncommon in the United States to detect MBLs in *Acinetobacter* spp.

Figure 1 summarizes the distribution of carbapenem MICs versus target (gene) identification. Both platforms identify target genes in *Acinetobacter* sp. strains within the susceptible and resistant ranges. However, most target gene identification occurred within the resistant *Acinetobacter* sp. population. Interestingly, very few isolates that demonstrate MICs near the breakpoint possessed *bla* carbapenemase genes.

Discrimination summaries and predictive values. Analyses presented in this section were conducted before discrepancy resolution. A phenotypic/genotypic isolate discrimination summary is provided in Table 4 as a cross-classification of the PCR/ESI-MS and MB results with the MIC results for the 200 isolates. For imipenem, the susceptibility and resistance sensitivities were 0.82 (95% confidence intervals [CI], 0.74, 0.89) and 0.95 (CI, 0.88, 0.98), respectively, for PCR/ESI-MS and were 0.92 (CI, 0.85, 0.97) and 0.88 (CI, 0.80, 0.94), respectively, for MB. Results for meropenem and doripenem were similar (Table 5 and Fig. 2).

SPVs and RPVs are displayed as a function of the prevalence of susceptibility (Fig. 3). Assuming 40% national imipenem susceptibility (60% resistance), the SPVs were 91% (CI, 85%, 98%) and 83% (CI, 76%, 91%) for PCR/ESI-MS and MB, respectively, while RPVs were 89% (CI, 85%, 93%) and 94% (CI, 91%, 98%) for PCR/ESI-MS and MB, respectively. Results for meropenem and doripenem were similar.

Discrepancy analyses. Discrepancies were observed due to (i) differences between platform results on a genotypic level; (ii) differences between genotype and predicted CR phenotype; and (iii) differences due to non-*baumannii Acinetobacter* species identification on the PCR/ESI-MS platform. To resolve these issues, discrepant analysis was performed on 63 isolates using a carbapenemase multiplex PCR as a third identification method, and the results are reported in Table 6. In this manner, we evaluated for false-positive or false-negative genotypes, inconsistencies in CR, and identification of non-*A. baumannii* species. We found that the number of false positives was higher using PCR/ESI-MS than MB. In contrast, false negatives were more common when testing with MB.

