Annals of Clinical Microbiology and Antimicrobials

BRIEF REPORT

Open Access

Activity of imipenem/relebactam against KPC-producing Klebsiella pneumoniae and the possible role of Ompk36 mutation in determining resistance: an Italian retrospective analysis

Emanuele Palomba[1,10*], Agnese Comelli[2], Francesca Saluzzo[3,4], Federico Di Marco[3], Elisa Matarazzo[5], Noemi Lo Re[5,6], Alessandra Bielli[5], Chiara Silvia Vismara[5], Antonio Muscatello[2], Marianna Rossi[7], Daniela Maria Cirillo[3], Alessandra Bandera[2,8] and Andrea Gori[1,9,10]

Abstract

Background Antimicrobial resistance in *Enterobacterales* represents a substantial threat in modern clinical practice and the collection of data on the efficacy of new molecules is of paramount importance. Our study aimed to analyse the in vitro activity of imipenem/cilastatin/relebactam (IMI/REL) against KPC-producing *Klebsiella pneumoniae* (KPC-Kp) and investigate the genetic determinants of resistance to this agent.

Methods A total of 603 KPC-Kp strains, which were randomly collected during a multicentre study in northern Italy in the period 2016–2018, were analysed retrospectively. Antibiotic susceptibility testing was performed using a commercial broth microdilution. IMI-REL-resistant KPC-Kp strains were further analysed by whole genome sequencing to identify resistance determinants.

Results Ninety-eight percent of KPC-Kp (591/603) showed in vitro susceptibility to IMI/REL, with a minimum inhibitory concentration below the EUCAST cut-off. Different mutations in OmpK36 were found in all 12 IMI/REL-resistant strains, which belonged to MLST STs 258 (3 isolates), 307 (8 isolates) and 512 (1 isolate), but no clonal relatedness was detected by the minimum spanning tree analysis, except for 2 strains isolated in the same hospital. Equal distribution of bla_{KPC-2} (6/12) and bla_{KPC-3} (6/12) was found, and in 11 isolates the presence of genetic variants associated with the production of beta-lactamases was also identified. KPC-Kp resistant to IMI/REL retained susceptibility to meropenem/vaborbactam (MVB, 12/12, 100%) and ceftazidime/avibactam (CZA, 11/12, 91.7%). Only one strain of 603 was resistant to either MVB and CZA but susceptible to IMI/REL with a MIC of 2 mg/L; 4/603 (0.7%) were resistant to CZA but susceptible to IMI/REL and MVB.

*Correspondence:
Emanuele Palomba
palomba.emanuele@asst-fbf-sacco.it

Full list of author information is available at the end of the article

Conclusions IMI/REL showed good in vitro activity against the KPC-Kp strains analysed. All the IMI/REL-resistant strains displayed a mutation in porin OmpK36 and produced carbapenemases, with KPC-2 and KPC-3 being equally distributed. MVB and CZA maintained good activity against IMI/REL resistant isolates.

Keywords Imipenem/relebactam, KPC, Carbapenem-resistant, Klebsiella pneumoniae

Introduction

Antimicrobial resistance in *Klebsiella pneumoniae* is an increasing threat to public health. Recent European surveillance data show a continuing upward trend in carbapenem-resistant strains, with even more worrying reports from southern countries such as Italy, where up to one in four isolates show this susceptibility pattern [1]. Resistance to carbapenems is often associated with the production of carbapenemases, of which *Klebsiella pneumoniae* carbapenemases (KPC) are the most common, accounting for more than 80% of resistant isolates retrieved from Italian bloodstream infections [2]. Furthermore, the loss or modification of key porin channels in the outer cell membrane, such as OmpK35 and OmpK36, can lead to multidrug resistance. This occurs by reducing the inner pore diameter, which restricts the uptake of various classes of antibiotics, presenting a significant challenge for clinicians [3].

Novel beta-lactam-beta-lactamases-inhibitor combinations (BLBLIC) represent a valuable option for the management of infections caused by these pathogens. Relebactam (REL) is a novel beta-lactamase inhibitor capable of restoring the susceptibility of bacteria producing class A carbapenemases (e.g. KPC) and class C cephalosporinases (e.g. AmpC) to imipenem-cilastatin (IMI) [4]. IMI/REL is approved in the US and Europe for the treatment of hospital-acquired and ventilator-associated pneumonia and other Gram-negative infections with limited treatment options, including complicated urinary tract infections and intra-abdominal infections. It has become a viable therapeutic option in Italy since 2021, when it was first licensed.

