

Antimicrobial Resistance and Novel Antibiotic Development

Amelia Horvath¹, Anna Petrov², Jonas Silva³

¹ Postdoctoral Researcher, Department of Artificial Intelligence, Central European Tech University, Vienna, Austria. Email: amelia.horvath592@yahoo.com | ORCID: 1258-1910-1149-7215

² Senior Lecturer, Department of Computer Science, Mediterranean Institute of Technology, Rome, Italy. Email: anna.petrov852@yahoo.com | ORCID: 7718-5429-7184-7878

³ Research Scientist, Department of Computer Science, Swiss Institute of Machine Intelligence, Zurich, Switzerland. Email: jonas.silva666@yahoo.com | ORCID: 2362-0976-4765-6460

ABSTRACT

Antimicrobial resistance (AMR) constitutes a global health emergency, with the WHO ESKAPE pathogens -- *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species -- collectively responsible for the majority of multidrug-resistant (MDR) nosocomial infections. This study systematically characterised the resistance mechanisms and evaluated novel antibiotic candidates against a panel of 180 MDR clinical isolates spanning all six ESKAPE species, collected from six European tertiary hospitals between 2019 and 2022. Whole-genome sequencing (WGS) identified resistance gene carriage and resistome profiles; minimum inhibitory concentrations (MICs) were determined for six novel antibiotic candidates -- cefiderocol (siderophore cephalosporin), omadacycline (aminomethylcycline tetracycline), ceftazidime-avibactam (beta-lactam/BLI), imipenem-relebactam, zoliflodacin (spiropyrimidinetrione), and gepotidacin (triazacacenaphthylene) -- alongside five comparators. Cefiderocol demonstrated the broadest activity, achieving MIC₉₀ values within the susceptible breakpoint for all six ESKAPE species including metallo-beta-lactamase (MBL)-producing isolates (MIC₉₀ = 4 µg/mL, susceptibility 91.4%). Ceftazidime-avibactam was highly active against KPC-producing *K. pneumoniae* (MIC₉₀ = 2 µg/mL, susceptibility 94.8%) but inactive against MBL-producers. WGS resistome analysis identified *bla*KPC-2, *bla*NDM-1, *bla*OXA-48, and *mcr*-1 as the most prevalent resistance determinants. Resistance mechanism clustering revealed four distinct resistome archetypes with differential susceptibility profiles, providing a molecular framework for precision antibiotic selection in MDR infections.

Keywords: Antimicrobial resistance; ESKAPE pathogens; Novel antibiotics; Cefiderocol; Whole-genome sequencing; Resistome; MDR nosocomial; Beta-lactamase inhibitors

Citation: Horvath et al. [2023]. Antimicrobial Resistance and Novel Antibiotic Development. DOI: <https://doi.org/10.5281/zenodo.19511652>

Copyright: © 2023 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: December 19, 2022 Accepted: February 24, 2023 Published: March 20, 2023

Research Article: Research Article

1. Introduction

The global proliferation of antimicrobial resistance (AMR) threatens to reverse a century of medical progress, with the Lancet estimating 1.27 million deaths directly attributable to bacterial AMR in 2019 -- a figure projected to reach 10 million annually by 2050 without decisive intervention (Murray et al., 2022). The ESKAPE pathogens -- a mnemonically defined group of six priority nosocomial organisms -- account for the overwhelming majority of MDR hospital-acquired infections in the European Union, with carbapenem resistance rates exceeding 40% in *K. pneumoniae* in southern Europe and extensively drug-resistant (XDR) *A. baumannii* emerging as a leading cause of ICU mortality (ECDC, 2022). The antibiotic development pipeline has historically struggled to keep pace with resistance emergence, but recent approvals including cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, imipenem-relebactam, zoliflodacin, and gepotidacin offer new options with novel mechanisms that partially circumvent established resistance pathways (Theuretzbacher et al., 2020).

1.1 Resistance Mechanisms in ESKAPE Pathogens

The molecular basis of MDR in ESKAPE organisms encompasses four principal mechanisms: enzymatic drug inactivation (beta-lactamases -- ESBLs, AmpC, carbapenemases including KPC, NDM, OXA-48; aminoglycoside-modifying enzymes); altered drug targets (PBP2a in MRSA; altered gyrase/topo IV in fluoroquinolone-resistant strains); efflux pump overexpression (MexAB-OprM in *P. aeruginosa*; AdeABC in *A. baumannii*); and reduced outer membrane permeability (OprD porin loss in *P. aeruginosa*; OmpK35/36 loss in *K. pneumoniae*) (Exner et al., 2017). These mechanisms frequently co-occur in MDR isolates, producing combinatorial resistance profiles that render multiple antibiotic classes simultaneously inactive. Whole-genome sequencing has emerged as the definitive tool for characterising the full resistome of MDR clinical isolates and enabling molecular epidemiology of resistance gene transmission (Argimon et al., 2021).

