Journal of Antimicrobial Chemotherapy

J Antimicrob Chemother 2018; **73**: 2738–2747
doi:10.1093/jac/dky276 Advance Access publication 24 July 2018

Performance of the EUCAST disc diffusion method and two MIC methods in detection of Enterobacteriaceae with reduced susceptibility to meropenem: the NordicAST CPE study

Bjørg Haldorsen[1], Christian G. Giske[2], Dennis S. Hansen[3], Kristjan Orri Helgason[4], Gunnar Kahlmeter[5], Iren H. Löhr[6], Erika Matuschek[5], Monica Österblad[7], Kaisu Rantakokko-Jalava[8], Mikala Wang[9], Lars Småbrekke[10], Ørjan Samuelsen[1,10] and Arnfinn Sundsfjord[1,11]* on behalf of the NordicAST CPE Study Group†

[1]Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway; [2]Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden; [3]Department of Clinical Microbiology, Herlev and Gentofte Hospital, Herlev, Denmark; [4]Department of Clinical Microbiology, Landspitali University Hospital, Reykjavik, Iceland; [5]EUCAST Development Laboratory, Växjö, Sweden; [6]Department of Medical Microbiology, Stavanger University Hospital, Stavanger, Norway; [7]Bacterial Infections Unit, National Institute for Health and Welfare, Turku, Finland; [8]Clinical Microbiology Laboratory, Turku University Hospital, Turku, Finland; [9]Department of Clinical Microbiology, Aarhus University Hospital, Aarhus, Denmark; [10]Department of Pharmacy, Faculty of Health Sciences, UiT – the Arctic University of Norway, Tromsø, Norway; [11]Department of Medical Biology, Faculty of Health Sciences, UiT – the Arctic University of Norway, Tromsø, Norway

*Corresponding author. Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, 9037 Tromsø, Norway. Tel +47-90616118 or +47-95217477; E-mail: arnfinn.sundsfjord@uit.no
†Additional members are listed in the Acknowledgements section.

Received 20 March 2018; returned 22 May 2018; revised 1 June 2018; accepted 15 June 2018

Objectives: To examine performance of EUCAST disc diffusion and supplementary MIC methods for detection of Enterobacteriaceae with reduced susceptibility to meropenem using EUCAST screening recommendations.

Methods: Sixty-one Nordic laboratories delivered data on EUCAST disc diffusion ($n = 61$), semi-automated meropenem MIC ($n = 23$; VITEK2, $n = 20$ and Phoenix, $n = 3$) and gradient meropenem MIC ($n = 58$) methods. The strains ($n = 27$) included the major carbapenemase classes (A, $n = 4$; B, $n = 9$; D, $n = 6$) involved in the global spread of carbapenemase-producing Enterobacteriaceae (CPE) and non-CPE strains ($n = 8$) covering a range of broth microdilution (BMD) meropenem MICs.

Results: A triplicate *Klebsiella variicola* (meropenem MIC 0.5 mg/L) harbouring OXA-48 and *Escherichia coli* ATCC 25922 showed an overall good precision. Meropenem zone diameters below the EUCAST screening cut-off (<27 mm) were reported for strains with MIC ≥1 mg/L ($n = 21$), irrespective of resistance mechanism. For three strains (MIC = 0.5 mg/L) with OXA-48/-181, eight laboratories provided meropenem zone diameters above the screening cut-off. Very major errors (VMEs) were not observed. The overall distributions of major errors (MEs) and minor errors (mEs) were 9% and 36% (disc diffusion), 26% and 18% (VITEK2) and 7% and 20% (gradient MIC), respectively. Differences in ME and mE distributions between disc diffusion and MIC gradient tests compared with semi-automated methods were significant ($P < 0.0001$), using BMD MICs as a reference for categorization.

Conclusions: The EUCAST disc diffusion method is a robust method to screen for CPE but isolates with meropenem MICs <1 mg/L pose challenges. The high ME rate in semi-automated methods might deter appropriate use of carbapenems in CPE infections with limited therapeutic options.

Introduction

With ever-increasing antimicrobial resistance, accurate and rapid antimicrobial susceptibility testing (AST) is essential for implementing appropriate therapy and timely infection control measures. This is particularly relevant when infections are caused by MDR Enterobacteriaceae, for which carbapenems remain a crucial therapeutic option.

The efficacy of carbapenems is jeopardized by the global increase in carbapenem-resistant Enterobacteriaceae (CRE).[1–5] Molecular mechanisms of carbapenem resistance in Enterobacteriaceae can be framed in two categories, which are not mutually exclusive:[6,7] (i) combined synergy between ESBLs and/or AmpC cephalosporinases (AmpC) and chromosomal mutations mediating porin deficiency, drug efflux and/or alterations in PBPs; and (ii) carbapenem hydrolysis by acquired carbapenemase enzymes. Carbapenemase-producing Enterobacteriaceae (CPE) have transmissible carbapenem resistance as the carbapenemase-encoding genes are mostly located on mobile genetic elements, a key factor in their global spread.[5] Particular CPE are now endemic in many parts of the world, including European countries such as Greece, Italy and Romania.[8]

The worldwide distribution of acquired carbapenemases in clinical strains of Enterobacteriaceae is dominated by five families within three Ambler classes: KPC (class A), NDM, VIM and IMP (class B) and OXA-48-like (class D).[5,8] This is consistent with the situation in the Nordic countries.[9–14] The phenotypic expression of carbapenemase production in terms of carbapenem MICs varies with species, strain and enzyme characteristics.[4,15–17] NDM-/KPC-producing strains of Enterobacteriaceae are mostly associated with moderate to high meropenem MICs.[15,16,18] In contrast, OXA-48-like-producing Enterobacteriaceae, *Escherichia coli* in particular, exhibit lower carbapenem MICs due to their relatively weak carbapenemase activity.[15,17–20] However, in combination with permeability deficiencies, OXA-48-like CPE may express high-level carbapenem resistance.[19,20] The diversity in carbapenem MIC distribution among CPE strains challenges the performance and interpretation of current phenotypic antimicrobial susceptibility tests. Various carbapenems have been evaluated for optimal detection of CPE, and meropenem seems to provide the best balance between sensitivity and specificity for screening purposes.[21]