Given the limited Italian data available on the activity of IMI/REL against KPC-producing *K. pneumoniae* (KPC-Kp) and the mechanisms underlying resistance, our study aimed to investigate the in vitro susceptibility of carbapenem-resistant strains from samples collected in a multicentre study enrolling patients in a northern Italian region. Additionally, whole genome sequencing (WGS) was used to analyse IMI/REL-resistant KPC-Kp to identify genetic determinants possibly related with elevated IMI/REL minimum inhibitory concentrations (MICs).

Methods

Bacterial isolates

KPC-Kp isolates were randomly selected from strains collected in a multicentre cohort study enrolling patients from June 2016 to April 2018. It included adult patients hospitalised in 15 institutions in Lombardy that had at least one positive KPC-Kp isolate during their hospital stay [5].

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using a commercial broth microdilution method (Sensititre™: EUMDRXXF; Thermo Fisher Diagnostics, USA). The molecules included in the EUMDRXXF panel were: aztreonam, colistin, imipenem, cefepime, amikacin, cefiderocol, meropenem/vaborbactam, imipenem/relebactam, ceftazidime/avibactam, eravacycline, ceftalozane/tazobactam, piperacillin/tazobactam, tobramycin, fosfomycin + glucose-6-phosphate, tigecycline. All panels were evaluated manually using the last EUCAST guidelines, version 12.0 [6]. Strains with MIC ≥ 2 mg/L were retested with the same method.

Analysis of genetic determinants

IMI-REL-resistant KPC-Kp were further analysed by WGS performed on Illumina NextSeq 500 platform with paired-end Nextera XT library. Sequencing data was analysed using the Snippy (v4.6) Audio Volume Mute pipeline, using the *Klebsiella pneumoniae* annotated reference genome from NCBI (Accession code NC_016845.1). Antibiotic resistance genes were identified using the NCBI AMRfinderPlus software (v3.11) [7] and kleborate (v3.1) [8]. MLST typing was performed using the software mlst (v2.23). Virulence determinants and Plasmid identification was performed using Abricate (v1.0.1) with the respective reference databases VFDB [9] and Plasmidfinder [10].

Minimum spanning tree analysis

Sequencing data was assembled with a custom pipeline including unicycler (v0.5.0) [11] and multiple sequence alignment of alleles was performed with kSNP (v3.1) [12]. Minimum spanning tree was computed using Grapetree (v2.2) [13]. Isolates with genetic distances lower than 21 SNPs were considered as clonally related.

Statistical analysis

Downstream analyses were performed in R, assuming a significant level at $p < 0.05$ applying Bonferroni correction for multiple testing. The occurrence of OmpK36 and OmpK35 aminoacidic mutations among the genomes of

the isolates was compared with phenotypic profile performing Fisher exact test for each mutation.

Data availability

Sequencing data was uploaded on NCBI with accession code PRJNA1162767. The genome sequences accession numbers are presented in Supplementary Table 1.

Results

In vitro susceptibility to IMI/REL was analysed for a total of 603 KPC-Kp isolates. More than 97% of KPC-Kp (591/603, 98%) showed a MIC ≤ 2 mg/L, while 12 isolates (12/603, 2%) had a MIC above the susceptibility threshold. The MICs distribution for IMI/REL is depicted in Fig. 1a.

Susceptibility to other antimicrobials was also tested for the same isolates. Colistin was active in 84% of KPC-Kp (507/603, 84.1%), while approximately 99% of the strains were susceptible to MVB and CZA (602/603, 99.9% and 597/603, 99%, respectively, Fig. 1b and c).

IMI/REL was active against 5 of the 6 KPC-Kp strains resistant to at least one of the new BLBLIC, specifically: 1/5 resistant to MVB and CZA and 4/5 resistant to CZA but susceptible to MVB (Supplementary Table 2).

Table 1 summarises the characteristics of the 12 IMI/REL-resistant strains.

