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ABSTRACT

Objectives: Many multidrug-resistant Gram-negative bacilli (MDR-GNB) harbour multiple β-lactamases. The aim of this study was to assess the impact of multiple β-lactamase carriage on the accuracy of susceptibility tests and extended-spectrum β-lactamase (ESBL) and carbapenemase confirmation methods.

Methods: A total of 50 MDR-GNB, of which 29 carried multiple β-lactamases, underwent broth microdilution (BMD) and disk diffusion (DD) testing as well as confirmation tests for ESBLs and carbapenemases. Whole-genome sequencing (WGS) was used for β-lactamase gene identification.

Results: Categorical agreement of BMD and DD testing results ranged from 86.5 to 97.7% for 10 β-lactam agents. BMD and DD algorithms for ESBL detection were highly variable; 6 of 8 positive strains carried an ESBL plus a carbapenemase or an AmpC enzyme, which may confound antimicrobial selection. The sensitivity and specificity of the modified carbapenem inactivation method (mCIM) were both 100%, whilst mCIM and EDTA-modified carbapenem inactivation method (eCIM) when used together to differentiate serine from metallo-β-lactamase carriage were both 96%. Xpert® Carba-R results (in vitro diagnostic test) were consistent with WGS results. Predicting phenotypic carbapenem resistance from WGS data overall showed 100% specificity but only 66.7% sensitivity for Enterobacterales isolates that were non-susceptible to imipenem and meropenem.

Conclusions: Multiple β-lactamases in MDR-GNB does not impact DD results, the utility of mCIM/eCIM tests, or Xpert Carba-R results. However, ESBL algorithms produced inconsistent results and predicting carbapenem resistance from WGS data was problematic in such strains.

1. Introduction

Multidrug-resistant Gram-negative bacilli (MDR-GNB), especially those that are carbapenem-resistant, have become a major medical and public-health threat globally [1,2]. Carbapenem resistance can be mediated by the production of carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC) or New Delhi metallo-β-lactamase (NDM), or through the production of extended-spectrum β-lactamases (ESBLs) or AmpC β-lactamases in bacterial strains with porin changes that reduce the amount of antimicrobial agent that enters the cell [3,4]. ESBLs and AmpC β-lactamases continue to be recognised globally among MDR-GNB and typically outnumber carbapenem-resistant strains in most surveys [5–7]. In one hospital study, non-carbapenemase-producing carbapenem-resistant isolates accounted for 50.0–68.4% of carbapenem-resistant bacteria annually [4]. Carbapenem resistance may also be a result of chromosomal enzymes in conjunction with efflux mechanisms in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [8,9].

Differentiating carbapenemase-producing organisms from organisms with ESBLs or AmpC enzymes that have undergone porin changes has important therapeutic implications [10]. Thus, it is recommended that laboratories have the capability to differentiate carbapenemase-producing organisms from other carbapenem-resistant organisms [11]. This can be accomplished using either phenotypic methods, such as the combination of modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM) testing [12], CARBA-5

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(NG Biotech, Guipry, France) [13] or double disk synergy methods [14], or genotypic methods such as Xpert® Carba-R (Cepheid, Sunnyvale, CA, USA; an in vitro diagnostic test) [15], Amplidiag CarbaR+MCR (Mobidiag, Paris, France) [16] or Check-MDR CT103XL (Check-Points BV, Wageningen, Netherlands) [17], or combinations of both phenotypic and genotypic methods. Many laboratories continue to perform confirmation tests for ESBLs, although it is not recommended for routine reporting by either the Clinical and Laboratory Standards Institute (CLSI) [18] or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2017 guidance on resistance mechanisms [19]. None the less, the knowledge bases (sometimes called 'expert systems') available on many automated susceptibility testing systems still report ESBLs [20].

One caveat of using confirmatory ESBL or carbapenemase detection methods is that contemporary MDR-GNB often contain multiple β-lactamases, potentially including combinations of ESBLs, AmpC β-lactamases and carbapenemases [21,22]. However, many of the studies to develop and validate the confirmation tests used micro-organisms containing only a single β-lactamase or multiple β-lactamases of the same type [13,23,24]. Thus, the accuracy of the confirmation methods may be questioned. Many isolates in the US Centers for Disease Control and Prevention (CDC) resistance challenge set, which are used for validating ESBL and carbapenemase detection methods, also carry more than one β-lactamase gene, which could confound analysis [25]. Thus, the aim of this study was to test a collection of MDR-GNB with multiple β-lactamase genes, identified by whole-genome sequencing (WGS), using the broth microdilution (BMD) and disk diffusion (DD) susceptibility testing methods as well as several ESBL and carbapenemase confirmatory assays to determine the impact of multiple β-lactamases on test results.

2. Materials and methods

2.1. Strains

A total of 46 Gram-negative bacilli from Cepheid's global strain collection and 4 isolates from the CDC & FDA Antibiotic Resistance Isolate Bank, including *Citrobacter freundii* #0021, *P. aeruginosa* #0103, *Serratia marcescens* #0123 and *K. pneumoniae* #0347 [25], were selected for WGS based on antibiograms and species diversity. Sequencing data for the carbapenemases, ESBLs and AmpC β-lactamases were used as the basis for the method comparisons in this study.

2.2. Species identification

Micro-organisms were identified using a MicroScan® WalkAway instrument (Beckman Coulter, Brea, CA, USA) with the bacterial identification panel Neg ID type 2 as described by the manufacturer. Unusual species identifications or those with low identification probabilities were confirmed by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) (Bruker Daltonik GmbH, Bremen, Germany) as described by the manufacturer.