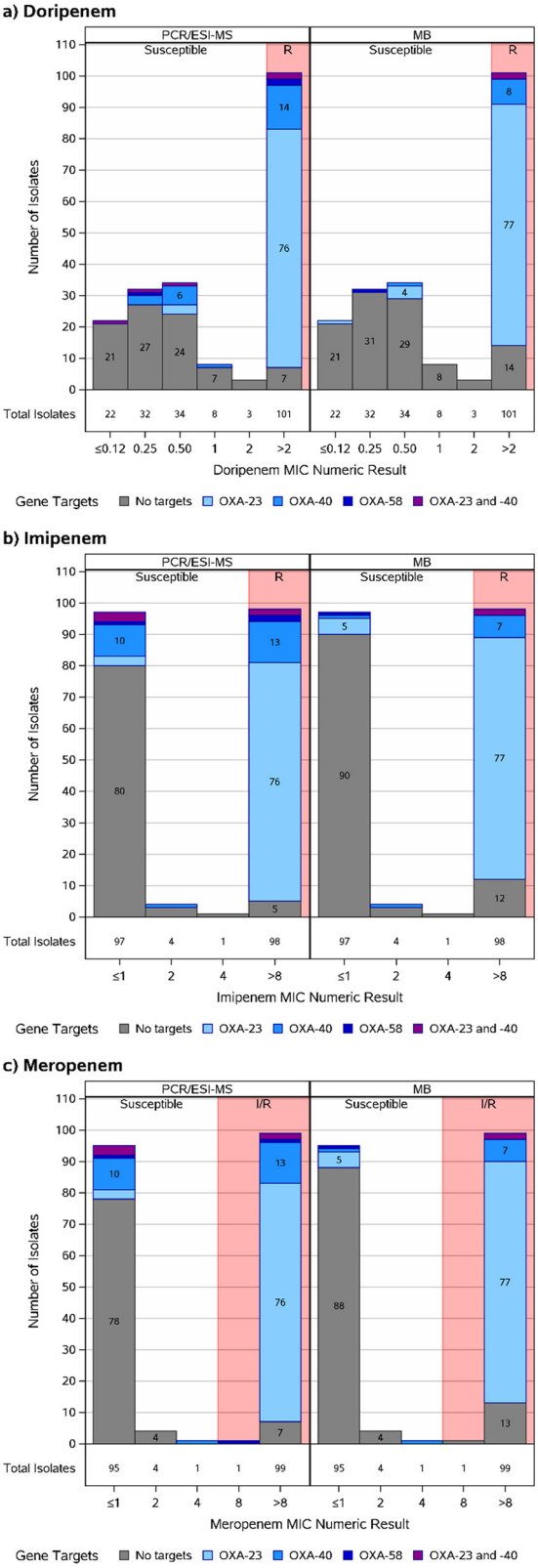

FIG 1 Distribution of *Acinetobacter* sp. carbapenem MICs versus target (gene) identification for PCR/ESI-MS and MB platforms. All gene targets were examined; only identified targets are presented. Abbreviations: I/R, intermediate/resistant; R, resistant.

TABLE 4 Phenotypic/genotypic isolate discrimination summary

Test	No. of isolates resistant or susceptible to[a]:					
	Imipenem		Meropenem		Doripenem	
	Resistant	Susceptible	Resistant	Susceptible	Resistant	Susceptible
Total (MIC)	98	102	100	100	101	99
PCR ESI/MS						
Positive	93	18*	93	18*	94	17*
Negative	5**	84	7**	82	7**	82
MB						
Positive	86	8*	86	8*	87	7*
Negative	12**	94	14**	92	14**	92

[a]*, Overtreatment; **, undertreatment; no asterisk, appropriate treatment.

DISCUSSION

CR *Acinetobacter* sp. infections are among the most challenging to treat, as clinicians are often limited to the use of polymyxins and tigecycline as the only effective therapies. Delaying the use of effective therapy against CR *Acinetobacter* spp. may lead to poor outcomes, whereas the unnecessary use of polymyxin and tigecycline may lead to adverse events associated with these drugs. In PRIMERS I and II, we developed an approach to inform clinicians on how to interpret RMDs that detect beta-lactam resistance in *E. coli* and *K. pneumoniae* in areas with different levels of prevalence. In the PRIMERS III study, our goal was to develop a similar approach to guide the best empiric therapy for *Acinetobacter* sp. infection, as clear guidance in this area is limited.

Using a collection of isolates for which a significant portion was CR, mirroring current Centers for Disease Control and Prevention and World Health Organization estimates of CR prevalence in various areas, MB and PCR/ESI-MS discriminated between CS and CR, thus demonstrating their potential to inform empiric antimicrobial therapy against *Acinetobacter* spp. In these analyses, clinicians can be confident >85% of the time, using either of these two platforms, that results indicating susceptibility or resistance based on gene detection are accurate, thus contributing to better initial antibiotic treatment decisions regarding *Acinetobacter* infections. Considering the epidemiological data present in the United States and worldwide, this improvement in