Screening recommendations for detection of CPE are provided by CLSI and EUCAST, but optimal screening cut-offs are the subject of debate and may depend on local epidemiology. A recent study of clinical isolates of Enterobacteriaceae ($n = 1022$) submitted to a reference laboratory for confirmation of carbapenemase production ($n = 188/1022$; 18.4%) showed that 14% and 1.6% of the CPE isolates would have been missed by CLSI and EUCAST screening breakpoints, respectively.[15]

The objective of this study was to examine the performance of the EUCAST disc diffusion method and supplementary MIC methods in detection of Enterobacteriaceae with reduced susceptibility to meropenem in a multicentre format using the EUCAST screening recommendations. We invited all Nordic clinical microbiology laboratories to blindly examine a well-characterized, genetically diverse collection of carbapenemase- and non-carbapenemase-producing clinical isolates of Enterobacteriaceae with reduced susceptibility to meropenem (non-WT; MIC >0.12 mg/L).

Materials and methods

Study design

The study was organized through the Nordic Committee on Antimicrobial Susceptibility Testing (NordicAST) network (www.nordicast.org). All Danish ($n = 11$), Finnish ($n = 24$), Icelandic ($n = 1$), Norwegian ($n = 22$) and Swedish ($n = 26$) clinical microbiology laboratories were invited. The study protocol included: (i) bacterial identification and recording of methods; (ii) AST by the EUCAST disc diffusion method using the following panel: meropenem (10 μg), cefotaxime (5 μg), ceftazidime (10 μg) and piperacillin/tazobactam (30/6 μg); (iii) meropenem MIC determination using broth microdilution and/or an MIC gradient strip test; (iv) reporting of AST results from semi-automated AST devices by those laboratories routinely using them; (v) interpretation of results using the NordicAST algorithm based on the EUCAST 2013 guidelines,[22] which were operational during the study period; and (vi) reporting of inhibition zone diameters, meropenem MIC, clinical breakpoint interpretation (SIR) and potential carbapenemase production (yes/no). The laboratories were asked to carry out the analyses as part of their routine diagnostic workflow. Genotypic confirmative testing was not allowed.

For detection of CPE, the EUCAST 2013 guidelines recommended screening cut-offs using meropenem MIC >0.12 mg/L and/or a < 25 mm meropenem (10 μg) disc diffusion zone diameter, with an increased meropenem zone diameter cut-off (the EUCAST screening cut-off) of <27 mm recommended for areas where OXA-48 producers are endemic. Notably, the meropenem EUCAST screening cut-off is different to the clinical breakpoint used for categorization of meropenem susceptibility. The operational NordicAST algorithm recommended that Enterobacteriaceae with meropenem zone diameters of 25–26 mm and piperacillin/tazobactam interpreted as intermediate or susceptible (unlikely OXA-48 producers) did not require carbapenemase confirmation tests.

Comparison of methods was carried out with meropenem broth microdilution (BMD) as the reference method. Performance criteria were calculated according to ISO 20776-2 (https://www.iso.org/obp/ui/#iso:std:iso:20776:-2:ed-1:v1:en) based on EUCAST clinical breakpoints v.6.0, which remain valid for meropenem. Essential agreement (EA), categorical agreement (CA), very major error (VME), major error (ME) and minor error (mE) rates were calculated.

Strain collection

The strain collection (Table 1) consisted of carbapenemase-positive ($n = 19$) and carbapenemase-negative ($n = 8$) clinical isolates of Enterobacteriaceae with reduced susceptibility to meropenem (MIC range 0.25 to ≥16 mg/L) obtained during 2007–14. Reference MICs were determined using BMD (Sensititre, Trek Diagnostic Systems, Cleveland, USA). *E. coli* ATCC 25922 was used as a meropenem-susceptible quality control (QC) strain. The carbapenemase-positive isolates were selected from a previously characterized Norwegian strain collection, mostly associated with import.[10] The carbapenemase-negative isolates were characterized as part of this study. All strains were identified by MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) and characterized by WGS as described previously.[10] Carbapenemase-encoding resistance genes were determined using ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) and MLST was performed using the CGE MLST server (https://cge.cbs.dtu.dk/services/MLST/).

The clinical strains included *Klebsiella* spp. ($n = 11$), *E. coli* ($n = 7$), *Enterobacter* spp. ($n = 5$), *Citrobacter* sp. ($n = 1$), *Proteus mirabilis* ($n = 2$) and *Providencia rettgeri* ($n = 1$). The carbapenemases included: class A, bla_{KPC} ($n = 3$) and bla_{IMI} ($n = 1$); class B, bla_{NDM} ($n = 5$), bla_{VIM} ($n = 3$) and bla_{IMP} ($n = 1$); and class D, $bla_{OXA\text{-}48\text{-}like}$ ($n = 6$). One $bla_{OXA\text{-}48}$-positive *Klebsiella variicola* (meropenem MIC 0.5 mg/L) was provided in triplicate and one *Klebsiella pneumoniae* isolate harboured both bla_{NDM} and $bla_{OXA\text{-}181}$. The strains covered a range of meropenem MICs: 0.25 mg/L ($n = 2$), 0.5 mg/L ($n = 6$; including the triplicate), 1.0 mg/L ($n = 5$), 2.0 mg/L ($n = 5$), 4.0 mg/L ($n = 3$), 8.0 mg/L ($n = 4$) and ≥16 mg/L ($n = 4$).

