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Differences in antimicrobial susceptibility testing complicating management of IMP carbapenemase-producing Enterobacterales infection

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ABSTRACT

Objectives: IMP-type carbapenemases are rarely detected in Europe and limited information is available to guide the treatment of infections caused by carbapenemase-producing Enterobacterales (CPE) producing these carbapenemases. Accurate antimicrobial susceptibility testing (AST) results are essential for optimal antibiotic management. Here we report discrepancies in AST of IMP-producing Enterobacterales (IMP-CPE) complicating the management of severe sepsis.
Methods: Antimicrobial susceptibilities were analysed by in-house VITEK® 2, Etest and broth microdilution (BMD). Carbapenemase-encoding genes were detected by PCR. Whole-genome sequencing (WGS) was performed using an Illumina MiSeq platform.
Results: Minimum inhibitory concentrations (MICs) determined by VITEK® 2 for *Enterobacter hormaechei* and *Klebsiella oxytoca* blood culture isolates were ≥16 mg/L for meropenem and ≤0.5 mg/L for ertapenem. In contrast, Etest analysis and BMD returned MICs of 2 mg/L and 1 mg/L, respectively. Both isolates tested positive for IMP carbapenemase-encoding genes by PCR. WGS revealed that both isolates carried the same bla_{IMP-4} gene. Based on VITEK® 2 susceptibilities, initial treatment was with tigecycline and amikacin. After subsequent deterioration, the patient was successfully treated with ertapenem and amikacin.
Conclusion: This case highlights that automated AST by VITEK® 2 can over-report meropenem resistance for IMP carbapenemase-producers compared with Etest and BMD. Clinicians need to be cautious deciding against carbapenem treatment based on VITEK® 2 susceptibility testing results for IMP-positive Enterobacterales. Tigecycline was inferior to carbapenem treatment for pyelonephritis caused by isolates expressing IMP carbapenemases, however specific evidence guiding the treatment of these infections is lacking.

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1. Introduction

The emergence and global dissemination of multidrug-resistant bacteria, including carbapenemase-producing Enterobacterales (CPE), is a serious threat to the delivery of modern healthcare. Imipenemase (IMP) enzymes, a subclass of metallo-β-lactamases (MBLs), are carbapenemases first described in *Serratia marcescens* in Japan in 1991 [1]. Since then, IMP-producing Enterobacterales, *Pseudomonas* and *Acinetobacter* have been reported worldwide. IMP-producing Enterobacterales (IMP-CPE) are now endemic in Japan and Taiwan, and IMP-4 is the most common CPE found in Australia [2]. In Europe, the IMP prevalence remains sporadic with no reported outbreaks or regional spread [3]. Similarly in Ireland, IMP-producing strains are uncommon, accounting for only 3.5% (15/433) of new cases of CPE detected in 2017 [4].

Considering the ability of CPE to successfully transmit among patients in healthcare settings, sensitive laboratory detection methods in conjunction with accurate and precise antimicrobial

AST, antimicrobial susceptibility testing; BMD, broth microdilution; MIC, minimum inhibitory concentration; WGS, whole-genome sequencing; CPE, carbapenemase-producing Enterobacterales; IMP, imipenemase; MBL, metallo-b-lactamase.

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susceptibility testing (AST) are crucial. Following detection, immediate infection prevention and control precautions can be instituted and appropriate antimicrobial treatment initiated if required. Recent guidelines and reviews on the management of multidrug- and carbapenem-resistant Gram-negative bacteria have been published, but much of the evidence to date is derived from studies in which *Klebsiella pneumoniae* carbapenemase (KPC)-producing strains of CPE predominated [5,6]. Little published evidence exists on the management of IMP-CPE infections to guide treatment decisions in these cases.

Here we report a case of severe sepsis caused by IMP-producing *Enterobacter hormaechei* and *Klebsiella oxytoca* where in-house AST by VITEK® 2 led to a categorical error in meropenem reporting and a clinically relapsed infection. We then describe and discuss the results of subsequent phenotypic and molecular testing and whole-genome sequencing (WGS) of the isolates in question. Our case highlights the importance of adequate AST for optimal management of CPE infections.