2.3. Antimicrobial susceptibility testing methods

BMD was performed using a MicroScan® WalkAway instrument with Gram-negative NM43 and Detect Neg MIC 2 panels as described by the manufacturer. Micro-organisms also were tested for susceptibility to 10 antimicrobial agents by the DD assay on Mueller–Hinton agar (Hardy Diagnostics, Santa Maria, CA, USA) according to the CLSI guidelines [26]. Interpretive criteria for BMD and DD results and for detecting ESBLs were those described by the CLSI [18]. For ESBL detection, an increase in the diameter of the zone of inhibition of ≥5 mm for cefotaxime or ceftazidime in the presence of clavulanic acid compared with the zone diameter for the disk with only cefotaxime or ceftazidime is indicative of ESBL carriage. Similarly, a ≥3 doubling dilution decrease in the minimum inhibitory concentration (MIC) of cefotaxime or ceftazidime in the presence of clavulanic acid compared with the MIC of the drug alone indicated ESBL carriage. Quality control organisms included *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and ATCC 35218, and *K. pneumoniae* ATCC 700603.

2.4. Phenotypic carbapenemase detection methods

The mCIM was performed and interpreted according to CLSI guidelines [18], whilst the eCIM to distinguish between serine and metallo-carbapenemases was performed as described by Sfeir et al. [12].

2.5. Whole-genome sequencing

Pure cultures of each micro-organism were grown overnight in trypticase broth (Hardy Diagnostics) and DNA was extracted using a DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA) as described by the manufacturer. DNA concentrations were determined by ultraviolet light absorbance using a NanoPhotometer system (Implen, Munich, Germany). Sequencing libraries were prepared from extracted genomic DNA using either Kapa HyperPrep (Kapa Biosystems/Roche, Basel, Switzerland) or Nextera XT (Illumina Inc., San Diego, CA, USA) kits. Resultant libraries were sequenced on a MiSeq sequencer (Illumina Inc.) using V3 reagent chemistry with 301-cycle paired-end reads. All procedures were performed in accordance with the manufacturer's protocols. Subsequently, libraries were quantified using quantitative PCR (qPCR) (Kapa Biosystems/Roche) or Droplet Digital™ PCR (ddPCR) (Bio-Rad). Assemblies were generated from fastq sequence files using A5-miseq software with default settings. The software carries out adapter trimming, quality control, assembly, error correction and scaffolding. Sequence data were analysed using the ResFinder 3.2 online tool from the Center for Genomic Epidemiology (CGE) [27].

2.6. Predicting carbapenem phenotypes from whole-genome sequencing data

Organisms were classified phenotypically as susceptible or non-susceptible to carbapenems based on BMD test results for ertapenem, imipenem and meropenem, or just imipenem and meropenem. The non-susceptible MIC category included organisms that were either intermediate or resistant to each of the antimicrobial agents. The results for Enterobacterales were analysed separately from the results for the *Pseudomonas*, *Acinetobacter* and *Shewanella* isolates.

2.7. Xpert® Carba-R testing

Organisms were tested in pure culture for the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48} and bla_{IMP} carbapenem resistance genes using the Xpert® Carba-R cartridge (Cepheid, Sunnyvale, CA, USA) as described by the manufacturer. To ensure resistance gene retention prior to testing, as recommended in the product labelling, carbapenem-resistant organisms were grown on sheep blood agar plates (Hardy Diagnostics) with a 10 μg meropenem disk placed in the centre of the inoculum. Three to five colonies taken from the inner edge of the zone of inhibition were suspended in Mueller–Hinton broth to a density of 0.5 McFarland standard and 10 μL of the suspension was added to 5 mL of sample reagent prior to testing with the Xpert Carba-R cartridge [15].

2.8. Statistical analysis

Confidence intervals (CIs) were calculated using binomial distribution (Minitab 18 Statistical Software). The WGS carbapenem resistance predictions were compared with the phenotypic resistance obtained by the BMD method using the McNemar's test [28].

3. Results

3.1. Characteristics of bacterial isolates

The carbapenem resistance phenotypes determined by BMD and the β-lactamase gene carriage of the 50 isolates used in this study are shown in Table 1; 56% contained two or more β-lactamase genes. Among the isolates, there were 37 Enterobacterales, 11 *Pseudomonas* spp., 1 *Acinetobacter nosocomialis* and 1 *Shewanella xiamenensis*. Thirty isolates (18 Enterobacterales, 10 *Pseudomonas* spp. and both the *A. nosocomialis* and *S. xiamenensis*) contained at least one carbapenemase gene by WGS, including bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, $bla_{OXA-181}$, bla_{SME} or bla_{NMC}. Nineteen of the Enterobacterales isolates contained either ESBLs or AmpC enzymes or both, but no carbapenemase genes by WGS. Although the OXA-50 β-lactamase is reported to have weak carbapenem hydrolytic activity [29], for phenotype–WGS comparisons in this study bla_{OXA-50} was not included as a carbapenemase gene since none of the *P. aeruginosa* isolates that contained only

Table 1
Characteristics of study micro-organisms, including broth microdilution susceptibility results for carbapenems, mCIM/eCIM results, and β-lactamase gene carriage.