Different mutations in *ompK36* were found in all 12 IMI/REL-resistant strains. All the identified mutations, are summarized in Table 2. The analysis of correlation with resistance was performed against all the mutations in *ompk36* identified in the dataset in analysis. Only one mutation in *ompk36* appear to be significantly correlated with resistance (Asp357fs; p value = 0,008372). No mutation with significant correlation with resistance was found for *ompk35* and *ompk37*.

The minimum spanning tree analysis demonstrated that the IMI/REL resistant strains were not part of the same clone, except for 2 strains, isolated in the same hospital, that resulted related (7 SNPs distance).

All IMI/REL-resistant KPC-Kp maintained susceptibility to at least one new BLBLIC tested, with MVB being the most active (12/12, 100%), followed by CZA (11/12, 91.7%).

Discussion

In our study analysing 603 KPC-Kp from a multicentric Italian cohort, 98% of the isolates were susceptible to IMI/REL. Within the IMI/REL-resistant KPC-Kp, all strains displayed a mutation in porin OmpK36, whereas all isolates produced carbapenemases, with KPC-2 and KPC-3 being equally distributed. Other BLBLICs, namely MVB and CZA, maintained activity in IMI/REL-resistant

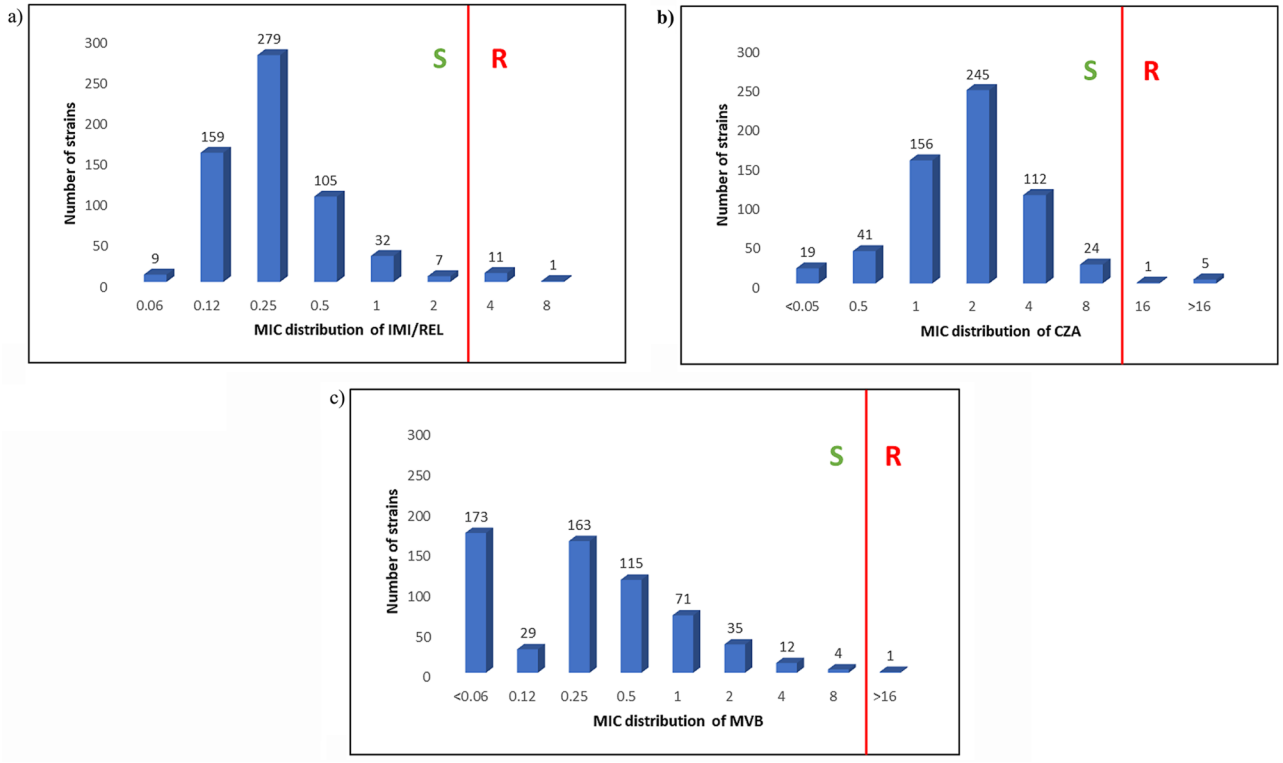

Fig. 1 Distribution of minimum inhibitory concentration for imipenem/cilastatin/relebactam (**a**), ceftazidime/avibactam (**b**) and meropenem/vaborbactam (**c**) within the 603 KPC-producing *Klebsiella pneumoniae* strains analysed in the study