2. Materials and methods

2.1. Clinical case

A 46-year-old female was admitted with sepsis having presented with fever, right flank pain, hypotension and tachycardia. She had a urinary catheter in situ and a history of recurrent urinary tract infections caused by an obstructing renal calculus. Successful fluid resuscitation was carried out in the intensive care unit and empirical treatment for urosepsis was commenced with meropenem 2 g three times daily and amikacin 15 mg/kg once daily.

The following day, admission blood cultures grew *Enterobacter cloacae* and *K. oxytoca* identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS). AST carried out using a VITEK® 2 automated instrument (bioMérieux, Marcy-l'Étoile, France) reported both isolates as resistant to meropenem [minimum inhibitory concentration (MIC) $\geq$16 mg/L] and susceptible to ertapenem (MIC $\leq$ 0.5 mg/L) using European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints. In light of this, meropenem was changed to tigecycline (100 mg loading dose, followed by 50 mg twice daily) and amikacin was continued. *Enterobacter cloacae* complex with the same susceptibility pattern was similarly isolated from midstream urine. The patient's condition improved, however on Day 9 of admission the patient became unwell again with pyrexia, hypotension and tachycardia. Ertapenem 1 g once daily was added to the above regimen and resolution of the septic episode followed. Tigecycline was discontinued after another 4 days. No microbial growth was identified from blood cultures drawn at this time.

2.2. Antimicrobial susceptibility testing (AST)

In-house AST was performed with a VITEK® 2 system. VITEK® 2 AST cards N297 and N254 were used for susceptibility testing. Elevated carbapenem MICs were reinvestigated by in-house Etest (bioMérieux) and were sent to a reference laboratory for confirmatory broth microdilution (BMD) testing. Isolates with elevated carbapenem MICs were further evaluated using the modified Hodge test.

A commercially available diagnostic kit (Rosco Diagnostica A/S, Taastrup, Denmark) consisting of disks containing 10 μg of meropenem alone and in combination with the β-lactamase inhibitors phenylboronic acid (Class A inhibitor), dipicolinic acid (Class B inhibitor) and cloxacillin (AmpC inhibitor), along with a disk containing temocillin (to detect OXA-48), was used to phenotypically distinguish CPE isolates.

Isolates grown on the original culture plate were sent to an external hospital (Hospital 2), which also used VITEK® 2 with N351 as susceptibility testing card for confirmation of AST results.

Following phenotypic testing, PCR was performed using a Check-Points Check-Direct CPE assay (Check-Points, Wageningen, The Netherlands). This is a real-time multiplex PCR assay that detects KPC, NDM/VIM and OXA-48 but not IMP genes. As the in-house CPE PCR did not detect a CPE gene, the isolates were then referred to the National CPE Reference Laboratory for further molecular testing using an in-house PCR assay.

2.3. Whole-genome sequencing (WGS)

Genomic DNA (gDNA) was purified from overnight cultures using an UltraClean® Microbial DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. gDNA was assessed using NanoDrop. Total gDNA from each isolate was diluted to 20 ng/μL (<500 ng total in 26 μL) confirmed by fluorometric quantification using a Qubit fluorometer and dsDNA HS (High Sensitivity) Assay Kit (Thermo Fisher Scientific). Libraries were prepared using diluted gDNA with an Ultra II FS DNA Library Prep Kit for Illumina (New England Biolabs) and were sequenced using a MiSeq platform (Illumina Inc.) with a MiSeq Reagent Kit V3 (2 $\times$ 300 bp paired-end). Library quantification was assessed using a Qubit fluorometer, and fragment length distribution was assessed using an Agilent Bioanalyzer 2100 with the High Sensitivity DNA 1000 Kit (Agilent).

2.4. Genomic analysis

The quality of reads was assessed using FastQC v.0.11.5. Error correction was performed using BFC v.r181. A relaxed quality trim was performed using Trimmomatic v.0.36 before the genome was de novo assembled using SPAdes v.3.9.1. The quality of the subsequent assemblies was assessed using Bandage v.0.8.1 and QUAST v.4.3. All assemblies were annotated using Prokka v.1.11.