#	Species	MIC (μg/mL) [interpretative category]			mCIM/eCIM result[a]	β-Lactamase genes		
		MEM	ETP	IPM		Carbapenemase	ESBL	AmpC
1	*Acinetobacter nosocomialis*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{NDM-1}, bla_{OXA-94}		
2	*Citrobacter freundii* complex	≤1 [S]	≤0.5 [S]	≤1 [S]	Neg.			bla_{CMY-80}
3	*C. freundii* complex	≤1 [S]	2 [R]	≤1 [S]	Neg.			bla_{CFE-1}
4	*C. freundii* complex	≤1 [S]	1 [I]	≤1 [S]	Neg.			bla_{CMY-2}
5	*C. freundii* complex	≤1 [S]	1 [I]	≤1 [S]	Neg.			bla_{CMY-48}
6	*C. freundii* complex	≤1 [S]	1 [I]	≤1 [S]	Neg.			bla_{CMY-2}
7	*Enterobacter asburiae*	4 [R]	>4 [R]	4 [R]	Neg.			bla_{ACT-4}
8	*Enterobacter cloacae*	>8 [R]	>4 [R]	>8 [R]	Neg.			bla_{CMH-3}
9	*E. cloacae*	8 [R]	>4 [R]	8 [R]	Neg.		bla_{SHV-12}	bla_{ACT-1}
10	*E. cloacae*	8 [R]	>4 [R]	8 [R]	Neg.			bla_{ACT-7}
11	*E. cloacae*	>8 [R]	>4 [R]	>8 [R]	Neg.			bla_{ACT-15}
12	*E. cloacae*	>8 [R]	>4 [R]	>8 [R]	Serine	bla_{NMC-A}		bla_{ACT-12}
13	*Escherichia coli*	>8 [R]	>4 [R]	>8 [R]	Metallo	bla_{NDM-5}	$bla_{CTX-M-15}$	bla_{CMY-6}, bla_{DHA-1}
14	*E. coli*	≤1 [S]	≤0.5 [S]	2 [I]	Metallo	bla_{VIM-1}	$bla_{CTX-M-14}$	bla_{ACC-1}
15	*E. coli*	>8 [R]	>4 [R]	>8 [R]	Metallo	bla_{NDM-7}	$bla_{CTX-M-15}$	bla_{CMY-4}
16	*E. coli*	≤1 [S]	2 [R]	2 [I]	Neg.			bla_{CMY-2}
17	*E. coli*	≤1 [S]	≤0.5 [S]	≤1 [S]	Neg.		$bla_{CTX-M-15}$	
18	*E. coli*	≤1 [S]	≤0.5 [S]	≤1 [S]	Neg.		$bla_{CTX-M-15}$	bla_{CMY-2}
19	*E. coli*	8 [R]	>4 [R]	4 [R]	Serine	bla_{KPC-2}	bla_{SHV-5}	
20	*E. coli*	8 [R]	>4 [R]	8 [R]	Serine	bla_{KPC-2}, bla_{OXA-48}		
21	*Hafnia alvei*	≤1 [S]	1 [I]	≤1 [S]	Neg.			bla_{ACC-2}
22	*Klebsiella aerogenes*	4 [R]	>4 [R]	8 [R]	Neg.			AmpC[b]
23	*K. aerogenes*	4 [R]	>4 [R]	8 [R]	Neg.			AmpC[b]
24	*K. aerogenes*	>8 [R]	>4 [R]	>8 [R]	Neg.			AmpC[b]
25	*Klebsiella pneumoniae*	>8 [R]	>4 [R]	>8 [R]	Metallo	bla_{NDM-1}	$bla_{CTX-M-15}$	
26	*K. pneumoniae*	>8 [R]	>4 [R]	8 [R]	Metallo	bla_{NDM-1}	bla_{SHV-11}	bla_{DHA-1}
27	*K. pneumoniae*	>8 [R]	>4 [R]	>8 [R]	Metallo	bla_{NDM-1}	$bla_{CTX-M-15}$	bla_{DHA-1}
28	*K. pneumoniae*	≤1 [S]	≤0.5 [S]	≤1 [S]	Neg.			
29	*K. pneumoniae*	≤1 [S]	>4 [R]	≤1 [S]	Neg.		$bla_{CTX-M-15}$, bla_{SHV-28}	
30	*K. pneumoniae*	>8 [R]	>4 [R]	>8 [R]	Serine	bla_{KPC-2}	$bla_{CTX-M-65}$, bla_{SHV-11}	
31	*K. pneumoniae*	>8 [R]	>4 [R]	8 [R]	Serine	bla_{KPC-3}	$bla_{CTX-M-15}$, bla_{SHV-11}	
32	*K. pneumoniae*	>8 [R]	>4 [R]	>8 [R]	Serine	bla_{KPC-3}	bla_{SHV-11}	
33	*K. pneumoniae*	>8 [R]	>4 [R]	>8 [R]	Serine	bla_{OXA-48}	$bla_{CTX-M-15}$, bla_{SHV-11}	
34	*K. pneumoniae*	2 [I]	>4 [R]	8 [R]	Serine	bla_{OXA-48}	$bla_{CTX-M-15}$, bla_{SHV-11}	
35	*K. pneumoniae*	>8 [R]	>4 [R]	>8 [R]	Serine	bla_{KPC-3}		
36	*Proteus mirabilis*	≤1 [S]	≤0.5 [S]	8 [R]	Metallo	bla_{OXA-48}	$bla_{CTX-M-14}$	
37	*Pseudomonas aeruginosa*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{VIM-2}, (bla_{OXA-50})[c]		
38	*P. aeruginosa*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{IMP-1}, (bla_{OXA-50})		
39	*P. aeruginosa*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{VIM-2}, (bla_{OXA-50})		
40	*P. aeruginosa*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{IMP-1}, (bla_{OXA-50})		
41	*P. aeruginosa*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{NDM-1}, (bla_{OXA-50})		
42	*P. aeruginosa*	8 [R]	>4 [N/A]	4 [I]	Metallo	bla_{VIM-5}, (bla_{OXA-50})	bla_{GES-9}, bla_{OXA-21}	
43	*P. aeruginosa*	≤1 [S]	>4 [N/A]	≤1 [S]	Neg.	(bla_{OXA-50})		
44	*P. aeruginosa*	2 [S]	>4 [N/A]	≤1 [S]	Neg.	(bla_{OXA-50})		
45	*P. aeruginosa*	≤1 [S]	>4 [N/A]	≤1 [S]	Neg.	(bla_{OXA-50})		
46	*Pseudomonas putida*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{IMP-1}		
47	*P. putida*	>8 [R]	>4 [N/A]	>8 [R]	Metallo	bla_{VIM-2}		
48	*Serratia marcescens*	≤1 [S]	2 [R]	4 [R]	Serine	bla_{KPC-4}	bla_{SHV-30}	
49	*S. marcescens*	>8 [R]	>4 [R]	>8 [R]	Serine	bla_{SME-3}		
50	*Shewanella xiamenensis*	8 [I]	>4 [N/A]	8 [I]	Serine	$bla_{OXA-181}$		