QC

QC assessment was based on EUCAST QC tables.[23]

Statistical analysis

Differences in categorical and continuous data between groups were examined by χ^2 tests and *t*-tests, respectively. $P < 0.05$ was considered significant.

Table 1. Bacterial strains ($n = 27$) used in this study and their relevant characteristics

Carbapenemase class/species	Meropenem MIC (mg/L)	Carbapenemases	Other β-lactamases	Isolate no.
Class A (serine) ($n = 4$)				
K. pneumoniae	1	KPC-2	SHV-11; TEM-1	4
E. cloacae complex	≥16	KPC-2	CTX-M-15; OXA-1; TEM-1	19
E. cloacae complex	8	KPC-2	ACT-6[v[a]]; OXA-9-p[b]; TEM-1	28
E. cloacae complex	1	IMI-9	ACT[v]	3
Class B (MBL) ($n = 9$)				
K. pneumoniae	≥16	NDM-1 + OXA-181[d]	CTX-M-15; OXA-1; SHV-11; TEM-1	2
K. pneumoniae	≥16	NDM-1	CTX-M-15; CMY-6; OXA-1; SHV-11	5
P. mirabilis	0.25	NDM-1	CMY-16; OXA-10	8
E. coli	2	NDM-5	SHV-12; TEM-1	9
Citrobacter sp.	4	NDM-1	CMY-4; OXA-10; SED-1	11
E. coli	1	VIM-29	CTX-M-15; CMY-4; OXA-1	7
K. pneumoniae	2	VIM-1	SHV-12	27
K. pneumoniae	≥16	VIM-27	SHV-11	29
E. coli	1	IMP-26	CTX-M-15; TEM-1	24
Class D (OXA-48-like) ($n = 6$[c])				
K. variicola[c]	0.5	OXA-48	LEN-16	6, 10, 25
K. pneumoniae	2	OXA-48	CTX-M-15; OXA-1; SHV-76; TEM-1	1
K. pneumoniae	0.5	OXA-48	SHV-11	14
E. coli	1	OXA-48	CTX-M-24; TEM-1	17
E. coli	0.5	OXA-181[d]	CTX-M-15; CMY-2; OXA-1; TEM-1	23
K. pneumoniae	2	OXA-162[d]	CTX-M-15, SHV-100, TEM-1	26
CPE-negative ($n = 8$)				
K. pneumoniae	4	—	CTX-M-15; SHV-11; OXA-1	12
E. coli	8	—	CMY-42; OXA-1; TEM-1	13
K. pneumoniae	4	—	CTX-M-15; OXA-1; SHV-27	15
E. cloacae complex	0.25	—	ACT-15	16
E. coli	8	—	CTX-M-15; OXA-1	18
P. mirabilis	0.5	—	—	20
Enterobacter aerogenes	8	—	—	21
P. rettgeri	2	—	PER-1	22

[a]v = variant gene.
[b]p = partial gene.
[c]The *K. variicola* strain was provided in triplicate.
[d]OXA-181 and OXA-162 belong to the OXA-48-like group.

Results

Participation

Sixty-one laboratories (73%), from Denmark ($n = 9$; 82%), Finland ($n = 15$; 63%), Iceland ($n = 1$; 100%), Norway ($n = 14$; 64%) and Sweden ($n = 22$; 85%), delivered data on bacterial identification ($n = 61$), EUCAST disc diffusion ($n = 61$), meropenem MIC ($n = 60$) and semi-automated AST ($n = 23$; 38%).

Bacterial identification

All but one of the isolates were correctly identified to genus level. Minor discrepancies were observed in identification to species level. Most laboratories ($n = 56$; 92%) used MALDI-TOF MS for identification; MALDI Biotyper ($n = 44$; Bruker Daltonik) and VITEK MS ($n = 12$; bioMérieux, Marcy l'Étoile, France). Five laboratories (8%) used VITEK2 (bioMérieux) and/or API20E (bioMérieux).

Materials for AST

Disc manufacturers included BD ($n = 3$; Becton Dickinson, NY, USA), MAST ($n = 5$; MAST Group, Bootle, UK), Oxoid ($n = 50$; Oxoid, Basingstoke, UK) and ROSCO ($n = 3$; ROSCO Diagnostica, Taastrup, Denmark). Suppliers of Mueller–Hinton agar (MHA) included BD ($n = 20$), Bio-Rad ($n = 1$; Bio-Rad Clinical Diagnostics, Oslo, Norway), E&O labs ($n = 1$; E&O laboratories Ltd, UK), Lab M ($n = 2$; Lab M Ltd, UK) and Oxoid ($n = 37$). Meropenem MIC determination was performed using gradient strips ($n = 58$), in-house BMD ($n = 1$) or commercial BMD ($n = 1$; Sensititre). MIC gradients included Etest ($n = 34$; bioMérieux), MIC Test strip (MTS) ($n = 18$; Liofilchem, Italy) or M.I.C.Evaluator (M.I.C.E.) ($n = 6$; Oxoid).

Twenty-three laboratories performed semi-automated AST including VITEK2 AST ($n = 20$; bioMérieux) and Phoenix ($n = 3$; BD). VITEK2 panels included: AST-N230 ($n = 6$), AST-N218 ($n = 5$) and AST-N209 ($n = 9$). The Phoenix NMIC-93 ($n = 2$) and 25 NMIC/ID90 ($n = 1$) panels were used.