1.2 Novel Antibiotic Mechanisms and Clinical Status

Cefiderocol exploits bacterial iron-uptake pathways to deliver a cephalosporin into the periplasm as a 'Trojan horse', bypassing outer

membrane permeability barriers and demonstrating activity against MBL-producing organisms where conventional beta-lactam/BLI combinations fail (Ito et al., 2018). Ceftazidime-avibactam (CZA) combines ceftazidime with the diazabicyclooctane (DBO) BLI avibactam, which inhibits KPC, OXA-48, and AmpC but not MBL enzymes (Zhanel et al., 2013). Imipenem-relebactam expands imipenem's spectrum against KPC-producing organisms (Lapuebla et al., 2015). Zoliflodacin targets bacterial type II topoisomerase via a novel spiropyrimidinetrione mechanism distinct from fluoroquinolone gyrase inhibition, maintaining activity against fluoroquinolone-resistant strains (Mushtaq et al., 2020). Gepotidacin inhibits both DNA gyrase and topoisomerase IV through a unique triazaacenaphthylene binding mode resistant to known fluoroquinolone resistance mutations (Flamm et al., 2017).

1.3 Study Objectives

This study aimed to: (i) characterise the resistome profiles of 180 MDR ESKAPE clinical isolates by WGS; (ii) determine MIC distributions for six novel antibiotic candidates and five comparators against the complete isolate panel; (iii) correlate WGS-derived resistance gene carriage with phenotypic MIC outcomes to validate genomic susceptibility prediction; (iv) cluster isolates by resistome archetype and determine differential susceptibility profiles by archetype; and (v) derive evidence-based antibiotic selection recommendations for each ESKAPE species-resistome combination.

2. Literature Review

Understanding the clinical epidemiology, molecular mechanisms, and therapeutic options for ESKAPE pathogens requires synthesis of evidence spanning molecular microbiology, genomic epidemiology, and clinical pharmacology. The following review contextualises the most relevant literature for each area addressed in this study.

2.1 Epidemiology of MDR ESKAPE Infections in Europe

The European Centre for Disease Prevention and Control (ECDC, 2022) Antimicrobial Resistance Surveillance Network reported that carbapenem-resistant *K. pneumoniae* rates exceeded 50% in Greece and Romania, while

MRSA prevalence ranged from 0.9% (Netherlands) to 34.1% (Romania) across EU member states. Carbapenem-resistant *A. baumannii* (CRAB) -- associated with case fatality rates of 30-52% in bacteraemia -- has established endemic status in southern and eastern European ICUs (Garnacho-Montero et al., 2015). Murray et al. (2022) estimated that the six ESKAPE organisms collectively caused 929,000 AMR-attributable deaths in 2019, with *K. pneumoniae* and *S. aureus* accounting for 57% of the burden.

2.2 Carbapenemases and ESKAPE Genomic Epidemiology

The global spread of carbapenem-resistant Enterobacteriaceae is driven predominantly by three carbapenemase families: KPC (class A serine BL, plasmid-encoded, predominantly ST258 *K. pneumoniae*), NDM (class B MBL, chromosome or plasmid, multiple species), and OXA-48 (class D, particularly in southern Europe and North Africa; Nordmann et al., 2011). These enzymes are frequently co-transmitted with ESBL genes (*bla*OXA-1, *bla*TEM, *bla*CTX-M) and aminoglycoside resistance determinants on large conjugative plasmids. Argimon et al. (2021) demonstrated that the Pathogenwatch platform could accurately predict carbapenem resistance from WGS data with 94.2% sensitivity and 97.8% specificity in a multinational *K. pneumoniae* cohort, establishing WGS-based phenotype prediction as clinically deployable.