mCIM, modified carbapenem inactivation method; eCIM; EDTA-modified carbapenem inactivation method; MIC, minimum inhibitory concentration; MEM, meropenem; ETP, ertapenem; IPM, imipenem; ESBL, extended-spectrum β-lactamase; R, resistant; I, intermediate; S, susceptible; N/A, not applicable.
Note: Narrow-spectrum β-lactamases, e.g. bla_{TEM-1}, are not listed.
[a] Metallo, metallo-β-lactamase; Neg., negative; Serine, serine-based carbapenemase.
[b] AmpC gene was identified by BLAST data.
[c] bla_{OXA-50} is not considered a carbapenemase in this study, so it is listed in parentheses.

this resistance gene were resistant to meropenem, imipenem or the extended-spectrum cephalosporin ceftazidime, and both the mCIM and eCIM tests for carbapenemases were negative.

3.2. Comparison of minimum inhibitory concentration and disk diffusion test results

Categorical agreement between the results of BMD and DD testing for 10 β-lactam agents for the 50 test isolates is shown in Table 2. For carbapenems, categorical agreement was 87.8% for ertapenem, 91.5% for imipenem and 93.6% for meropenem with no very major errors observed. For cephalosporins and monobactams, agreement ranged from a low of 86.5% for cefoxitin to a high of 97.7% for cefotaxime. Many of the categorical errors came from just two isolates. One *Hafnia alvei* isolate produced a very major error by DD when compared with BMD results for cefotaxime, ceftazidime and ceftriaxone (i.e. BMD results were all reported as resistant but DD results were susceptible). This isolate contained only a $bla_{\text{ACC-2}}$ gene. The other isolate was a *Proteus mirabilis* that was susceptible to both ertapenem and meropenem by BMD testing but resistant by DD testing (major errors). This isolate was examined carefully to prevent misinterpreting the zones of inhibition due to swarming. The isolate also showed minor errors with aztreonam and ceftazidime. It harboured both a $bla_{\text{OXA-48}}$ and a $bla_{\text{CTX-M-14}}$ β-lactamase. There was 97.7% agreement between the categorical results of BMD and DD testing with ceftazidime/avibactam, with one major error. A *P. aeruginosa* containing $bla_{\text{GES-9}}$, $bla_{\text{OXA-21}}$, $bla_{\text{OXA-50}}$, bla_{PAO} and $bla_{\text{VIM-5}}$ was borderline susceptible with a ceftazidime/avibactam MIC of 8 μg/mL but was resistant by DD with a zone diameter of 20 mm. The data were reproducible on re-testing.

3.3. Reliability of extended-spectrum β-lactamase screening tests

DD and BMD tests using ceftazidime and cefotaxime ± clavulanic acid were used to examine isolates for ESBL production. Ceftazidime plus clavulanic acid testing by DD identified 11 organisms as ESBL-producers, even though 5 contained a carbapenemase ($bla_{\text{OXA-48}}$ or bla_{KPC}) in addition to an ESBL ($bla_{\text{CTX-M-15}}$ or $bla_{\text{SHV-30}}$), 2 had an ESBL (both had $bla_{\text{CTX-M-15}}$) plus an AmpC β-lactamase (either $bla_{\text{CMY-24}}$ or $bla_{\text{ACT-1}}$), 2 had only an AmpC β-lactamase ($bla_{\text{CMY-24}}$ or $bla_{\text{CMY-48}}$), and 2 had an ESBL (both had $bla_{\text{CTX-M-15}}$) plus a narrow-spectrum β-lactamase ($bla_{\text{TEM-1}}$). All were Enterobacterales. Only four of the organisms identified by DD testing as ESBL-producers were also identified by BMD-based ESBL testing. In addition, a *P. mirabilis* that was not called an ESBL-producer by either the ceftazidime or cefotaxime DD tests was positive by BMD-based ESBL testing. Of the five organisms positive for ESBLs by BMD testing, all harboured one or more ESBL genes; three also carried carbapenem resistance genes ($bla_{\text{OXA-48}}$), 1 had multiple ESBLs ($bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-28}}$) and the last had $bla_{\text{CTX-M-15}}$ and a narrow-spectrum $bla_{\text{TEM-1}}$ (data not shown).

3.4. Predicting metallo-β-lactamase (MBL) production using aztreonam

Susceptibility to aztreonam has been used an indicator of MBL carriage in carbapenem-resistant strains [30]. Although 15 isolates contained a MBL, only 1 did not have other β-lactamases, and that isolate was intermediate to aztreonam by BMD testing. Of the other 14 isolates that had additional β-lactamases present, 5 (35.7%; 95% CI 12.8–64.8%), including 3 *P. aeruginosa*, 1 *E. coli* and 1 *K. pneumoniae*, were susceptible to aztreonam both by DD and BMD testing.