QC

All laboratories except one reported meropenem zone diameters in agreement with the accepted QC range for *E. coli* ATCC 25922 (28–35 mm), with 25 laboratories (42%) on target values (31–32 mm), with no significant differences in zone diameters between Oxoid and BD MHA ($P = 0.6$). For the discs, no formal statistical testing was possible due to a limited number of comparative observations. For piperacillin/tazobactam, cefotaxime and ceftazidime, 93%, 97% and 98% of the laboratories, respectively, reported zone diameters in agreement with the accepted QC range for *E. coli* ATCC 25922. All except two laboratories (3%) reported meropenem MICs within the accepted QC range for *E. coli* ATCC 25922 (0.008–0.06 mg/L), with 55 laboratories (92%) on target values (0.016–0.03 mg/L).

There was good reproducibility (precision) for the OXA-48-producing *K. variicola* triplicate with 59/61 laboratories reporting meropenem zone diameters within 5 mm. A total of 60 laboratories performed 180 MIC tests on this triplicate with a median MIC value of 0.5 mg/L. Of these, 109 (61%) were on the target value (0.5 mg/L) or within 0.25–1 mg/L (which equals target ± 1 dilution). Twenty-one of 60 laboratories (35%) obtained a value within this range on all three occasions. For the most commonly used methods, 34, 18 and 6 laboratories used Etest, MTS and M.I.C.E., respectively. Of these, 12 of 34 (35%), 5 of 18 (28%) and 3 of 6 (50%), respectively, obtained a value within the range on all triplicates. Fifteen results (8%) were outside the target of ± 2 dilutions.

Performance of the EUCAST disc diffusion method in detection of reduced susceptibility to meropenem

The results are based on the observations ($n = 1566$) displayed in Table 2 and those obtained with ROSCO tablets ($n = 81$; Table S1, available as Supplementary data at *JAC* Online); a total of 1647 observations, counting the *K. variicola* triplicate only once. ROSCO tablets have a larger diameter (9 mm) than BD, MAST and Oxoid discs (6 mm) and are therefore presented separately.

For strains with meropenem MIC ≥ 1 mg/L ($n = 21$), all laboratories reported meropenem zone diameters below the EUCAST screening cut-off, irrespective of resistance mechanism (with one exception; isolate 18). For strains with a meropenem MIC of 0.25 or 0.5 mg/L, one or several (range 8–60) laboratories reported meropenem zone diameters above the EUCAST screening cut-off, also affecting the interpretation of OXA-48-like carbapenemase strains.

CA between meropenem disc diffusion results compared with BMD meropenem MIC using EUCAST clinical breakpoints for categorization

Zone diameters ($n = 1566$) from individual laboratories ($n = 58$) using BD, MAST and Oxoid discs and corresponding SIR categories (Table 2) plus observations from three laboratories using ROSCO tablets ($n = 81$ observations) were compared with the SIR category as defined by the actual BMD meropenem MIC (Table 3). The overall CA was 55%. No VMEs were observed. The overall proportions of MEs and mEs were 9% and 36%, respectively, varying between Ambler classes and carbapenemase-negative strains.

Reporting potential carbapenemase production (Table 4)

Forty-four laboratories (72%) interpreted their results using the recommended EUCAST screening cut-off, taking into account the piperacillin/tazobactam susceptibility profile. Notably, eight laboratories (13%) used a <25 mm cut-off and seven laboratories (11%) interpreted the potential presence of carbapenemase production according to clinical breakpoints. Two laboratories did not report their interpretation criteria and were excluded from the analysis.

Among the class A carbapenemase-producing strains ($n = 4$), the three bla_{KPC}-positive strains were classified as potential carbapenemase producers by 98%–100% of the laboratories. The bla_{IMI}-positive *Enterobacter cloacae* complex strain (median meropenem zone diameter 18 mm) was not reported as a carbapenemase producer by seven laboratories (11%).

Eight of the class B carbapenemase-positive isolates ($n = 9$) were interpreted as possible carbapenemase producers by 97%–100% of the laboratories. Fifteen laboratories (25%) did not report the bla_{NDM}-positive *P. mirabilis* (median meropenem zone diameter of 22 mm) as a putative carbapenemase producer.

The triplicate OXA-48-producing *K. variicola* isolates were not classified as potential carbapenemase producers by 11 (18%), 10 (16%) and 12 (20%) laboratories, respectively. The median meropenem zone diameters were 24, 23 and 24 mm, respectively. It is notable that 2%–5% of the laboratories using the EUCAST screening cut-off, 13%–38% of those using <25 mm and 86% of those using the clinical breakpoints did not suspect carbapenemase production in the *K. variicola* triplicate.

For the other OXA-48-like producers ($n = 5$) with a meropenem median zone diameter ranging between 20 and 24 mm, 80%–98% of the laboratories suspected carbapenemase production. As for the triplicate strain, failure proportions in suspecting carbapenemase production depended on the screening criteria used.

Among the carbapenemase-negative strains ($n = 8$), six were suspected as carbapenemase producers by 90%–98% of the laboratories. The *E. cloacae* complex strain (meropenem MIC 0.25 mg/L) was suspected to be a carbapenemase producer by 13 (21%) of the laboratories, while none suspected carbapenemase production in the *P. mirabilis* strain (meropenem MIC 0.5 mg/L). The median meropenem zone diameters for these two strains were 28 and 30 mm, respectively.