Antimicrobial resistance-encoding genes were identified using ResFinder 3.1 (version 2018-10-11). Plasmid replicon typing was performed using PlasmidFinder 1.3. Antimicrobial resistance genes and plasmid genes were queried using the ABRicate script (https://git.lumc.nl/bvhhornung/antibiotic-resistance-pipeline/tree/master/tools/abricate). Sequences from these databases were identified within the genomes of all isolates using BLAST+ v.2.5.0 and Biopython v.1.68. Mash was used to query the assemblies and to identify the closest related sequences from RefSeq. Multilocus sequence typing (MLST) and serotyping were performed in silico using the SRST2 tool.

3. Results

We encountered a major categorical error in meropenem AST results using VITEK® 2 that resulted in clinical failure. Initial testing by in-house VITEK® 2 with AST cards N297 and N254 reported meropenem resistance in both isolates (*E. cloacae* complex meropenem MIC $\geq$ 16 mg/L and *K. oxytoca* MIC $\geq$ 16 mg/L) but susceptibility was subsequently demonstrated by Etest, VITEK® 2 with N351 in Hospital 2, and BMD testing in the reference laboratory (Table 1A,B). This major error led to a discontinuation of meropenem treatment in favour of a combination of tigecycline and amikacin. On the diagnosis of clinically relapsed infection, however, ertapenem was added to tigecycline and amikacin was continued. Tigecycline was then discontinued after a further 4 days. A sustained clinical improvement followed and ertapenem was continued for a total of 14 days.

Following the identification of high meropenem MICs, the isolates were tested phenotypically for CPE production. Although the

Table 1A
Carbapenem susceptibility test results of IMP carbapenemase-producing *Enterobacter cloacae* complex and *Klebsiella oxytoca*

Isolate	Carbapenem	MIC (mg/L)			
		VITEK® 2, in-house [a]	Etest, in-house	BMD, reference laboratory	VITEK® 2, Hospital 2 [a]
E. cloacae complex	Meropenem	≥16	2	2	8
	Ertapenem	≤0.5	0.5	0.25	≤0.5
K. oxytoca	Meropenem	≥16	1	0.5 [b]	4
	Ertapenem	≤0.5	0.125	1	0.25

MIC, minimum inhibitory concentration; BMD, broth microdilution.
[a] VITEK® 2 susceptibility testing was performed with cards N297 and N254 in-house and with card N351 in Hospital 2.
[b] The isolate failed meropenem BMD susceptibility testing and the MIC was determined by gradient testing.

Table 1B
Antimicrobial susceptibilities determined by VITEK® 2 of IMP carbapenemase-producing *Enterobacter cloacae* complex and *Klebsiella oxytoca*

Antimicrobial agent	MIC (mg/L) [susceptibility]	
	E. cloacae complex	*K. oxytoca*
AMP	≥32 [R]	≥32 [R]
AMC	≥32 [R]	≥32 [R]
TZP	≥16 [R]	8 [S]
CXM	≥64 [R]	≥64 [R]
CTX	≥64 [R]	8 [R]
CAZ	≥64 [R]	16 [R]
FEP	4 [I]	≤1.0 [I]
ETP	≤0.5 [S]	≤0.5 [S]
MEM	≥16 [R]	≥16 [R]
IPM	2.0 [I]	≥16 [R]
GEN	≥16 [R]	≥16 [R]
AMK	4 [S]	≤2.0 [S]
CIP	1 [I]	≤0.25 [S]
FOS	64 [R]	≥16 [S]
TMP	≥16 [R]	≥16 [R]
TIG	≤0.5 [S]	≤0.5 [S]
ATM	≥64 [R]	16 [R]
COL	≤0.5 [S]	≤0.5 [S]
CHL	16 [R]	≤2.0 [S]
SXT	≥32 [R]	≥32 [R]