TABLE 5 Sensitivities and predictive value summary

Parameter and antimicrobial agent (*n*)	Value determined by[b]:	
	PCR/ESI-MS	MB
Susceptibility sensitivities		
Doripenem (99)	0.83 (0.74, 0.90)	0.93 (0.86, 0.97)
Imipenem (102)	0.82 (0.74, 0.89)	0.92 (0.85, 0.97)
Meropenem (100)	0.82 (0.73, 0.89)	0.92 (0.85, 0.96)
Resistance sensitivities		
Doripenem (101)	0.93 (0.86, 0.97)	0.86 (0.78, 0.92)
Imipenem (98)	0.95 (0.88, 0.98)	0.88 (0.80, 0.94)
Meropenem (100)	0.93 (0.86, 0.97)	0.86 (0.78, 0.92)
Susceptibility predictive values[a]		
Doripenem	0.89 (0.82, 0.96)	0.82 (0.74, 0.89)
Imipenem	0.91 (0.85, 0.98)	0.83 (0.76, 0.91)
Meropenem	0.89 (0.81, 0.96)	0.81 (0.74, 0.89)
Resistance predictive values[a]		
Doripenem	0.89 (0.85, 0.93)	0.95 (0.91, 0.98)
Imipenem	0.89 (0.85, 0.93)	0.94 (0.91, 0.98)
Meropenem	0.89 (0.84, 0.93)	0.94 (0.90, 0.98)

[a]Assuming 40% susceptibility.
[b]Estimates with 95% confidence intervals are presented.

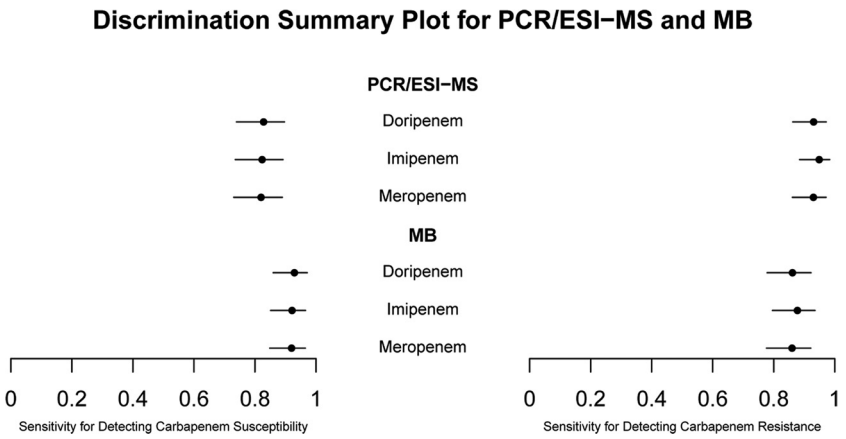

FIG 2 Estimates of the susceptibility and resistance sensitivities displayed using discrimination summary plots. Results are presented with 95% confidence intervals for PCR/ESI-MS and MB.

clinical decision-making can have profound positive consequences on patient care. Simply put, these methods can be a helpful tool for clinicians with regard to treatment decision-making.

However, RMDs are not perfect. In a setting where the level of CR *Acinetobacter* spp. is low, there is an increased likelihood for the RMD to inaccurately identify resistance (Fig. 3). This could lead to overprescribing with an associated increase in cost. However, the monetary cost in that circumstance is not great, and clinicians feel it is better to overtreat than undertreat where there is uncertainty that can lead to incorrect treatment decisions. The setting in which rapid identification of resistance determinants would have the greatest impact is in an area of high prevalence of CR *Acinetobacter* spp. with access to a laboratory that operates continuously.

The complexity of the CR phenotype (e.g., CarO mutations, efflux pumps, uncharacterized OXAs) may help explain misclassified isolates. We found using this heterogeneous collection that the presence of porins, efflux pumps, or different *bla* genes not included in our selection can confound the interpretation of results. Fortunately, this imprecision is still relatively minor.

We also showed that after CR strains are identified using RMDs, tigecycline and polymyxins (colistin) can be used for effective empiric therapy in ~90% of cases. Minocycline is the only orally available agent to be considered, but resistance rates were surprisingly high (62%) in this collection. The extremely high rates of amikacin, ampicillin-sulbactam, and gentamicin resistance also merit further molecular analysis. These data highlight the major clinical challenge posed by CR *Acinetobacter* spp., as isolates are frequently resistant to all agents used to treat CS *Acinetobacter* spp.