Agreement between meropenem MIC measured by BMD and semi-automated susceptibility testing

We recorded MIC observations using VITEK2 ($n = 539$) and Phoenix ($n = 81$) from 20 and 3 laboratories, respectively (Table 3). The overall EA between BMD and VITEK2 was 65%. Fifty percent, 61% and 25% of the MICs from the VITEK2 systems were higher (>2-fold) than the BMD MIC for class A, class B and class D

Table 2. Distributions ($n = 1682$[b]) of meropenem zone diameters (mm) using EUCAST disc diffusion method with BD, MAST or Oxoid discs

				Distribution of meropenem zone diameters (mm)																													
				R (<16 mm)											I							S (≥22 mm); screening breakpoint < 27 mm											
Carbapenemases	Isolate no.	Species	MEM MIC (mg/L)	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Class A (serine) ($n = 4$)																																	
KPC ($n = 3$)	4	*K. pneumoniae*	1			1		1	1	2	1	5	6	9	13[a]	11	4	2		1				1									
	19	*E. cloacae* complex	≥16	57[a]	1																												
	28	*E. cloacae* complex	8	7		4		3	2	4	4	14[a]	12	3	4	1																	
IMI ($n = 1$)	3	*E. cloacae* complex	1	12		2		1	3	4	2	1	1	2		5[a]	5	7	3	7	2		1										
Class B (MBL) ($n = 9$)																																	
NDM ($n = 5$)	2	*K. pneumoniae*	≥16	57[a]	1																												
	5	*K. pneumoniae*	≥16	9		4	3	16[a]	16	6	2	2																					
	8	*P. mirabilis*	0.25								1				1	3	3	11	6	12[a]	6	9	4	1	1								
	9	*E. coli*	2	9	1	1		2	1	3	3	5	8[a]	11	6	5	3																
	11	*Citrobacter* sp.	4	3		1	1		2	6	6	4	10[a]	8	4	1	5	1	2			2											
VIM ($n = 3$)	7	*E. coli*	1								3	1	1	5	6	18[a]	10	8	5			1											
	27	*K. pneumoniae*	2	1								3	5	7	9	8[a]	8	2	4	7	2	2											
	29	*K. pneumoniae*	≥16	53[a]	1	4																											
IMP ($n = 1$)	24	*E. coli*	1								1		1		2	3	12	15[a]	14	6	2	2											
Class D (OXA-48-like) ($n = 6$)	6	*K. variicola*[b]	0.5														1	3	2	9	11	19[a]	9	4									
	10	*K. variicola*	0.5	1												1		1	7	10	13[a]	13	9	3									
	25	*K. variicola*	0.5														1	3	3	6	13	11[a]	12	5	2		2						
	1	*K. pneumoniae*	2	3				2					2	2	1	7	7	17[a]	13	2	2												
	14	*K. pneumoniae*	0.5															2	2	7	12	15[a]	9	8	3								
	17	*E. coli*	1						1									6	6	15	18[a]	8	2	2									
	23	*E. coli*	0.5															2	5	6	8	18[a]	13	5	1								
	26	*K. pneumoniae*	2						1	1						2	1	4	11	12[a]	15	8	2	1									
CPE-negative ($n = 8$)	12	*K. pneumoniae*	4									1	1	4	4	2	15	22[a]	9														
	13	*E. coli*	8						1	4	6	4	9	9[a]	8	5	3	6	3														
	15	*K. pneumoniae*	4	1						1	2	3	6	12	23[a]	6	2	1	1														
	16	*E. cloacae complex*	0.25													1				2	1	1	3	5	8	15[a]	15	6	1				
	18	*E. coli*	8	1						1			2	8	3	14	7[a]	6	10	3	1	1								1			
	20	*P. mirabilis*	0.5																					1	1	3	5	21[a]	14	8	1	4	
	21	*E. aerogenes*	8	1									5	6	7	17[a]	5	6	3	6	1		1										
	22	*P. rettgeri*	2	1								1	3	2	8	7	12[a]	13	9	2													

MEM, meropenem.
[a]Median observation.
[b]The *K. variicola* strain was provided in triplicate (isolates 6, 10 and 25). Only one *K. variicola* isolate (isolate 6) was included in the further analyses. Consequently, a total of 1566 distributions of zone diameters using BD, MAST and Oxoid discs were included in the analyses in Table 3.

Table 3. Agreement (%) between meropenem MIC determined by BMD and disc diffusion, semi-automated susceptibility testing or gradient tests and corresponding CA (%) in clinical SIR categorization

		Agreement category[a] and *n* (%) of instances						
Method/no. of observations	Group	EA	>BMD MIC	<BMD MIC	VME	ME	mE	CA
Disc diffusion								
1647	total	NA	NA	NA	0	153 (9)	594 (36)	900 (55)
244	class A	NA	NA	NA	0	48 (20)	117 (48)	79 (32)
549	class B	NA	NA	NA	0	69 (13)	226 (41)	254 (46)
366	class D	NA	NA	NA	0	26 (7)	114 (31)	226 (62)
488	CPE-negative	NA	NA	NA	0	10 (2)	137 (28)	341 (70)
Automated ASTs								
VITEK2								
539	total	347 (65)	180 (33)	12 (2)	0	141 (26)	98 (18)	300 (56)
80	class A	40 (50)	40 (50)	0	0	40 (50)	20 (25)	20 (25)
179	class B	69 (39)	110 (61)	0	0	98 (55)	12 (7)	69 (38)
120	class D	90 (75)	30 (25)	0	0	3 (2.5)	34 (28)	83 (69.5)
160	CPE-negative	148 (92.5)	0	12 (7.5)	0	0	32 (20)	128 (80)
Phoenix								
81	total	61 (75)	18 (22)	2 (3)	0	11 (14)	12 (15)	58 (71)
12	class A	9 (75)	3 (25)	0	0	2 (16)	2 (16)	8 (68)
27	class B	14 (52)	12 (44)	1 (4)	0	9 (33)	4 (15)	14 (52)
18	class D	15 (83)	3 (17)	0	0	0	3 (17)	15 (83)
24	CPE-negative	23 (96)	0	1 (4)	0	0	3 (12.5)	21 (87.5)
MIC gradient tests[b]								
1566	total	1105 (70)	278 (18)	183 (12)	0	114 (7)	319 (20)	1133 (73)
232	class A	161 (69)	65 (28)	6 (3)	0	40 (17)	64 (28)	128 (55)
522	class B	384 (74)	122 (23)	16 (3)	0	43 (8)	99 (19)	380 (73)
348	class D	226 (65)	54 (15.5)	68 (19.5)	0	12 (3)	28 (8)	308 (89)
464	CPE-negative	334 (72)	36 (8)	94 (20)	0	19 (4)	128 (28)	317 (68)