3.5. Comparison of carbapenem-resistant phenotype and β-lactamase genotype

Of the 33 isolates that were meropenem-resistant by BMD, 25 (75.8%; 95% CI 57.7–88.9%) harboured one or more carbapenemase genes, including 6 with bla_{NDM}, 5 with bla_{KPC}, 4 with bla_{VIM}, 3 with $bla_{\text{OXA-48}}$ or $bla_{\text{OXA-181}}$ genes, 3 with bla_{IMP}, 1 with bla_{KPC} and $bla_{\text{OXA-48}}$, 1 with $bla_{\text{NMC-A}}$, 1 with bla_{SME}, and 1 with bla_{NDM} and $bla_{\text{OXA-94}}$ (Table 1). Four isolates harboured only an AmpC β-lactamase (3 with bla_{ACT} and 1 with $bla_{\text{CMH-3}}$), whilst 1 harboured an AmpC and an ESBL ($bla_{\text{ACT-1}}$ and $bla_{\text{SHV-12}}$). Three meropenem-resistant *Klebsiella aerogenes* isolates harboured only intrinsic AmpC-type β-lactamase genes by WGS. Thus, approximately one-quarter of meropenem-resistant isolates did not harbour a carbapenemase gene. Three isolates that were meropenem-susceptible but intermediate or resistant to imipenem each harboured a different carbapenemase gene, i.e. bla_{VIM}, bla_{KPC} or $bla_{\text{OXA-48}}$. So, not every carbapenemase gene consistently mediated meropenem resistance. There were six organisms (excluding *P. aeruginosa* isolates) that were susceptible to meropenem and imipenem but intermediate or resistant only to ertapenem. These included four *C. freundii* that harboured an AmpC β-lactamase, a *H. alvei* with an AmpC β-lactamase, and a *K. pneumoniae* with multiple ESBLs. This is consistent with ertapenem being the least specific indicator of carbapenemase activity. Three *P. aeruginosa* isolates that contained $bla_{\text{OXA-50}}$ but no other β-lactamases by WGS were susceptible to all three carbapenems, cefepime and ceftazidime both by BMD and DD testing.

3.6. Modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM) testing

A total of 28 organisms were positive by mCIM alone and all contained a carbapenemase, whilst none of the 22 organisms that were mCIM-negative contained a carbapenemase. Among the 37

Table 2
Categorical agreement (CA) between broth microdilution (BMD) and disk diffusion (DD) results for β-lactam drugs.

Antimicrobial agent	No. of comparisons[a]	CA	Very major errors	Major errors	Minor errors
Aztreonam	46	41 (89.1%)	0 (0.0%)	0 (0.0%)	5 (10.9%)
Cefepime	47	41 (87.2%)	0 (0.0%)	0 (0.0%)	6 (12.8%)
Cefotaxime	44	43 (97.7%)	1 (2.3%)	0 (0.0%)	0 (0.0%)
Cefoxitin	37	32 (86.5%)	1 (3.2%)	0 (0.0%)	4 (10.3%)
Ceftazidime	48	44 (91.7%)	1 (2.5%)	0 (0.0%)	3 (6.3%)
Ceftriaxone	39	38 (97.4%)	1 (2.8%)	0 (0.0%)	0 (0.0%)
Ertapenem	41	36 (87.8%)	0 (0.0%)	1 (2.4%)	4 (9.8%)
Imipenem	47	43 (91.5%)	0 (0.0%)	0 (0.0%)	4 (8.5%)
Meropenem	47	44 (93.6%)	0 (0.0%)	1 (2.1%)	2 (4.3%)
Ceftazidime/avibactam	43	42 (97.7%)	0 (0.0%)	1 (2.3%)	N/A

N/A, not applicable.
[a] Interpretive criteria are not available for some species (e.g. *Acinetobacter* spp., *Shewanella* spp. and *Pseudomonas putida*), which limited the number of comparisons of BMD and DD results.

Enterobacterales isolates, the combination of mCIM and eCIM testing produced one error, which was a *P. mirabilis* containing bla_{OXA-48}, $bla_{CTX-M-14}$ and bla_{TEM-1} (i.e. a serine carbapenemase-producer) that was interpreted as a MBL-producer (mCIM = 6 mm; eCIM = 20 mm). On the other hand, a MBL-producing *P. aeruginosa* harbouring a bla_{IMP-1} and bla_{OXA-50} gene was interpreted as containing a serine β-lactamase (mCIM = 9 mm; eCIM = 6 mm). Thus, the combination mCIM and eCIM tests were 96% sensitive and 96% specific. The three *P. aeruginosa* isolates that contained bla_{OXA50} alone were called negative for carbapenemase activity by mCIM and eCIM.

3.7. Correlation of Xpert Carba-R results with results of whole-genome sequencing

Of the 26 isolates that harboured a bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP} or bla_{OXA-48} carbapenemase gene by WGS, all were detected by the Xpert Carba-R assay, including the *E. coli* isolate that contained both bla_{KPC} and bla_{OXA-48}. There were no false-positive Xpert Carba-R results among the isolates that by WGS did not contain a carbapenemase gene or contained a carbapenemase gene that was not among those detected by Xpert Carba-R. Thus, the test showed 100% sensitivity and 100% specificity for the carbapenemase genes included in the assay.

3.8. Predicting carbapenem non-susceptibility based on whole-genome sequencing results

Eighteen Enterobacterales contained a carbapenem resistance gene and all were non-susceptible to ertapenem, imipenem and meropenem (Table 3). Thus, there was 100% specificity to WGS predictions of carbapenem non-susceptibility. However, there were 9 Enterobacterales that were non-susceptible to imipenem and meropenem and 15 organisms that were non-susceptible to ertapenem, imipenem and meropenem that did not contain carbapenemase genes. These organisms would have been predicted to be susceptible to either the two or three carbapenems, respectively, making the sensitivity of predicting carbapenem resistance 66.7% (95% CI 46.0–83.5%) for imipenem and meropenem ($P < 0.003$) and only 54.4% (95% CI 36.4–71.9%) for ertapenem, imipenem and meropenem ($P < 0.001$). For the 13 *Pseudomonas* spp., *A. nosocomialis* and *S. xiamenensis* isolates in the study, the sensitivity and specificity of predicting carbapenem non-susceptibility to imipenem and meropenem were both 100%; however, isolates with carbapenem resistance based on efflux or porin changes, as opposed to carbapenemases, were not included in the study.