In conclusion, we show that the groundwork established in PRIMERS I and II can be extended to another Gram-negative MDR pathogen, *Acinetobacter* spp. Compared with conventional susceptibility testing done in clinical microbiology laboratories, RMD platforms that can identify bla_{OXA} carbapenemase genes can have a significant impact on the empiric decision to use specific agents. Our analysis also demonstrates that if CR is found, clinicians can use colistin, polymyxin B, or tigecycline in 85 to 90% of cases and choose a correct empiric treatment provided the prevalence of resistance to those agents is low. However, we cannot say this will result in better outcomes. We also cannot address the complexity of single versus combination chemotherapy. Considerations such as these require more detailed analytical studies on a larger number of isolates with diverse phenotypes.

Unfortunately, as Tables 4 and 6 show, these analyses and RMDs have limitations. First, these analyses assume knowledge of the *Acinetobacter* spp. Many laboratories still misidentify the species of *Acinetobacter*. Second, the consequences of false-positive/-negative results loom large, as we have discussed above and in PRIMERS I and II (1). In

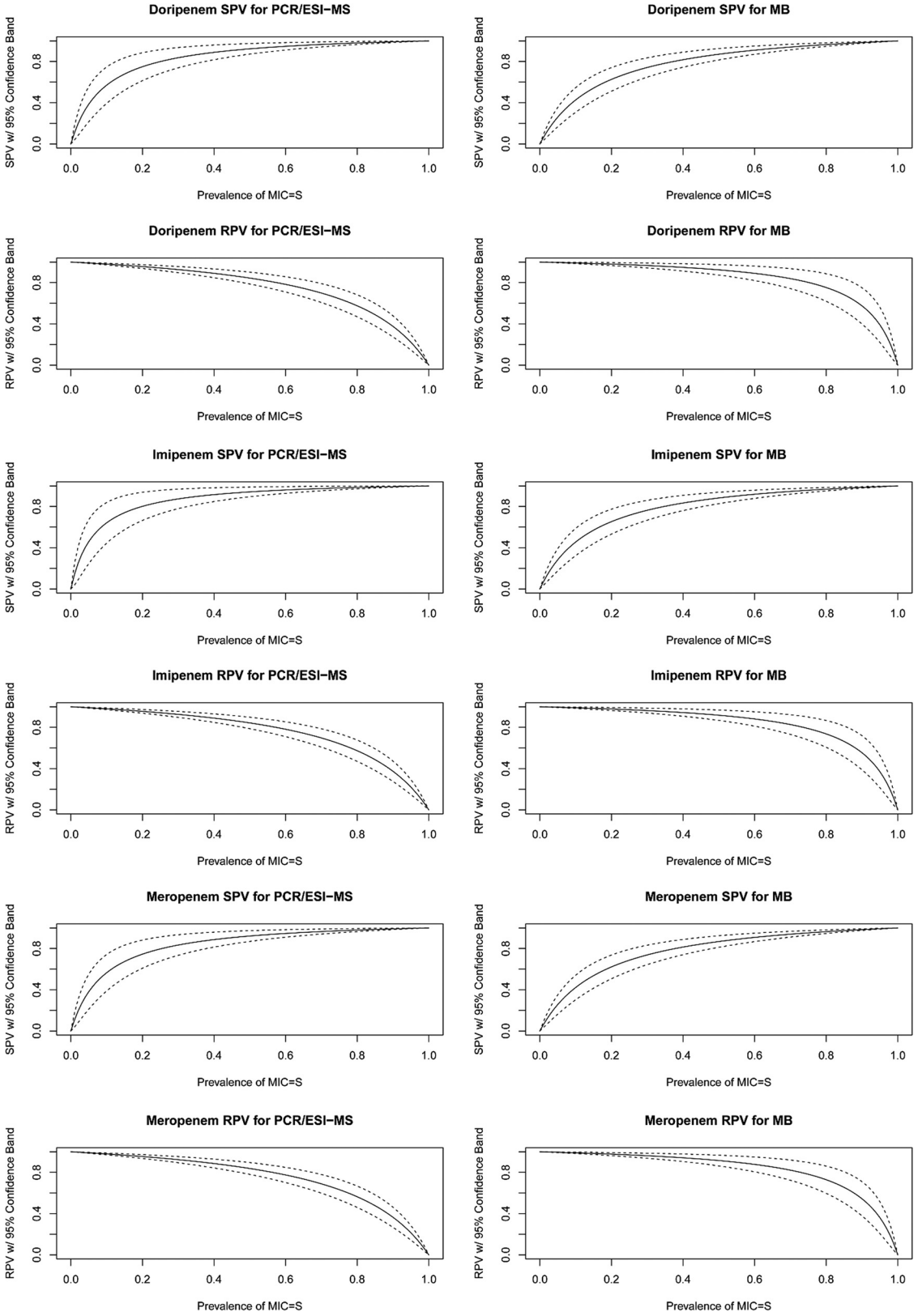

FIG 3 Susceptibility predictive value (SPV) and resistance predictive value (RPV) plots with 95% confidence bands by drug and platform.

TABLE 6 Discrepancy resolution

Carbapenemase gene	No. detected by:		No. of positive results after discrepancy resolution via carbapenemase multiplex PCR[a]
	MB	PCR/ESI-MS	
$bla_{OXA-24/40}$	11 (1 false positive, 4 false negatives)	29 (15 false positives)	14
bla_{OXA-23}	84 (8 false positives, 3 false negatives)	84 (7 false positives, 2 false negatives)	79
bla_{OXA-58}	1 (1 false positive, 2 false negatives)	3 (1 false positive)	2
bla_{IMP}	0	0	0
bla_{KPC}	0	0	0
bla_{NDM}	0	0	0
bla_{VIM}	0	0	0

[a]Results are from the OXA carbapenemase multiplex PCR on crude lysates to resolve observed discrepancies due to differences between platform results on a genotypic level, differences between genotype and predicted CR phenotype, and examining those isolates in which a non-*baumannii* species identification was obtained on the PCR/ESI-MS platform. An independent third method, the carbapenemase multiplex PCR, was used to evaluate false-positive or false-negative genotypes and inconsistencies in CR.