NA, not applicable.
[a]EA, results within one 2-fold MIC dilution; CA, agreement in SIR category; VME, false susceptibility; ME, false resistance; mE, susceptible or resistant interpreted as intermediate or vice versa.
[b]MIC gradient tests from bioMérieux ($n = 34$), Liofilchem ($n = 18$) and Oxoid ($n = 6$).

carbapenemase-producing strains, respectively. Notably, laboratories did not report lower VITEK2 meropenem MICs compared with BMD for carbapenemase producers, in contrast to carbapenemase-negative strains.

Overall CA between BMD and VITEK2 was 56%. No VMEs were observed. Overall proportions of MEs and mEs were 26% and 18%, respectively, varying with resistance mechanism. No MEs were observed for carbapenemase-negative strains. The difference in distributions of MEs and mEs between disc diffusion and VITEK2 was statistically significant ($P < 0.0001$).

For the Phoenix system ($n = 81$), MICs were higher than the BMD MICs for the class A (25%), class B (44%) and class D (17%) carbapenemase-producing strains (Table 3); none of the carbapenemase-negative strains measured above the BMD MIC. There was no statistical significant difference in overall results between VITEK2 and Phoenix ($P = 0.132$).

Overall CA between BMD and Phoenix was 71% with no VMEs. The total proportions of MEs and mEs were 14% and 15%, varying between strains with different carbapenemase classes. No MEs were reported for class D and carbapenemase-negative strains.

Agreement between meropenem MIC determined by BMD and gradient tests

We recorded 1566 gradient MIC observations with Etest ($n = 918$), MTS ($n = 486$) and M.I.C.E. strips ($n = 162$), from 34, 18 and 6 laboratories, respectively (Table S2). All class A and class B carbapenemase-producing strains expressed gradient meropenem MIC above the EUCAST MIC screening cut-off value (>0.12 mg/L). In contrast, 18 laboratories (18/60; 30%) reported meropenem MIC below the EUCAST MIC screening cut-off value for the triplicate strains of the OXA-48-producing *K. variicola*, all reporting 0.125 mg/L. Nine laboratories (15%) reported meropenem MIC below the cut-off (0.125 mg/L) for three of the other OXA-48-like strains. The median gradient test MIC for these strains was 0.5 mg/L.

The meropenem MIC agreement between BMD and gradient tests is presented in Table 3. Overall EA was 70%. In contrast to VITEK2, the gradient tests did record meropenem MIC values below the BMD reference MIC for class A, B and D carbapenemase-producing strains. The overall results between VITEK2 and MIC gradient tests were significantly different ($P < 0.0001$).

Table 4. Reporting potential carbapenemase production from the laboratories ($n = 59$) based on the use of different meropenem zone diameter screening cut-off criteria; the recommended (<27 mm), <25 mm and <22 mm (clinical breakpoint)

Carbapenemase	Isolate no.	Species	MEM MIC (mg/L)	Suspect CPE (%)	<27 mm ($n = 44$)[a]		<25 mm ($n = 8$)		<22 mm ($n = 7$)	
					yes (%)	no (%)	yes (%)	no (%)	yes (%)	no (%)
Class A (serine)										
KPC ($n = 3$)	4	*K. pneumoniae*	1	98	100	0	87	13	100	0
	19	*E. cloacae* complex	≥16	100	100	0	100	0	100	0
	28	*E. cloacae* complex	8	98	98	2	100	0	100	0
IMI ($n = 1$)	3	*E. cloacae* complex	1	89	95	5	62	38	86	14
Class B (MBL)										
NDM ($n = 5$)	2	*K. pneumoniae*	≥16	100	100	0	100	0	100	0
	5	*K. pneumoniae*	≥16	100	100	0	100	0	100	0
	8	*P. mirabilis*	0.25	75	89	11	75	25	14	86
	9	*E. coli*	2	100	100	0	100	0	100	0
	11	*Citrobacter* sp.	4	98	100	0	100	0	100	0
VIM ($n = 3$)	7	*E. coli*	1	98	100	0	100	0	86	14
	27	*K. pneumoniae*	2	98	100	0	100	0	100	0
	29	*K. pneumoniae*	≥16	100	100	0	100	0	100	0
IMP ($n = 1$)	24	*E. coli*	1	97	100	0	100	0	86	14
Class D (OXA-48-like) ($n = 6$)	6	*K. variicola*[b]	0.5	82	95	5	87	13	14	86
	10	*K. variicola*	0.5	84	98	2	87	13	14	86
	25	*K. variicola*	0.5	80	95	5	62	38	14	86
	1	*K. pneumoniae*	2	98	100	0	100	0	100	0
	14	*K. pneumoniae*	0.5	80	93	7	87	13	14	86
	17	*E. coli*	1	89	98	2	100	0	29	71
	23	*E. coli*	0.5	87	98	2	87	13	43	57
	26	*K. pneumoniae*	2	85	95	5	100	0	29	71
CPE-negative ($n = 8$)	12	*K. pneumoniae*	4	97	98	2	100	0	86	14
	13	*E. coli*	8	95	98	2	100	0	71	29
	15	*K. pneumoniae*	4	98	98	2	100	0	100	0
	16	*E. cloacae complex*	0.25	21	25	75	0	100	0	100
	18	*E. coli*	8	95	98	2	87	13	86	14
	20	*P. mirabilis*	0.5	0	0	100	0	100	0	100
	21	*E. aerogenes*	8	93	95	5	100	0	100	0
	22	*P. rettgeri*	2	90	95	5	100	0	71	29

MEM, meropenem.
[a]The number shown is the number of laboratories.
[b]The *K. variicola* strain was provided in triplicate (isolates 6, 10 and 25).