Table 1 Characteristics of the 12 KPC-producing *Klebsiella pneumoniae* strains resistant to Imipenem/cilastatin/relebactam

ID	Sequence type (ST)	MIC value for specific antibiotics (S/I/*R*)						Carbapenem resistance determinants	Beta-lactams resistant determinants
		IMI/REL (0.06/4–8/4)	IMI (1–8)	MEM (0.12-16)	MVB (0.06/8–16/8)	C/T (0.25/4–8/4)	CZA (0.25/4–16/4)		
I091	512	4	> 8	> 16	8	> 8	16	bla_{KPC-3}	bla_{SHV-11}; bla_{TEM-1},bla_{OXA-1}
G007	307	4	> 8	> 16	2	> 8	8	bla_{KPC-3}	bla_{SHV-28}; bla_{TEM-1},bla_{OXA-1}
G003	307	4	> 8	> 16	4	> 8	4	bla_{KPC-3}	bla_{TEM},bla_{OXA-1}
E195	258	4	> 8	> 16	2	> 8	1	bla_{KPC-2}	bla_{SHV-12}
H117	307	4	> 8	> 16	4	> 8	2	bla_{KPC-2}	bla_{SHV-28},bla_{OXA-1}
A157/2	307	4	> 8	> 16	2	> 8	2	bla_{KPC-2}	bla_{SHV-28},bla_{OXA-1}
G019	307	4	> 8	> 16	1	> 8	2	bla_{KPC-3}	bla_{SHV-28}; bla_{TEM-1},bla_{OXA-1}
G021	307	4	> 8	> 16	1	> 8	4	bla_{KPC-3}	bla_{SHV-28}; bla_{TEM-1},bla_{OXA-1}
G025	258	4	> 8	> 16	4	> 8	2	bla_{KPC-2}	bla_{SHV-12}
C053	258	4	> 8	> 16	4	> 8	2	bla_{KPC-2}	bla_{SHV-12}
B040	307	4	> 8	> 16	2	> 8	2	bla_{KPC-2}	bla_{SHV-28}; $bla_{TEM-1;}$ bla_{OXA-1}
B100	307	8	> 8	> 16	4	> 8	4	bla_{KPC-3}	bla_{SHV-28}; bla_{TEM-1},bla_{OXA}

MIC: minimum inhibitory concentration, S: susceptibile, I: increased exposure; R: resistant; IMI/REL: imipenem/cilastatin/relebactam; IMI: imipenem; MEM: meropenem; MVB: meropenem/vaborbactam; C/T: ceftolozane/tazobactam; CZA: ceftazidime/avibactam. Resistance determinants are reported according to the NCBI AMRFinderPlus categorization at the time of analysis. No mutations in *OmpK36* were identified. Complete analysis of resistance, virulome and identified plasmids is reported in the Supplementary materials

Table 2 Protein mutations identified in the strains resistant to to Imipenem/cilastatin/relebactam. Correlation analysis with resistance was performed with all the Ompk36 mutations identified in all the Kp-KPC dataset (603 samples)

Protein	Mutation	*p* value
Ompk36	p.Asp357fs	0,008372
Ompk36	p.Asp135_Thr136insGlyAsp	0,071530
Ompk36	p.Tyr201fs	0,457700
Ompk36	p.Trp125*	0,906200
Ompk36	p.Ala183_Leu184delinsThr	1
Ompk36	p.Asn221His	1
Ompk36	p.AspAsnSer344GluAsnAsp	1
Ompk36	p.HisAsn349ArgArg	1
Ompk36	p.Ile315Leu	1
Ompk36	p.Leu307Ile	1
Ompk36	p.Thr192Gly	1
Ompk36	p.Tyr201Phe	1

strains, while IMI/REL was active against 5/6 isolates resistant to MVB and/or CZA.