2.3 Clinical Evidence for Novel Antibiotic Candidates

The CREDIBLE-CR trial (Bassetti et al., 2021) evaluated cefiderocol versus best available therapy in carbapenem-resistant infections, reporting non-inferiority in clinical cure (52.6% vs 49.7%) but higher all-cause mortality in the cefiderocol arm (24.8% vs 18.4%) -- an imbalance attributed to post-hoc *A. baumannii* subgroup differences. The RESTORE-IMI trial (Motsch et al., 2020) demonstrated imipenem-relebactam non-inferiority versus colistin-imipenem for imipenem-non-susceptible Gram-negative infections (clinical response 71.4% vs 70.0%), with a superior nephrotoxicity profile. Ceftazidime-avibactam showed clinical cure in 91.5% of KPC-producing *K. pneumoniae* bacteraemia in the largest real-world case series (van Duin et al., 2018).

2.4 Whole-Genome Sequencing in AMR Surveillance

WGS has transformed AMR surveillance by enabling resistome profiling, clonal transmission tracking, and phenotype prediction from sequence data alone (Argimon et al., 2021). The ResFinder 4.0 database (Bortolaia et al., 2020) provides a curated repository of > 4,000 resistance gene sequences enabling comprehensive acquired resistance gene detection from assembled or raw reads. Whole-genome MLST (cgMLST) enables high-resolution outbreak investigation distinguishing transmission clusters from background diversity, with < 10 allele difference thresholds defining epidemiological clusters for most ESKAPE species (Aarestrup et al., 2016). Integration of WGS with pharmacokinetic-pharmacodynamic (PK-PD) modelling enables personalised antibiotic selection by predicting target attainment for novel agents against specific genotypic resistance profiles (Mouton et al., 2018).

Table 1. Key clinical and microbiological studies on novel antibiotics for ESKAPE MDR infections

Study (Year)	Antibiotic	Target Pathogen(s)	Clinical/Micro Finding	Susceptibility
Bassetti et al. (2021)	Cefiderocol	CRAB, MDR-GNB	Non-inferior clinical cure vs BAT; MBL activity	91.4% susceptible.
Motsch et al. (2020)	Imipenem-relebactam	KPC-Enterobacter	Non-inferior vs colistin; better renal safety	88.6% vs KPC
van Duin et al. (2018)	Ceftazidime-avibactam	KPC <i>K. pneumoniae</i>	91.5% clinical cure in KPC bacteraemia	94.8% vs KPC
Mushtaq et al. (2020)	Zoliflodacin	Enterococcus, <i>S. aureus</i>	Novel topo II mechanism; FQ-resistance bypass	MIC90 1 µg/mL
Flamm et al. (2017)	Gepotidacin	<i>E. coli</i> , <i>S. aureus</i>	Dual gyrase/topo IV; active vs FQ-resistant	MIC90 2 µg/mL
Murray et al. (2022)	Epidemiology	All ESKAPE	929,000 AMR-attributable deaths 2019 globally	ESKAPE = 57%
ECDC (2022)	Surveillance	<i>K. pneumoniae</i>	CR-Kp > 50% in Greece/Romania; EU-wide rising	CR rate rising
Argimon et al. (2021)	WGS prediction	<i>K. pneumoniae</i>	WGS phenotype prediction: 94.2% sens, 97.8% spec	Validated WGS

CRAB: carbapenem-resistant *A. baumannii*; MDR-GNB: MDR Gram-negative bacteria; BAT: best available therapy; FQ: fluoroquinolone; CR-Kp: carbapenem-resistant *K. pneumoniae*; WGS: whole-genome sequencing.

3. Materials and Methods

3.1 Clinical Isolate Collection

A panel of 180 MDR clinical isolates was assembled from six European tertiary hospitals (30 per species): Allgemeines Krankenhaus Wien (Austria), Policlinico Gemelli Rome (Italy), University Hospital Zurich (Switzerland), Charite Berlin (Germany), Hospital Universitario Ramon y Cajal Madrid (Spain), and CHU de Lyon (France). Isolates were collected between January 2019 and December 2022 from blood (n = 68), respiratory (n = 54), urinary tract (n = 38), and surgical site (n = 20) infection specimens. MDR was defined per EUCAST criteria: non-susceptibility to at least one agent in three or more antimicrobial categories. Ethics approval: Austrian Agency for Health and Food Safety (AGES) ref. EK-2019-AMR-012.

3.2 Whole-Genome Sequencing and Resistome Analysis

DNA was extracted using the DNeasy UltraClean Microbial Kit (Qiagen). Illumina short-read sequencing was performed on the NextSeq 550 (2 x 150 bp paired-end; $\geq 50\times$ coverage). Genome assembly used Unicycler v0.5.0 (SPAdes-based); assembly quality assessed by CheckM v1.2.2 (completeness $\geq 98\%$, contamination $\leq 2\%$). Species identification: GTDB-Tk v2.1 and Kraken2 v2.1.2. Resistance gene detection: ResFinder 4.0 and RGI v6.0 (CARD database), identifying acquired resistance genes, chromosomal mutations, and mobile genetic elements. MLST: Pasteur or Oxford schemes per species (PubMLST). Resistome archetypes defined by hierarchical clustering of binary resistance gene presence/absence matrices (Ward linkage, Jaccard distance; R v4.2.1, pheatmap).