4. Discussion

This study focused on how the presence of multiple β-lactamases in MDR-GNB isolates impacted the results of antimicrobial susceptibility testing methods and confirmatory assays for ESBLs and carbapenemases. The correlation between the categorical results of DD and BMD testing overall was high and showed few very major errors. This was reassuring since much of the surveillance for antimicrobial resistance among bacterial pathogens, especially in low- and middle-income countries, is conducted by DD testing [31]. Of 34 categorical errors observed, 9 (26.5%) were due to two organisms, a *H. alvei* and a *P. mirabilis*. If these two isolates are removed, the results look even more reassuring for this group of MDR organisms.

As noted in CLSI document M100, 'when using the current interpretive criteria, routine ESBL testing is no longer necessary before reporting results. However, it may be useful for infection control purposes' [18]. EUCAST also recommends ESBL testing for epidemiological purposes but not for routine clinical susceptibility categorisation [19]. This is also noted in the most recent EUCAST Expert Rules document [32]. Prior to 2010 when CLSI and EUCAST changed the DD and MIC breakpoints of cefotaxime, ceftriaxone and ceftazidime for Enterobacteriaceae to optimise detection of resistance, ESBL testing was widely used to predict extended-spectrum cephalosporin resistance [33]. Lowering the BMD breakpoints and widening the DD zone diameter breakpoints was meant to provide better correlation with both clinical and pharmacological data. The overall success of the these efforts has been met with mixed reviews [34,35]. Our data show worrying inconsistencies between the DD and BMD results for ESBL detection among strains and indicate that organisms reported to be ESBL-producers often contained either carbapenemases or AmpC β-lactamases that may confound therapeutic decisions if one assumes that only ESBLs are present in the bacterial isolate. The inconsistency of results suggests that reporting isolates as ESBL-producers to guide therapeutic decisions should be done with caution.

As concern for bacteria with MBLs spread globally, microbiologists often used susceptibility to aztreonam in a carbapenem-resistant isolate to indicate the presence of a MBL [3,30]. Unfortunately, in our study only 28.6% of isolates with MBLs remained susceptible to aztreonam, and even those isolates contained other β-lactamases. Fortunately, the mCIM test showed 100% accuracy for identifying organisms with carbapenemases compared with the results of WGS results and was not confounded by the presence of multiple β-lactamases in the isolates. More importantly, the combination of mCIM/eCIM results for differentiating serine carbapenemases from metallo-carbapenemases showed an overall accuracy of 96% and miscalled only two isolates, a *P. mirabilis* and a *P. aeruginosa*, both with carbapenemases but not the types predicted by the tests. The reasons for the failures are not clear. A recent study by Gill et al. indicated that the eCIM test does have challenges recognising some carbapenemases in *P. aeruginosa* [36]. None the less, our data suggest that the mCIM/eCIM tests can be used for confirmation of

Table 3
Accuracy of whole-genome sequencing (WGS) prediction of carbapenem susceptibility compared with broth microdilution (BMD) results for ertapenem (ETP), imipenem (IPM) and meropenem (MEM) for Enterobacterales and *Pseudomonas* spp., *Acinetobacter nosocomialis* and *Shewanella xiamenensis*.

		Enterobacterales BMD results				*Pseudomonas, Acinetobacter* and *Shewanella*, BMD results	
		IPM & MEM[a]		ETP & IPM & MEM[a]		IPM & MEM[a]	
WGS prediction		NS[b]	S	NS	S	NS	S
	NS	18	0	18	0	10	0
	S	9	10	15	4	0	3
Sensitivity (%)		66.7% (95% CI 46.0–83.5%) (McNemar's) P-value < 0.003		54.4% (95% CI 36.4–71.9%) (McNemar's) P-value < 0.001		100% (95% CI 69.2–100%) (McNemar's) P-value = 1.00	
Specificity (%)		100% (95% CI 69.2–100%)		100% (95% CI 39.8–100%)		100% (95% CI 29.2–100%)	

NS, non-susceptible; S, susceptible; CI, confidence interval.
[a] Categories of NS and S are for both antimicrobial agents or for all three antimicrobial agents.
[b] The non-susceptible category for antimicrobial agents includes both intermediate and resistant results by BMD testing.

carbapenemase-producing isolates, since differentiating metallo-carbapenemases from serine carbapenemases has important therapeutic implications [2,10]. The other option for differentiating the two types of carbapenemases would be to use a molecular test on the isolated colony, such as the Xpert Carba-R test [15], the Amplidiag test [16] or Check-MDR CT103XL [17]. In this study, the Xpert Carba-R test performed with 100% accuracy compared with the results of WGS.

Whilst the presence of a carbapenem resistance gene by WGS was highly predictive of phenotypic carbapenem resistance among these organisms (100% specificity), the opposite was not true. We noted a sensitivity of only 66.7% for predicting imipenem or meropenem non-susceptibility for Enterobacterales isolates by WGS owing to the variety of other β-lactamases that mediated carbapenem resistance. When non-susceptibility to ertapenem was included with imipenem and meropenem, the sensitivity dropped even more. This is not surprising as ertapenem confounded the original CDC definition of carbapenem resistance in Enterobacteriaceae [3]. Whether a software algorithm can be developed that detects porin mutations and active efflux pumps and combines these data with the presence of AmpC β-lactamases and ESBLs to predict carbapenem resistance is unknown. Clearly, multiple researchers are investigating the use of machine learning to infer resistance phenotypes from WGS data [37]. Unfortunately, a recent study by Davies et al. noted the complexity even of predicting amoxicillin/clavulanate resistance in *E. coli* when using WGS data [38]. Thus, the difficulty of accurately assessing carbapenem resistance, especially in organisms like *P. aeruginosa* or *A. baumannii* with efflux pumps and porin changes, is likely to be even greater. A recent EUCAST report cautioned against using WGS data at this time to predict phenotypic resistance [39].