Carbapenem resistance in KPC-Kp is primarily due to KPC enzymes, though alterations or loss of key porin channels in the outer membrane can further elevate resistance levels. The main porins characterising *K. pneumoniae* are OmpK35 and OmpK36 [14]. With regard to KPC-Kp resistance to IMI/REL, mutations in *ompK36* have already been described as an important determinant of increased MICs, with Rogers et al. reporting an *ompK36* IS5 mutation in up to one third of KPC-Kp strains that were not susceptible to IMI/REL, while the novel BLBLIC was active on all *ompK36* wild-type isolates tested [3, 15].

Available literature data show good in vitro activity of IMI/REL against KPC-Kp strains. Delgado-Valverde et al. reported susceptibility of 98.5% of 264 KPC-3 producing KPC-Kp analysed, highlighting a slightly better efficacy than the comparator, CZA. Notably, their analysis of mutations in penicillin binding proteins and porin genes showed no differences between isolates susceptible and resistant to IMI/REL, with all isolates presenting wild type *ompK36*. Mutated *ompK35* and *ompK37* were detected in two isolates resistant to IMI/REL, however, these same mutations were also present in susceptible isolates [16]. Similarly, in a Greek collection of 266 carbapenem-resistant *K. pneumoniae*, three-quarter of which KPC-producing, IMI/REL displayed activity in 98.5% of KPC-Kp strains [17]. Our analysis seems to be consistent with these data, with a similar overall susceptibility rate. Interestingly, analysis of IMI/REL-resistant strains showed production of both KPC-2 and KPC-3 carbapenemases, equally distributed within these isolates.

Useful insights into the genetic determinants of IMI/REL resistance were highlighted in a recent report analysing KPC-Kp strains resistant to MVB and CZA. This study showed activity of IMI/REL in most isolates (11/13, 84.6%), and cross-resistance in two of six KPC-Kp resistant to both CZA and MVB (33.3%). The IMI/REL-resistant strains showed porin OmpK 35 and 36 mutations and increased bla_{KPC} copy number with different variants ($bla_{\text{KPC-3}}$, heteroresistant $bla_{\text{KPC-53}}$) [18].

These results, in line with our analysis, suggest that porin Ompk36 mutations play a pivotal role in KPC-Kp resistance to IMI/REL, independent of carbapenemases and beta-lactamases production. While no mutation

with significant correlation with resistance was found for porins Ompk35 and Ompk37.

Some limitations of our study should be acknowledged. First, The strains analysed were collected between 2016 and 2018, which may make them less representative of the current epidemiology and molecular characteristics of KPC-Kp strains. Furthermore, given the lack of therapeutic options at that time, no conclusions could be drawn about the effect on susceptibility after exposure to novel BLBLIC, and in particular to IMI/REL that was not available for clinicians during the study period. Moreover, our analysis did not include the number of copies of bla_{KPC} when expressed, and it has been noted that the number of copies can influence drug susceptibility. Finally, the study focused on the in vitro activity of IMI/REL and did not evaluate the clinical characteristics, therapeutic management and outcomes of infections caused by these microorganisms, as these variables fell outside the scope of the analysis. However, the samples were collected in an area endemic for carbapenem-resistant pathogens, and the in vitro susceptibility of such heterogeneous samples may provide valuable insights with implications for clinical management. Another strength of our analysis was the use of WGS, which allowed full characterisation of IMI/REL-resistant strains and provided information on porin mutations along with evidence of production of carbapenemases and beta-lactamases.

In conclusion, an improved understanding of the resistance determinants in carbapenem-resistant bacteria is paramount to tailor the place in therapy of novel BLBLICs. The collection of in vitro susceptibility information is a first step in this endeavour, which needs to be strengthened by larger cohorts, taking into account regional and national ecologies. Furthermore, the characterisation of resistance to less widely used molecules such as IMI/REL is crucial to inform fast microbiology techniques and optimise their use in clinical practice and streamlined empiric antibiotic therapy. Our findings confirm that IMI/REL may be an alternative option in the management of infections caused by KPC-Kp strains, especially when the isolates are not susceptible to the recommended first-line agents (i.e. CZA).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-025-00792-w.