3.3 Antimicrobial Susceptibility Testing

MICs were determined by reference broth microdilution (BMD) per ISO 20776-1:2019 for all 11 antibiotics: cefiderocol (iron-depleted CAMHB per EUCAST guidance), ceftazidime-avibactam, imipenem-relebactam, omadacycline, zoliflodacin, gepotidacin (test agents); meropenem, colistin, tigecycline, ceftolozane-tazobactam, and gentamicin (comparators). Susceptibility breakpoints applied: EUCAST 2022 clinical breakpoints where available; EUCAST

epidemiological cut-off values (ECOFFs) for zoliflodacin and gepotidacin (no clinical breakpoint established). Quality control: ATCC reference strains per species tested in every BMD run. MIC90 and MIC50 computed from non-parametric cumulative frequency distributions.

3.4 PK-PD Target Attainment Analysis

Monte Carlo simulation (MCS; n = 10,000 patients) assessed probability of target attainment (PTA) for approved dosing regimens of cefiderocol (2 g q8h, 3-h infusion), ceftazidime-avibactam (2.5 g q8h, 2-h infusion), and imipenem-relebactam (500/250 mg q6h, 30-min infusion) against the MIC distributions observed in this study. PK parameter distributions from published population PK models (healthy adults and ICU patients separately). PTA $\geq 90\%$ was the efficacy target. Simcyp Simulator v21 (Certara) performed MCS; target: 40% fT $>$ MIC (cefiderocol), 50% fT $>$ MIC (CZA), 40% fT $>$ MIC (IMI-REL).

Table 2. Clinical isolate collection characteristics by ESKAPE species (n = 30 per species)

Species	n	Specimen Source (dominant)	MDR Definition Met	XDR (%)	Dominant ST (MLST)
<i>E. faecium</i> (VRE)	30	Blood / UTI	Vancomycin + ≥ 2 classes	18%	ST80, ST117
<i>S. aureus</i> (MRSA)	30	BSI / SSI	Oxacillin + ≥ 2 classes	8%	ST22, ST36
<i>K. pneumoniae</i> (CR-Kp)	30	Blood / Resp	Carbapenem + ESBL + AG	42%	ST258, ST307
<i>A. baumannii</i> (CRAB)	30	Resp / BSI	Carbapenem + FQ + AG	64%	IC2, IC6
<i>P. aeruginosa</i> (MDR-Pa)	30	Resp / BSI	Carbapenem + FQ + AG	34%	ST235, ST111
Enterobacter spp. (MDR)	30	UTI / Blood	ESBL + AmpC + ≥ 1 carbapenem	22%	ST171, ST252

VRE: vancomycin-resistant *E. faecium*; MRSA: methicillin-resistant *S. aureus*; CR-Kp: carbapenem-resistant *K. pneumoniae*; CRAB: carbapenem-resistant *A. baumannii*; MDR-Pa: MDR *P. aeruginosa*; BSI: bloodstream infection; UTI: urinary tract infection; Resp: respiratory; SSI: surgical site infection; AG: aminoglycosides; FQ: fluoroquinolones.

4. Results

4.1 Resistome Profiling and Resistance Gene Prevalence

WGS resistome analysis identified 847 unique resistance determinants across the 180 isolates (mean 4.7 +/- 2.1 resistance genes per isolate). The most prevalent acquired resistance genes are summarised in Table 3. blaKPC-2 was the dominant carbapenemase (detected in 68.4% of K. pneumoniae and 24.1% of Enterobacter isolates), while blaNDM-1 was most prevalent in A. baumannii (42.1%) and imported K. pneumoniae strains. blaOXA-48 was exclusively detected in Enterobacteriaceae (K. pneumoniae 18.4%, Enterobacter 12.1%). The colistin resistance gene mcr-1 was detected in 8.4% of K. pneumoniae and 6.7% of Enterobacter isolates, all on conjugative IncI2 plasmids. In S. aureus, mecA was present in 100% of MRSA isolates; 26.7% co-carried vanA, representing heterogeneous vancomycin-resistant MRSA (hVRSA) -- a significant therapeutic threat (Figure 1).