MIC, minimum inhibitory concentration; AMP, ampicillin; AMC, amoxicillin/clavulanate; TZP, piperacillin/tazobactam; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; ETP, ertapenem; MEM, meropenem; IPM, imipenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin; FOS, fosfomycin; TMP, trimethoprim; TIG, tigecycline; ATM, aztreonam, COL, colistin; CHL, chloramphenicol; SXT, trimethoprim/sulfamethoxazole; S, susceptible; I, intermediate; R, resistant.

modified Hodge test was positive, the combination disk test was only suggestive but not conclusive for MBL production. PCR performed in the reference laboratory detected the presence of an IMP carbapenemase gene both in the *Klebsiella* and *Enterobacter* strains. Subsequent WGS identified this as a $bla_{\text{IMP-4}}$ gene that was associated with an integron (Fig. 1), but since we performed short-read sequencing we were unable to resolve the full integron. This IMP-4 encoding integron has high BLASTN identity to a class 1 integron found in nosocomial *K. pneumoniae* [7].

In addition, while MALDI-TOF/MS identified the *Enterobacter* isolate as *E. cloacae* complex, WGS revealed *E. hormaechei* subsp. *steigerwaltii* C309/ST110 as the closest sequence match for the *Enterobacter* isolate. In silico MLST identified *K. oxytoca* as ST50.

Sequencing analysis also revealed a similar resistance profile in both isolates, which included $bla_{\text{TEM1-B}}$ and $bla_{\text{OXA-1}}$ β-lactamase genes, *aac(6′)-Ib* gene encoding aminoglycoside acetyltransferase, *oqxB* multidrug efflux pump gene, *qnrB2_1* plasmid-mediated quinolone resistance gene, *catA* and *catB* chloramphenicol resistance determinants, and *tetD* and *sul1_5* tetracycline and sulfonamide resistance genes (Supplementary Table S1).

Plasmid replicon analysis identified IncHI1 and IncFII sequences both from the *E. hormaechei* and *K. oxytoca* isolates. As both strains shared the $bla_{\text{IMP-4}}$ gene together with several other antimicrobial resistance genes, a common plasmid host to the integron was likely transmitted between these isolates.

4. Discussion and conclusions

The finding of discrepant meropenem MICs found between in-house VITEK® 2, Etest and BMD AST on both isolates is concerning.

The influence of storage on phenotypic expression of resistance has been reported. It is recognised that resistance determinants such as plasmid-borne β-lactamases can be lost and MIC values can change on isolate storage [8]. However, it is unclear whether changes in resistance phenotype can occur on short-term storage and in the absence of freezing, as experienced in this study.

Differences in VITEK® 2 carbapenem MICs observed in the two hospitals could relate to different VITEK® 2 cards used in the laboratories at the time. Overestimation of carbapenem resistance in Enterobacterales by VITEK® 2 in comparison with BMD has been reported. Bobenchick et al. described finding carbapenem MICs >2 $\log_2$ dilutions higher than the reference method BMD in 1.7% of isolates of Enterobacterales tested [9]. Higher ertapenem MICs from VITEK® 2 compared with Etest and agar dilution in *E. cloacae* have also been described [10]; and in the setting of KPC-producing *K. pneumoniae*, meropenem Etest MICs were found to be in greater agreement with BMD relative to MICs obtained by VITEK® 2 (85% vs. 23%) [11].

Rapid and accurate microbiological identification of CPE and the underlying enzyme type is crucial both for clinical and infection control purposes.

Among commercially available phenotypic detection methods for the detection of IMP carbapenemases, carbapenem inactivation methods report high sensitivity and specificity, whereas immunochromatographic assays can miss some IMP producers [12]. The use of molecular methods for CPE detection is commonplace, however reliable detection of IMP producers is challenging owing to the range of mutations that give rise to a large number of gene variants. The commercially available molecular assay Carba-R (Cepheid) had a sensitivity of 44.4% [95% confidence interval (CI) 18.9–73.3%] for the detection of IMP enzymes overall, detecting IMP-1, -4 and -28 but not the other IMP variants included in the study, i.e. IMP-8, -13, -14, -22 and -50 [12]. It is important for clinical microbiology laboratories using this assay to be aware of the potential for false-negative results for IMP enzymes arising from failure to detect variants other than IMP-1. While IMP-producing Enterobacterales are uncommon in many parts of Europe, *E. hormaechei* carrying either VIM or IMP genes were recently reported to be endemic in Poland [13]. Furthermore, plasmid sequences IncHI1 and IncFII, which were identified from both our isolates by replicon analysis, have been associated with hospital outbreaks of CPE [3]. The isolates in our case, which were not related to each other yet shared the same plasmid sequences,