this testing exercise, PCR/ESI-MS showed a high false-positive rate, particularly for $bla_{OXA-24/40}$. Therapy that is targeted and effectively addresses the resistance pattern in certain cases is preferable to incorrect therapy, which can result in treatment failure and mortality. Further refinement is needed to help place this approach in the appropriate clinical context. Nevertheless, our results are encouraging and point to the successful introduction of RMDs in clinical practice for the correct diagnosis, effective treatment, and antibiotic stewardship of infections caused by MDR Gram-negative bacteria.

MATERIALS AND METHODS

Antimicrobial susceptibility testing (AST) and isolate selection. The MICs for each strain were used as a gold standard to define susceptibility or resistance for each beta-lactam antibiotic and other antimicrobial drugs. We assembled a panel of 102 CS and 98 CR *Acinetobacter* spp. from locations worldwide (9–11). We assessed susceptibility to the following antibiotics by broth microdilution: amikacin, gentamicin, tobramycin, ticarcillin-clavulanate, piperacillin-tazobactam, aztreonam, cefepime, ceftazidime, ciprofloxacin, levofloxacin, imipenem, meropenem, doripenem, tetracycline, minocycline, tigecycline, colistin, polymyxin B, and trimethoprim-sulfamethoxazole using Sensititre GNX2F trays (Thermo Fisher Scientific, Oakwood Village, OH) and ampicillin-sulbactam using MicroScan Neg BP combo panel type 34 trays (Beckman Coulter Inc., Brea, CA). Results were interpreted according to the 2014 Clinical and Laboratory Standards Institute (CLSI) guidelines (12). American Type Culture Collection (ATCC) control strains *Pseudomonas aeruginosa* 27853 and *Escherichia coli* 25922 were used as quality controls. Breakpoints for *Enterobacteriaceae* or *P. aeruginosa* were used when they were not available for *Acinetobacter* spp. Intermediate interpretations were considered resistant for analytical purposes.