Supplementary Material 1

Acknowledgements

Preliminary data on this study were presented as oral e-poster at ESCMID Global 2024 in Barcelona, Spain.The authors wish to thank the KPC-Kp Study Group collaborators: Floriana Gona, Giovanni Lorenzin, Matteo Moro and Paolo Scarpellini (IRCCS San Raffaele Scientific Institute, Milan, Italy); Daniele Castelli, Paolo Bonfanti, Annalisa Cavallero (S. Gerardo de'Tintori Hospital, Monza, Italy); Maddalena Casana, Paola Morelli and Daria Pocaterra (Humanitas Clinical and Research Centre, Milan, Italy); Chiara Aldieri and Andrea Cona (San Paolo Hospital, Milan, Italy); Roberto Martegani, Gioconda Brigante, Antonella Carducci and Marco Franzetti (Busto Arsizio Hospital, Busto Arsizio, Italy); Paolo A. Grossi, Daniela Dalla Gasperina and C. Rovelli (University of Insubria, ASST Sette Laghi-Varese, Italy); Giuliano Rizzardini, Sara G. Rimoldi and Pietro Olivieri (ASST Fatebenefratelli Sacco, Milan, Italy); Paola Brambilla, Angelo Pan and Alessia Zoncada (Istituti Ospedalieri of Cremona, Cremona, Italy); Alessandro Pandolfo and Stefania Piconi (Ospedale A. Manzoni, Lecco, Italy); Evelyn Van Hauwermeiren, Francesco Castelli and Ester Pollastri (University of Brescia and ASST Spedali Civili, Brescia, Italy); Massimo Puoti, Cecilia Del Curto, Giovanna Travi and Chiara Silvia Vismara (ASST Grande Ospedale Metropolitano Niguarda, Milan); Gianni Gattuso and Silvia Garilli (Carlo Poma Hospital, Mantova, Italy); Chiara Cerri, Manuela Piazza and Angelo Regazzetti (Hospital of Lodi, Lodi, Italy); Milena Arghittu and Rosaria Maria Colombo (IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy), Francesco Petri (University of Milano Bicocca, Monza, Italy); Sara Bigoni, Franca Averara and Francesca Vailati (Papa Giovanni XXIII Hospital, Bergamo, Italy), Antonella D'Arminio Monforte, Mario Raviglione (University of Milan).

Author contributions

EP, AC, MR, DMC and AG conceived the research question and study design; FS, FDM, EM provided and analysed the data; EP and FS wrote the first draft; DMC, AB and AG supervised the study; all authors contributed to and revised the submitted version of the manuscript.

Funding

This study was partially supported by a research grant from Investigator-Initiated Studies Program of Merck Sharp & Dohme LLC. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme LLC. MSD provided financial support.

Data availability

Sequencing data was uploaded on NCBI with accession code PRJNA1162767. Sequences respective codes are reported in Supplementary Table 1.

Declarations

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Infectious Diseases, Luigi Sacco Hospital, Via G.B. Grassi 74, 20157 Milan, Italy
[2]Infectious Diseases Unit, Foundation IRCCS Ca'Granda Ospedale Maggiore Policlinico, Milan, Italy
[3]IRCCS San Raffaele Scientific Institute, Milan, Italy
[4]Vita-Salute San Raffaele University, Milan, Italy
[5]Clinical Microbiology Unit, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy
[6]Università Cattolica del Sacro Cuore, Rome, Italy
[7]Infectious Diseases Unit, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy
[8]Department of Pathophysiology and Transplantation, University of Milano, Milan, Italy
[9]Department of Biomedical and Clinical Sciences "L.Sacco", University of Milano, Milan, Italy
[10]Centre for Multidisciplinary Research in Health Science (MACH), University of Milano, Milan, Italy

Received: 22 July 2024 / Accepted: 27 March 2025
Published online: 13 April 2025

References

1. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual epidemiological report 2020. Stockholm: ECDC; 2022. https://doi.org/10.1136/vr.g2500.