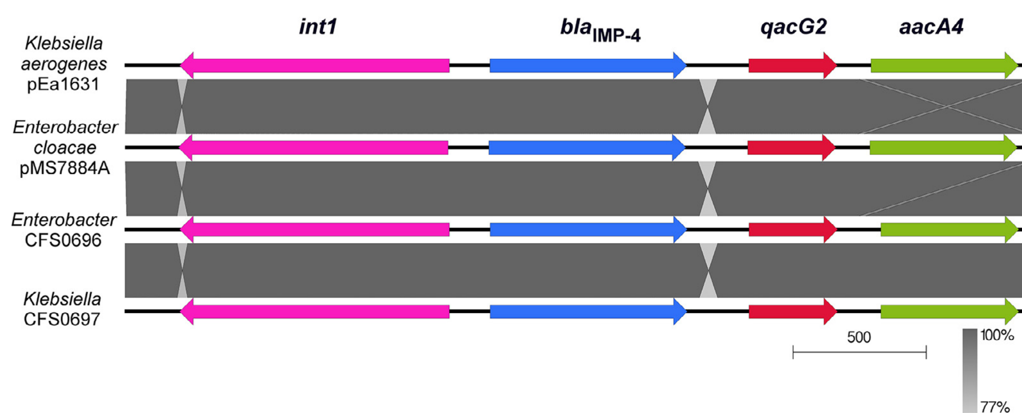

Fig. 1. Class 1 integrons in *Enterobacter* spp. (CFS0696) and *Klebsiella oxytoca* (CFS0697) are identical to the In*809* integron found in plasmids pEa1631 (*Klebsiella aerogenes*) and pMS884A (*Enterobacter hormaechei*).

highlight the substantial mobility of plasmids carrying bla_{IMP-4} and its ability to spread between various Enterobacterales species.

IMP-4-producing Enterobacterales have been reported in Australia, associated with patients who have travelled abroad but were not correlated with particular sequence types [3].

Few descriptive and epidemiological studies to date have characterised the plasmids or other mobile genetic elements associated with respective CPE types. Recent WGS studies have provided detailed analysis on transferrable plasmids and integrons associated with the spread of CPE, including bla_{IMP} genes [14].

IMP carbapenemases are unusual in Ireland and our patient had no relevant travel history. The mortality rate associated with IMP-producing *E. cloacae* complex bloodstream infections is reported to be as high as 40%, making the institution of early and effective treatment crucial [15].

Evidence guiding treatment of IMP-CPE infections arises mostly from case reports, as no large prospective or retrospective study has been performed looking specifically at this type of carbapenemase. Nakakura et al. reported on two patients with IMP-6 bloodstream infections treated successfully with amikacin and high-dose prolonged-infusion meropenem (2 g infused over 3 h twice a day) [16]. A further case report of a patient with mixed IMP-8 *K. oxytoca* and *Staphylococcus epidermidis* bloodstream infection described successful treatment with high-dose fosfomycin, amikacin and vancomycin [17]. The use of combination therapy has not been specifically studied in relation to IMP-CPE, but there is some evidence that it improves outcomes in patients with serious or invasive CPE infections.

A large retrospective cohort study of patients with CPE bloodstream infections by Gutiérrez-Gutiérrez et al. described a lower 30-day mortality in high-risk patients treated with two or more active agents in comparison with those treated with monotherapy [30/63 (48%) vs. 64/103 (62%); adjusted hazard ratio = 0.56, 95% CI 0.34–0.91, $P = 0.02$] [18].