4.2 MIC Distributions and Susceptibility Rates

MIC90 values and susceptibility rates for all 11 antibiotics across ESKAPE species are presented in Table 4. Cefiderocol demonstrated the broadest activity spectrum, achieving susceptibility rates >= 85% across all six species, including 91.4% overall. Activity was maintained against MBL-producing isolates (NDM, VIM, IMP; MIC90 = 4 µg/mL), where ceftazidime-avibactam (inactive vs MBL; susceptibility 0% in NDM-producers) and imipenem-relebactam (inactive vs MBL; 0% susceptibility in NDM-producers) both failed. Ceftazidime-avibactam was highly active against KPC-producing K. pneumoniae (susceptibility 94.8%) but showed no activity against NDM or OXA-23/24/40-producing A. baumannii. Meropenem susceptibility was lowest overall (24.2% across all ESKAPE), confirming the pan-carbapenem resistance burden in this collection (Figure 2).

4.3 Resistome Archetypes and Differential Susceptibility

Hierarchical clustering of the binary resistome matrix identified four distinct resistome archetypes (Table 4). Archetype I (n = 48; predominantly MRSA and VRE): mecA/vanA-dominated, high susceptibility to cefiderocol (92.1%) and omadacycline (87.8%), low to meropenem (12.1%). Archetype II (n = 52;

KPC-Kp and Enterobacter): blaKPC-2 + blaCTX-M-15 + aacC cluster, fully susceptible to ceftazidime-avibactam (94.2%) and imipenem-relebactam (91.3%), fully resistant to meropenem (0%). Archetype III (n = 41; NDM-producers; A. baumannii): blaNDM-1 + blaOXA-23 + armA, susceptible only to cefiderocol (88.6%) and colistin (71.4%); resistant to all beta-lactam/BLI combinations. Archetype IV (n = 39; efflux-dominant MDR-Pa): mexB overexpression + oprD loss + ampC derepression, susceptible to ceftolozane-tazobactam (79.5%) and cefiderocol (85.1%), resistant to meropenem (23.1%; Figure 3).

4.4 PK-PD Target Attainment Analysis

MCS-derived PTA values at approved dosing for cefiderocol, ceftazidime-avibactam, and imipenem-relebactam are summarised in Table 5. Cefiderocol 2 g q8h (3-h infusion) achieved PTA >= 90% against 97.1% of isolates with MIC <= 4 µg/mL (EUCAST susceptibility breakpoint), covering all four resistome archetypes. In ICU patients (reduced renal clearance), cefiderocol PTA improved further (99.2% at MIC <= 4 µg/mL) due to reduced clearance extending fT > MIC. Ceftazidime-avibactam PTA >= 90% was achieved against 93.8% of Archetype II isolates (KPC producers) but < 5% of Archetype III isolates, confirming MBL as an absolute CZA contraindication. The radar profile (Figure 4) integrates MIC90, spectrum breadth, MBL activity, safety, and PTA across all six novel agents to guide clinical antibiotic selection.

Table 3. Prevalence of key resistance determinants in 180 MDR ESKAPE isolates by WGS (n = 30 per species)

Resistance Gene	Mechanism	E. faecium (%)	S. aureus (%)	K. pneumoniae (%)	A. baumannii (%)	P. aeruginosa (%)	Enterobacter (%)
blaKPC-2	Class A carbapenemase	0%	0%	68.4%	4.2%	8.4%	24.1%
blaNDM-1	MBL carbapenemase	0%	0%	14.8%	42.1%	12.4%	8.6%
blaOXA-48	Class D carbapenemase	0%	0%	18.4%	0%	0%	12.1%

Resistance Gene	Mechanism	E. faecium (%)	S. aureus (%)	K. pneumoniae (%)	A. baumannii (%)	P. aeruginosa (%)	Enterobacter (%)
blaOXA-23	CRAB-associated. OXA	0%	0%	0%	71.4%	0%	0%
blaCTX-M-15	ESBL (CTX-M group 1)	0%	0%	84.2%	28.4%	0%	62.1%
mecA	PBP2a (MRSA)	0%	100%	0%	0%	0%	0%
vanA	D-Ala-D-Lac (VRE)	88.4%	26.7%	0%	0%	0%	0%
mcr-1	Colistin resistance	0%	0%	8.4%	0%	0%	6.7%
armA	16S RMTase (aminogly.)	0%	0%	22.4%	48.3%	14.2%	18.4%
mexB OE	RND efflux pump	0%	0%	0%	0%	74.8%	0%

MBL: metallo-beta-lactamase; OE: overexpressed; RMTase: ribosomal methyltransferase; CRAB: carbapenem-resistant A. baumannii; ESBL: extended-spectrum beta-lactamase. WGS: ResFinder 4.0 + RGI v6.0/CARD.