2. Istituto Superiore di Sanità. CRE: National surveillance of bloodstream infections due to Carbapenem-Resistant enterobacterales. Data 2022. Rome: Istituto Superiore di Sanità; 2023.
3. Rogers TM, Kline EG, Griffith MP, Jones CE, Rubio AM, Squires KM, et al. Impact of ompk36 genotype and KPC subtype on the in vitro activity of Ceftazidime/avibactam, Imipenem/relebactam and Meropenem/vaborbactam against KPC-producing K. pneumoniae clinical isolates. JAC-Antimicrobial Resist. 2023;5. https://doi.org/10.1093/JACAMR/DLAD022.
4. Motsch J, De Oliveira CUM, Stus V, Kö Ksal I, Lyulko O, Boucher HW, et al. RESTORE-IMI 1: A multicenter, randomized, doubleblind trial comparing efficacy and safety of Imipenem/Relebactam vs colistin plus Imipenem in patients with Imipenem-nonsusceptible bacterial infections. Clin Infect Dis. 2020;70:1799–808. https://doi.org/10.1093/cid/ciz530.
5. Rossi M, Chatenoud L, Gona F, Sala I, Nattino G, D'Antonio A, et al. Characteristics and clinical implications of carbapenemase-producing Klebsiella pneumoniae colonization and infection, Italy. Emerg Infect Dis. 2021;27:1416–26. https://doi.org/10.3201/eid2705.203662.
6. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 14.0, 2024. http://www.eucast.org/clinical_breakpoints/
7. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, et al. AMRFinderPlus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep 2021. 2021;111:11:1–9. https://doi.org/10.1038/s41598-021-91456-0.
8. Lam MMC, Wick RR, Watts SC, Cerdeira LT, Wyres KL, Holt KE. A genomic surveillance framework and genotyping tool for Klebsiella pneumoniae and its related species complex. Nat Commun 2021 121. 2021;12:1–16. https://doi.org/10.1038/s41467-021-24448-3.
9. Chen L, Zheng D, Liu B, Yang J, Jin Q. VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. Nucleic Acids Res. 2016;44(D694–7). https://doi.org/10.1093/NAR/GKV1239.
10. Carattoli A, Zankari E, Garciá-Fernández A, Larsen MV, Lund O, Villa L, et al. In Silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895–903. https://doi.org/10.1128/AAC.02412-14/SUPPL_FILE/ZAC007143013SO1.PDF.
11. Wick RR, Judd LM, Gorrie CL, Holt KE, Unicycler. Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Comput Biol. 2017;13:e1005595. https://doi.org/10.1371/JOURNAL.PCBI.1005595.
12. Gardner SN, Slezak T, Hall BG. kSNP3.0: SNP detection and phylogenetic analysis of genomes without genome alignment or reference genome. Bioinformatics. 2015;31:2877–8. https://doi.org/10.1093/BIOINFORMATICS/BTV271.
13. Zhou Z, Alikhan NF, Sergeant MJ, Luhmann N, Vaz C, Francisco AP, et al. GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Res. 2018;28:1395–404. https://doi.org/10.1101/GR.232397.117.
14. Wong JLC, Romano M, Kerry LE, Kwong HS, Low WW, Brett SJ, et al. OmpK36-mediated carbapenem resistance attenuates ST258 Klebsiella pneumoniae in vivo. Nat Commun 2019. 2019;101:10:1–10. https://doi.org/10.1038/s41467-019-11756-y.
15. Findlay J, Rens C, Poirel L, Nordmann P. In vitro mechanisms of resistance development to Imipenem-Relebactam in KPC-Producing Klebsiella pneumoniae. Antimicrob Agents Chemother 2022:1–8.
16. Delgado-Valverde M, Portillo-Calderón I, Alcalde-Rico M, Conejo MC, Hidalgo C, del Toro Esperón C, et al. Activity of Imipenem/relebactam and comparators against KPC-producing Klebsiella pneumoniae and imipenem-resistant Pseudomonas aeruginosa. Eur J Clin Microbiol Infect Dis. 2024;43:445–57. https://doi.org/10.1007/S10096-023-04735-1/TABLES/4.
17. Maraki S, Mavromanolaki VE, Magkafouraki E, Moraitis P, Stafylaki D, Kasimati A, et al. Epidemiology and in vitro activity of ceftazidime–avibactam, meropenem–vaborbactam, imipenem–relebactam, Eravacycline, Plazomicin, and comparators against Greek carbapenemase-producing Klebsiella pneumoniae isolates. Infection. 2022;50:467–74. https://doi.org/10.1007/S15010-021-01735-1/TABLES/3.
18. Lombardo D, Ambretti S, Lazzarotto T, Gaibani P. In vitro activity of imipenem-relebactam against KPC-producing Klebsiella pneumoniae resistant to ceftazidime-avibactam and/or meropenem-vaborbactam. Clin Microbiol Infect. 2022;28:749–51. https://doi.org/10.1016/J.CMI.2022.01.025.

Publisher's note