The recent British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association (BSAC/HIS/BIA) Joint Working Party guidance on the treatment of infections caused by multidrug-resistant Gram-negative bacteria advises a combination of colistin and meropenem for the treatment of susceptible KPC-producing *Klebsiella* spp. infections when the meropenem MIC is ≤8 mg/L, and higher-dose meropenem administered by prolonged infusion to be considered when the MIC is between 8 mg/L and 32 mg/L. In the setting of infections caused by strains harbouring carbapenemases other than KPC, or meropenem-resistant KPC strains, they recommend the use of colistin with an aminoglycoside or tigecycline [5]. Tigecycline is primarily excreted unchanged in the faeces, and as low drug levels are found in urine its use in the treatment of urinary tract infections is subject to debate [6]. This may explain why tigecycline failed to produce a sustained clinical response in our case of urinary tract-derived bloodstream infection, even though it was administered in combination with an aminoglycoside antibiotic.

Another recent review recommends treatment with ceftazidime/avibactam or meropenem with either colistin, tigecycline or fosfomycin depending on AST results for isolates with meropenem MIC ≤ 8 mg/L. They recommend colistin and either fosfomycin and/or an aminoglycoside when the meropenem MIC is >8 mg/L [6]. Interestingly, apart from KPC, neither review makes specific recommendations in relation to infections caused by other subtypes of CPE. This highlights the lack of evidence and clinical studies on the management of genotypically distinct CPE types and further reinforces the need for urgent research in this area. Successful management of MBLs was achieved with the combination of aztreonam and ceftazidime/avibactam in two patients with NDM-1 infections [19].

Cefiderocol is a novel siderophore cephalosporin stable to hydrolysis both by serine β-lactamases and MBLs and may prove a much needed new agent in the management of infections caused by MBL-producing CPE [20].

In relation to our case, specific evidence on the management of IMP-CPE has not been published. On examination of the systematic reviews already conducted, few if any include IMP-producing isolates. In the absence of specific guidance in relation to the treatment of infections caused by IMP carbapenemases, treatment decisions must be based on the results of in vitro susceptibility testing in conjunction with recommendations and guidelines derived from the study of infections with other CPE subtypes.

In conclusion, we caution clinicians regarding the interpretation of carbapenem MIC results using VITEK® 2 alone and demonstrate that testing with a second method is warranted in cases where high meropenem MICs are obtained. WGS analysis showed that a similar resistome was shared by both isolates with IMP-4 and shared plasmid replicons indicating a common plasmid host to the integron was likely responsible for the observed resistance in these isolates. Our patient was successfully treated with ertapenem and amikacin, but our review highlights the lack of specific evidence in relation to IMP infections. This reinforces the need for future studies to investigate optimum treatment strategies for CPE infections expressing rarer carbapenemases such as IMP.

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Competing interests

None declared.

Ethical approval

Written patient consent was obtained to write up this case.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2021.09.010.