Overall CA between BMD and gradient tests was 73%. No VMEs were observed. The overall proportions of MEs and mEs were 7% and 20%, varying between strains with different resistance mechanisms. The difference in distributions of MEs and mEs between MIC gradient tests and VITEK2 was significant ($P < 0.0001$).

Discussion

Accurate detection of CPE is essential to prevent further transmission. We have examined the multicentre performance of EUCAST disc diffusion and selected MIC methods in detection of Enterobacteriaceae with reduced meropenem susceptibility involving 61 Nordic clinical laboratories. The strains included the major bacterial species and carbapenemase classes involved in the global spread of CPE, as well as non-CPE strains, representing a range of meropenem MIC levels challenging the sensitivity and interpretation of the methods involved.

The overall results suggest that the EUCAST disc diffusion method is robust in detection of reduced susceptibility to meropenem in both CPE and non-CPE. No VMEs were observed and a relatively low overall ME rate (9%) compared with semi-automated systems (14%–26%), but in line with gradient tests (7%), was found. However, a relatively high mE rate (36%) related to semi-automated systems (15%–18%) and gradient tests (20%) was noted.

Meropenem zone diameters below the EUCAST screening cut-off were reported for all strains with meropenem MIC ≥1 mg/L irrespective of resistance mechanism. However, the sensitivity of the EUCAST disc diffusion method is challenged by strains expressing meropenem MICs <1 mg/L. This observation is illustrated with the meropenem zone diameter range crossing the screening breakpoint for all strains expressing BMD meropenem MICs of 0.25–0.5 mg/L. Our results are consistent with previous observations showing an overrepresentation of OXA-48-like CPE strains within this low but significant non-WT MIC category.[15,17]

The results illustrate the challenges in setting appropriate screening breakpoints for the detection of resistance mechanisms of clinical importance. The breakpoints have to balance the need for high sensitivity without compromising the predictive value of a positive test result and must take into consideration variabilities in the epidemiology of relevant carbapenemases. Thus, the EUCAST 2013 guidelines suggested increasing the zone diameter of the meropenem CPE-screening breakpoint due to increased occurrence of OXA-48-like CPE.[22] The present study was based on the 2013 EUCAST guidelines and supports the use of increased zone diameters to enhance sensitivity in CPE detection. Our study design did not allow assessment of specificity. The most recent EUCAST guidelines have further increased the screening cut-off, now including isolates with meropenem zone diameters of 25–27 mm if they are also resistant to piperacillin/tazobactam and/or temocillin.[21] The addition of criteria linked to piperacillin/tazobactam and temocillin resistance will contribute to higher specificity, particularly in low CPE-prevalence settings, but the overall specificity remains to be evaluated. Moreover, the screening methods are continuously being challenged by new carbapenemase variants with weak yet significant carbapenemase activity.[24–26]

A number of laboratories failed to report potential carbapenemase production in strains with meropenem MICs of 0.25–0.5 mg/L. These failures were mainly due to the choice of screening breakpoints and were most common in the OXA-48-like group, where 0%–86% of the laboratories using clinical breakpoints did not select those strains for carbapenemase detection. In contrast, only 0%–38% and 0%–7% of the laboratories using <25 or <27 mm as a screening cut-off, respectively, did not suspect carbapenemase production in the OXA-48-like group. Although the study protocol referred to the NordicAST algorithm based on the EUCAST guidelines, several laboratories ($n = 15$) deviated from the recommendations for unknown reasons. It is not clear whether the variable screening breakpoints used by laboratories was due to misinterpretation of the study protocol or reflected local routine practice at the time of the study. Nevertheless, our observations underline the importance of clear communication in recommended screening breakpoints to reduce the risk of misinterpretations.

We observed a significant trend towards overestimation of meropenem MIC for CPE and a potential underestimation of meropenem MIC for non-CPE strains with reduced susceptibility to meropenem in semi-automated systems compared with BMD. The inflated meropenem MIC, particularly for strains with class A and class B carbapenemases will lead to overcalling of resistance. In the past this would perhaps not be seen as a significant problem, due to the general approach of discouraging use of carbapenems. However, clinical studies have shown that, even when MICs are elevated, carbapenems still have an important role in the treatment of infections caused by CPE, usually in combination with other active agents.[27] Overcalling resistance may therefore discourage what would be appropriate use of meropenem for CPE that remain clinically susceptible to meropenem. In contrast, the significant trend towards underestimating meropenem MICs for non-CPE strains may encourage inappropriate use of meropenem in supposedly susceptible strains. Notably, we recorded no VMEs in the overall CA between meropenem MIC obtained by BMD and semi-automated systems. In contrast to the semi-automated systems, we did not observe any statistically significant trends in overestimation or underestimation of gradient test MICs compared with BMD. The overall CA and EA were high; 73% and 70%, respectively. As for semi-automated systems, we observed no VMEs. Significantly lower ME rates were observed for gradient test meropenem MIC as compared with semi-automated systems.