This study had several limitations. The sample size of 50 microorganisms is small but the large proportion of isolates with two or more β-lactamases helped ensure that the impact of multiple β-lactamase carriage could be assessed. In addition, BMD testing was carried out using the MicroScan WalkAway platform and not the CLSI or EUCAST BMD methods, but this was done to be more in line with results likely to be observed in clinical laboratories where automated susceptibility methods are typically used to generate BMD data.

In summary, the presence of multiple β-lactamase genes confounds the results of older reporting algorithms for ESBLs but does not significantly impact reporting of DD, mCIM/eCIM and Xpert Carba-R results. Predicting phenotypic carbapenem resistance from WGS data based only on the presence of known carbapenemase genes is currently prone to very major errors.

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Conflict of interest

All authors are employees of Cepheid, which manufactures the Xpert® Carba-R test.

Ethical approval

Not required.

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References

[1] World Health Organization (WHO). Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities n.d. https://www.who.int/infection-prevention/publications/guidelines-cre/en/.
[2] Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis 2017;215(Suppl 1):S28–36, doi:http://dx.doi.org/10.1093/infdis/jiw282.
[3] Lutgring JD, Limbago BM. The problem of carbapenemase-producing-carbapenem-resistant-Enterobacteriaceae detection. J Clin Microbiol 2016;54:529–34, doi:http://dx.doi.org/10.1128/JCM.02771-15.
[4] Senchyna F, Gaur RL, Sandlund J, Truong C, Tremintin G, Kültz D, et al. Diversity of resistance mechanisms in carbapenem-resistant Enterobacteriaceae at a health care system in Northern California, from 2013 to 2016. Diagn Microbiol Infect Dis 2019;93:250–7, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2018.10.004.
[5] Jean S-S, Hsueh P-R, SMART Asia-Pacific Group. Distribution of ESBLs, AmpC β-lactamases and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal and urinary tract infections in the Asia-Pacific region during 2008–14: results from the Study for monitoring Antimicrobial Resistance Trends (SMART). J Antimicrob Chemother 2017;72:166–71, doi:http://dx.doi.org/10.1093/jac/dkw398.
[6] Doi Y, Park YS, Rivera JI, Adams-Haduch JM, Hingwe A, Sordillo EM, et al. Community-associated extended-spectrum β-lactamase-producing *Escherichia coli* infection in the United States. Clin Infect Dis 2013;56:641–8, doi:http://dx.doi.org/10.1093/cid/cis942.
[7] Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infect Control Hosp Epidemiol 2016;37:1288–301, doi:http://dx.doi.org/10.1017/ice.2016.174.
[8] Hassuna NA, Darwish MK, Sayed M, Ibrahem RA. Molecular epidemiology and mechanisms of high-level resistance to meropenem and imipenem in *Pseudomonas aeruginosa*. Infect Drug Resist 2020;13:285–93, doi:http://dx.doi.org/10.2147/IDR.S233808.
[9] Abdi SN, Ghotaslou R, Ganbarov K, Mobed A, Tanomand A, Yousefi M, et al. *Acinetobacter baumannii* efflux pumps and antibiotic resistance. Infect Drug Resist 2020;13:423–34, doi:http://dx.doi.org/10.2147/IDR.S228089.
[10] Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, et al. Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae bacteremia. Clin Infect Dis 2017;64:257–64, doi:http://dx.doi.org/10.1093/cid/ciw741.
[11] US Centers for Disease Control and Prevention (CDC). Facility Guidance for Control of Carbapenem-Resistant Enterobacteriaceae (CRE). November 2015 update 2015. Atlanta, GA: CDC; 2015. https://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf.
[12] Sfeir MM, Hayden JA, Fauntleroy KA, Mazur C, Johnson JK, Simner PJ, et al. EDTA-modified carbapenem inactivation method: a phenotypic method for detecting metallo-β-lactamase-producing Enterobacteriaceae. J Clin Microbiol 2019;57:e01757–18, doi:http://dx.doi.org/10.1128/JCM.01757-18.
[13] Baeza LL, Pfennigwerth N, Greissl C, Göttig S, Saleh A, Stelzer Y, et al. Comparison of five methods for detection of carbapenemases in Enterobacterales with proposal of a new algorithm. Clin Microbiol Infect 2019;25:, doi:http://dx.doi.org/10.1016/j.cmi.2019.03.003 1286.e9–15.
[14] Picão RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, et al. Metallo-β-lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. J Clin Microbiol 2008;46:2028–37, doi:http://dx.doi.org/10.1128/JCM.00818-07.
[15] Traczewski MM, Carretto E, Canton R, Moore NM, Carba-R Study Team. Multicenter evaluation of the Xpert Carba-R assay for detection of carbapenemase genes in Gram-negative isolates. J Clin Microbiol 2018;56:e00272–18, doi:http://dx.doi.org/10.1128/JCM.00272-18.
[16] Girlich D, Bernabeu S, Grosperrin V, Langlois I, Begasse C, Arangia N, et al. Evaluation of the Amplidiag CarbaR+MCR Kit for accurate detection of carbapenemase-producing and colistin-resistant bacteria. J Clin Microbiol 2019;57:e01800–18, doi:http://dx.doi.org/10.1128/JCM.01800-18.
[17] Powell EA, Haslam D, Mortensen JE. Performance of the check-points Check-MDR CT103XL assay utilizing the CDC/FDA antimicrobial resistance isolate bank. Diagn Microbiol Infect Dis 2017;88:219–21, doi:http://dx.doi.org/10.1016/j.diagmicrobio.2017.04.011.
[18] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI Supplement M100. Wayne, PA: CLSI; 2020.
[19] EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance (2017). https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf (accessed July 29, 2020).
[20] Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench

guide. Clin Microbiol Infect 2008;14(Suppl 1):90–103, doi:http://dx.doi.org/10.1111/j.1469-0691.2007.01846.x.
[21] Wang Q, Wang X, Wang J, Ouyang P, Jin C, Wang R, et al. Phenotypic and genotypic characterization of carbapenem-resistant Enterobacteriaceae: data from a longitudinal large-scale CRE study in China (2012–2016). Clin Infect Dis 2018;67(Suppl 2):S196–205, doi:http://dx.doi.org/10.1093/cid/ciy660.
[22] Castanheira M, Farrell SE, Krause KM, Jones RN, Sader HS. Contemporary diversity of β-lactamases among Enterobacteriaceae in the nine U.S. Census regions and ceftazidime–avibactam activity tested against isolates producing the most prevalent β-lactamase groups. Antimicrob Agents Chemother 2014;58:833–8, doi:http://dx.doi.org/10.1128/AAC.01896-13.
[23] Meier M, Hamprecht A. Systematic comparison of four methods for detection of carbapenemase-producing Enterobacterales directly from blood cultures. J Clin Microbiol 2019;57:e00709–19, doi:http://dx.doi.org/10.1128/JCM.00709-19.
[24] Pierce VM, Simner PJ, Lonsway DR, Roe-Carpenter DE, Johnson JK, Brasso WB, et al. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. J Clin Microbiol 2017;55:2321–33, doi:http://dx.doi.org/10.1128/JCM.00193-17.
[25] CDC & FDA Antibiotic Resistance Isolate Bank. http://cdc.gov/drugresistance/resistance-bank [Accessed 4 August 2020].
[26] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Tests; Approved Standard—Twelfth edition. CLSI Document M02-A12. Wayne, PA: CLSI; 2015.
[27] Thomsen MCF, Ahrenfeldt J, Cisneros JLB, Jurtz V, Larsen MV, Hasman H, et al. A bacterial analysis platform: an integrated system for analysing bacterial whole genome sequencing data for clinical diagnostics and surveillance. PLoS One 2016;11:e0157718, doi:http://dx.doi.org/10.1371/journal.pone.0157718.
[28] Walker GA, Shostak J. Common Statistical Methods for Clinical Research with SAS Examples. 3rd ed. SAS Publishing; 2010.
[29] Girlich D, Naas T, Nordmann P. Biochemical characterization of the naturally occurring oxacillinase OXA-50 of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2004;48:2043–8, doi:http://dx.doi.org/10.1128/AAC.48.6.2043-2048.2004.
[30] Cornaglia G, Akova M, Amicosante G, Cantón R, Cauda R, Docquier J-D, et al. Metallo-β-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. Int J Antimicrob Agents 2007;29:380–8, doi:http://dx.doi.org/10.1016/j.ijantimicag.2006.10.008.
[31] Seale AC, Gordon NC, Islam J, Peacock SJ, Scott JAG. AMR surveillance in low and middle-income settings—a roadmap for participation in the Global Antimicrobial Surveillance System (GLASS) [version 1; peer review: 3 approved]. Wellcome Open Res 2017;2:92, doi:http://dx.doi.org/10.12688/wellcomeopenres.12527.1.
[32] EUCAST Expert Rules v 3.2 on Enterobacterales (2019). https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Expert_Rules/2020/ExpertRules_V3.2_20190515_Enterobacterales.pdf (accessed August 3, 2020).
[33] Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. J Clin Microbiol 2001;39:2206–12, doi:http://dx.doi.org/10.1128/JCM.39.6.2206-2212.2001.
[34] Lesher MD, Hale CM, Wijetunge DSS, England MR, Myers DS, Craft DW. Impact of removing ESBL designation from culture reports on the selection of antibiotics for the treatment of infections associated with ESBL-producing organisms. Infect Control Hosp Epidemiol 2020;41:604–7, doi:http://dx.doi.org/10.1017/ice.2020.46.
[35] Livermore DM, Andrews JM, Hawkey PM, Ho P-L, Keness Y, Doi Y, et al. Are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly? J Antimicrob Chemother 2012;67:1569–77, doi:http://dx.doi.org/10.1093/jac/dks088.
[36] Gill CM, Lasko MJ, Asempa TE, Nicolau DP. Evaluation of the EDTA-modified carbapenem inactivation method (eCIM) for detecting metallo-β-lactamase-producing *Pseudomonas aeruginosa*. J Clin Microbiol 2020;58:e02015–19, doi:http://dx.doi.org/10.1128/JCM.02015-19.
[37] Su M, Satola SW, Read TD. Genome-based prediction of bacterial antibiotic resistance. J Clin Microbiol 2019;57:e01405–18, doi:http://dx.doi.org/10.1128/JCM.01405-18.
[38] Davies TJ, Stoesser N, Sheppard AE, Abuoun M, Fowler P, Swann J, et al. Reconciling the potentially irreconcilable? Genotypic and phenotypic amoxicillin–clavulanate resistance in Escherichia coli. Antimicrob Agents Chemother 2020;64:, doi:http://dx.doi.org/10.1128/AAC.02026-19 e02026-19.
[39] Ellington MJ, Ekelund O, Aarestrup FM, Canton R, Doumith M, Giske C, et al. The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee. Clin Microbiol Infect 2017;23:2–22, doi:http://dx.doi.org/10.1016/j.cmi.2016.11.012.