References

[1] Osano E, Arakawa Y, Wacharotayankun R, Ohta M, Horii T, Ito H, et al. Molecular characterization of an enterobacterial metallo β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob Agents Chemother 1994;38:71–8.
[2] Sidjabat HE, Townell N, Nimmo GR, George NM, Robson J, Vohra R, et al. Dominance of IMP-4-producing *Enterobacter cloacae* among carbapenemase-producing Enterobacteriaceae in Australia. Antimicrob Agents Chemother 2015;59:4059–66.
[3] Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL, Koraqi A, et al. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill 2015;20. doi:10.2807/1560-7917.ES.2015.20.45.30062.
[4] National Carbapenemase Producing Enterobacterales Reference Laboratory (NCPERL). *NCPERLS annual report 2017. National Carbapenemase Producing Enterobacterales (CPE) Reference Laboratory Service.* https://saolta.ie/sites/default/files/publications/NCPERLS%20ANNUAL%20REPORT%202017%20final.pdf [accessed 10 November 2021] 2021.
[5] Hawkey PM, Warren RE, Livermore DM, McNulty CAM, Enoch DA, Otter JA, et al. Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party. J Antimicrob Chemother 2018;73(Suppl 3):iii2–ii78.
[6] Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, Pascual A. Treatment of infections caused by extended-spectrum-β-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. Clin Microbiol Rev 2018;31 e00079-17.
[7] Gorrie CL, Mirceta M, Wick RR, Judd LM, Wyres KL, Thomson NR, et al. Antimicrobial-resistant *Klebsiella pneumoniae* carriage and infection in specialized geriatric care wards linked to acquisition in the referring hospital. Clin Infect Dis 2018;67:161–70.
[8] Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, et al. CLSI Methods Development and Standardization Working Group best practices for evaluation of antimicrobial susceptibility tests. J Clin Microbiol 2018;56 e01934-17.
[9] Bobenchik AM, Deak E, Hindler JA, Charlton CL, Humphries RM. Performance of Vitek 2 for antimicrobial susceptibility testing of Enterobacteriaceae with Vitek 2 (2009 FDA) and 2014 CLSI breakpoints. J Clin Microbiol 2015;53:816–23.
[10] Pailhoriès H, Cassisa V, Lamoureux C, Chesnay A, Lebreton C, Lemarié C, et al. Discordance in the minimal inhibitory concentrations of ertapenem for *Enterobacter cloacae*: VITEK 2 system versus Etest and agar dilution methods. Int J Infect Dis 2014;18:94–6.
[11] Lat A, Clock SA, Wu F, Whittier S, Della-Latta P, Fauntleroy K, et al. Comparison of polymyxin B, tigecycline, cefepime, and meropenem MICs for KPC-producing *Klebsiella pneumoniae* by broth microdilution, VITEK 2, and Etest. J Clin Microbiol 2011;49:1795–8.
[12] 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 1286.e9–15.
[13] Izdebski R, Baraniak A, Żabicka D, Sękowska A, Gospodarek-Komkowska E, Hryniewicz W, et al. VIM/IMP carbapenemase-producing Enterobacteriaceae in Poland: epidemic *Enterobacter hormaechei* and *Klebsiella oxytoca* lineages. J Antimicrob Chemother 2018;73:2675–81.
[14] Matsumura Y, Peirano G, Bradford PA, Motyl MR, DeVinney R, Pitout JDD. Genomic characterization of IMP and VIM carbapenemase-encoding transferable plasmids of Enterobacteriaceae. J Antimicrob Chemother 2018;73:3034–8.
[15] Hayakawa K, Miyoshi-Akiyama T, Kirikae T, Nagamatsu M, Shimada K, Mezaki K, et al. Molecular and epidemiological characterization of IMP-type metallo-β-lactamase-producing *Enterobacter cloacae* in a large tertiary care hospital in Japan. Antimicrob Agents Chemother 2014;58:3441–50.
[16] Nakakura I, Ogawa Y, Sakakura K, Imanishi K, Hirota K, Shimatani Y, et al. IMP-6 carbapenemase-producing Enterobacteriaceae bacteremia successfully treated with amikacin–meropenem in two patients. Pharmacotherapy 2017;37:e96–102.
[17] Vergara-López S, Carmen Domínguez M, Carmen Conejo M, Pascual A, Rodríguez-Bano J. Prolonged treatment with large doses of fosfomycin plus vancomycin and amikacin in a case of bacteraemia due to methicillin-resistant *Staphylococcus epidermidis* and IMP-8 metallo-β-lactamase-producing *Klebsiella oxytoca*. J Antimicrob Chemother 2015;70:313–15.
[18] Gutiérrez-Gutiérrez B, Salamanca E, de Cueto M, Hsueh PR, Viale P, Paño–Pardo JR, et al. Effect of appropriate combination therapy on mortality of patients with bloodstream infections due to carbapenemase-producing Enterobacteriaceae (INCREMENT): a retrospective cohort study. Lancet Infect Dis 2017;17:726–34.
[19] Davido B, Fellous L, Lawrence C, Maxime V, Rottman M, Dinha A. Ceftazidime–avibactam and aztreonam, an interesting strategy to overcome β-lactam resistance conferred by metallo-β-lactamases in Enterobacteriaceae and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2017;61 e01008-17.
[20] Sato T, Yamawaki K. Cefiderocol: discovery, chemistry, and in vivo profiles of a novel siderophore cephalosporin. Clin Infect Dis 2019;69(Suppl 7):S538–43.