In summary, the EUCAST disc diffusion method is a robust method to screen for CPE, but isolates with low meropenem MICs (<1 mg/L) still pose challenges to laboratories, not least from the perspective of interpreting guidelines correctly. This study supports using an increased meropenem screening cut-off of <27 mm to achieve greater sensitivity. This is particularly important in the detection of OXA-48-like producing isolates. Further expansion of the zone diameter (<28 mm), as recommended in the EUCAST 2017 guidelines, may add to the increased sensitivity. However, the effect on specificity of new screening cut-offs remains to be determined and would be of high interest in low resistance prevalence settings. Using BMD as the reference method, we noted an overall higher EA and CA with gradient tests compared with semi-automated AST. Furthermore, we observed an overestimation of meropenem MIC for CPE when using semi-automated systems compared with BMD, resulting in high ME rates for class A and class B carbapenemase-producing strains. The tendency of semi-automated systems to overcall meropenem resistance in CPE might have the undesirable effect of deterring appropriate use of carbapenems in the treatment of infections caused by MDR Enterobacteriaceae with limited therapeutic options.

Acknowledgements

Parts of the data were presented at the Twenty-seventh European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017 (Abstracts EP0674 and P0153).

Additional members of the NordicAST CPE Study Group

Jurgita Samulioniene (Aalborg University Hospital, Aalborg, Denmark), Kristian Schønning (Hvidovre Hospital, Copenhagen, Denmark), Flemming Schønning Rosenvinge (Odense University Hospital, Odense, Denmark), Ulrik Stenz Justesen (Rigshospitalet, Copenhagen, Denmark), Frank Hansen (Statens Serum Institut, Copenhagen, Denmark), Turid Snekloth Søndergaard (Sønderjylland Hospital, Sønderborg, Denmark), Claus Østergaard (Lillebælt Hospital, Vejle, Denmark), Evaliina Tarkka (Helsinki University Hospital, Helsinki, Finland), Benita Forsholm-Helander (Kymenlaakso Hospital, Kotka, Finland), Anne-Mari Rissanen (ISLAB Kuopio, Kuopio, Finland), Pauliina Kärpänoja (Päijät-Häme Central Hospital, Lahti, Finland), Terhi Tuhkalainen (Etelä-Karjalan Keskussairaala, Lappeenranta, Finland), Jaana Kaupilla (NordLab Oulo, Oulo, Finland), Raija Manninen (Satakunnan Keskussairaala, Pori, Finland), Joanna Peltola (NordLab, Rovaniemi, Finland), Antti Nissinen (Synlab, Turku, Finland), Jari Hirvonen (Fimlab Laboratories, Finland), Marianne Gunell (TYKS-Sapa Microbiology and Genetics, Turku, Finland),

Päivi Kankkunen (VITA HealthCare Services, Helsinki, Finland), Kerttu Saha (Seinäjoki Central Hospital, Seinäjoki, Finland), Ólafía Svandís Grétarsdóttir (Landspitali University Hospital, Reykjavik, Iceland), Bjørn Odd Johnsen (Akershus University Hospital, Akershus, Norway), Nadine Pullar (Vestre Viken Hospital, Bærum, Norway), Einar Tollaksen Weme (Vestre Viken Hospital, Drammen, Norway), Torunn Haukeland (Haukeland University Hospital, Norway), Susanne Hartzen (Innlandet Hospital, Lillehammer, Norway), Heidi Aasen Tomren (Molde Hospital, Molde, Norway), Hege Elisabeth Larsen (Nordland Hospital, Bodø, Norway), Gorm Hansen (Oslo University Hospital, Rikshospitalet, Oslo, Norway), Anita Løvås Brekken (Stavanger University Hospital, Stavanger, Norway), Ståle Tofteland (Sørlandet Hospital, Kristiansand, Norway), Karianne Wiger Gammelsrud (Oslo University Hospital, Ullevål, Oslo, Norway), Kristina Papp (Unilabs Telelab, Skien, Norway), Brian Guennigsmann (University Hospital North-Norway, Tromsø, Norway), Dagfinn Skaare (Vestfold Hospital, Tønsberg, Norway), Ann-Katrin Larsson (Aleris/Medilab, Täby, Sweden), Frida Nilsson (Blekinge Hospital, Karlskrona, Sweden), Sotirios Oikonomou (Södra Älvsborgs Hospital, Borås, Sweden), Dalila Kartout Boukdir (Mälar Hospital, Eskilstuna, Sweden), Karin Sjöström (Folkhälsomyndigheten, Solna, Sweden), Kerstin Röhstö (Gävle Hospital, Gävle, Sweden), Sara Gianello (Sahlgrenska University Hospital, Gothenburg, Sweden), Ingegerd Sjögren (Hallands Hospital, Halmstad, Sweden), Sara Petersson (Länssjukhuset, Kalmar, Sweden), Anders Nyberg (Länssjukhuset, Sundsvall, Sweden), Margareta Granlund (Norrlands University Hospital, Umeå, Sweden), Anna-Karin Smekal (Academic Hospital, Uppsala, Sweden), Margareta Tholander (Visby Lasarett, Visby, Sweden), Marie Thelander (Falun Hospital, Falun, Sweden), Mimmi Blad (Länssjukhuset Ryhov - Hospital, Jönköping, Sweden), Torbjörn Kjerstadius (Central Hospital, Karlstad, Sweden), Inga Fröding (Karolinska University Hospital, Huddinge, Sweden), Ann-Cathrine Petersson (Region Skåne, Lund, Sweden), Eva Halldin (Vestmanlands Hospital, Västerås, Sweden), Cecilia Alexandersson, (Central Hospital, Växjö, Sweden) and Carina Thilesen (Unilabs/Skaraborgs Hospital, Skövde, Sweden).

Funding

This work was supported by and organized through the Nordic Committee on Antimicrobial Susceptibility Testing (NordicAST) network. The shipment of strains and overall data collection was coordinated by the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance and as such supported by the Northern Norway Regional Health Authority (Helse Nord RHF) and the Norwegian Directorate of Health. The two latter funders have not played any decision-making role in the design, execution, analysis or reporting of the research.

Transparency declarations

None to declare.

Supplementary data

Tables S1 and S2 are available as Supplementary data at *JAC* Online.

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