Analysis of *bla* genes using RMD platforms. As the number of carbapenemase genes is quite large in *Acinetobacter* spp., we focused upon the *bla* genes that are most relevant and prevalent in survey studies worldwide: bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-58}, bla_{NDM}, bla_{KPC}, bla_{VIM}, and bla_{IMP}.

PCR/ESI-MS and MB were used to evaluate isolates of *Acinetobacter* spp. for the presence or absence of the genetic targets (carbapenemase genes) that are known to be associated with CR, as previously described (13, 14). In brief, PCR/ESI-MS is a nucleic acid amplification technology that targets select genes using "smart primers," determines their exact mass, and then uses algorithms to define the target gene identified (1). The PCR/ESI-MS platform also provides genus- and species-level identification. MBs are fluorescently labeled oligonucleotide hybridization probes that can report the presence of specific nucleic acid targets in heterogeneous solutions, as previously described (1). The platform results were compared with AST results for each carbapenem. The RMD result was considered resistant when any of the targeted genes were detected; the RMD result was considered susceptible when none of the targets were detected.

OXA carbapenemase multiplex PCR for discrepancy resolution. We applied PCR methods to resolve various discrepancies that arose within the experiments. These discrepancies came about due to (i) differences between platform results, on a genotypic level, (ii) differences between genotype and predicted CR phenotype, and (iii) differences due to non-*baumannii Acinetobacter* species identification on the PCR/ESI-MS platform (see Table S1 in the supplemental material).

To resolve discrepancies resulting from i and ii above, an OXA carbapenemase multiplex PCR was performed on crude lysates to identify the OXA carbapenemases present (as a third method for discrepancy resolution). This multiplex PCR assay is able to detect multiple OXA carbapenemase genes on the basis of differential PCR product sizes: $bla_{OXA-143}$ (150 bp), $bla_{OXA-24/40}$ (264 bp), bla_{OXA-51} (353 bp),

bla_{OXA-23} (500 bp), bla_{OXA-58} (600 bp), and $bla_{OXA-235}$ (700 bp) (15–17). To resolve discrepancies resulting from iii above, select primers were used for more accurate species identification (18–21).

Statistical methods. Discrimination summary plots were used to display the 95% CI estimates of susceptibility sensitivity, defined as the probability that the platform result is susceptible when the MIC result is susceptible, and of resistance sensitivity, defined as the probability that the platform result is resistant when the MIC result is resistant (1). The primary analysis of the data presented in the manuscript was done prior to discrepancy resolution.

The susceptibility predictive value (SPV) is the probability that a MIC result would indicate susceptibility when the platform result indicates susceptibility, and the resistance predictive value (RPV) is the probability that a MIC result would indicate resistance when the platform result indicates resistance. The SPV and RPV are also functions of the prevalence of susceptibility. Since there are temporal and geographic variations in the prevalence of susceptibility of *Acinetobacter* spp. to carbapenems, the SPV and RPV were plotted as a function of the prevalence of susceptibility (with 95% confidence bands) to allow for interpretation across the spectrum of prevalence.

The sample size of 200 isolates was chosen based on estimating susceptibility and resistance sensitivities with desirable precision. Roughly half of the isolates were expected to be susceptible and half resistant and thus available for estimating susceptibility/resistance sensitivities. For example, a sample size of 90 isolates produces a two-sided 95% CI with a width of 0.13 when the observed susceptibility/resistance sensitivity is 90%.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01524-16.

Table S1, XLSX file, 0.01 MB.

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