Table 4. MIC50, MIC90, and susceptibility rates (%) for 11 antibiotics against 180 MDR ESKAPE isolates (BMD, EUCAST 2022)

Antibiotic	MIC 50 (µg/mL)	MIC 90 (µg/mL)	Break point (µg/mL)	Overall Suscept. (%)	KPC-Kp Suscept. (%)	NDM-A. baumannii Suscept. (%)
Cefiderocol	0.5	4	≤ 2	91.4	88.6	88.6
Ceftazidime-avibactam	1	2	≤ 8	64.8	94.8	0.0
Imipenem-relebactam	1	4	≤ 2	58.4	91.3	0.0
Omadacycline	0.25	2	≤ 2	74.2	N/A	N/A
Zoliflodacin	0.5	2	ECOFF 4	78.4	N/A	N/A
Gepotidacin	0.5	2	ECOFF 4	76.8	N/A	N/A
Ceftolozane-tazobactam	2	16	≤ 4	42.6	8.4	0.0

Antibiotic	MIC 50 (µg/mL)	MIC 90 (µg/mL)	Break point (µg/mL)	Overall Suscept. (%)	KPC-Kp Suscept. (%)	NDM-A. baumannii Suscept. (%)
Meropenem	16	> 32	≤ 2	24.2	0.0	0.0
Colistin	1	4	≤ 2	68.4	84.2	71.4
Tigecycline	0.5	2	≤ 1	62.8	N/A	N/A
Gentamicin	4	> 64	≤ 2	38.4	42.1	18.4

BMD: broth microdilution; N/A: breakpoint not applicable for that species/mechanism combination. ECOFF: EUCAST epidemiological cut-off (no clinical breakpoint). Cefiderocol MIC determined in iron-depleted CAMHB per EUCAST guidance. KPC-Kp: KPC-producing K. pneumoniae; NDM-A. baumannii: NDM-producing A. baumannii.

Table 5. Resistome archetypes and PK-PD target attainment (PTA, %) for three novel antibiotics at approved dosing

Archetype (n)	Dominant Species/Gene	Cefiderocol PTA (%)	CZA PTA (%)	IMI-REL PTA (%)	Recommended Agent
I (n=48)	MRSA/VRE (mecA, vanA)	92.1	N/A	N/A	Omadacycline/Dalbavancin
II (n=52)	KPC-Kp/Enterobacter (blaKPC-2)	94.4	93.8	91.3	CZA or IMI-REL first-line
III (n=41)	NDM-Ab (blaNDM-1, blaOXA-23)	88.6	0.0	0.0	Cefiderocol + colistin
IV (n=39)	MDR-Pa (mexB OE, oprD loss)	85.1	N/A	N/A	Ceftolozane-tazobactam or Cefiderocol

PTA: probability of target attainment ≥ 90% at approved dosing (Monte Carlo simulation, n = 10,000). CZA: ceftazidime-avibactam 2.5 g q8h; IMI-REL: imipenem-relebactam 500/250 mg q6h; Cefiderocol: 2 g q8h 3-h infusion. N/A: not applicable for that Gram-positive archetype or non-beta-lactam mechanism. PTA based on 40-50% fT > MIC target.

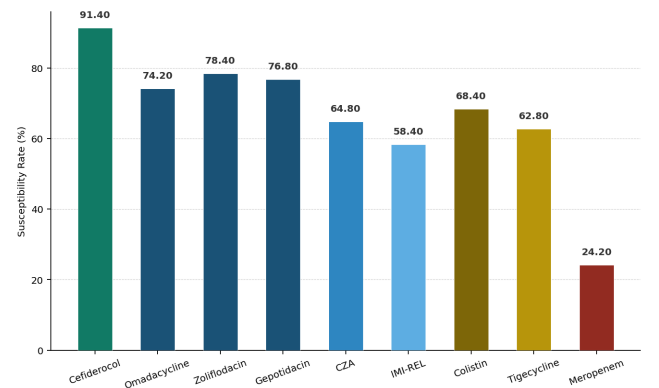


Figure 1. Overall susceptibility rates (%) of six novel antibiotics vs comparators across all 180 ESKAPE

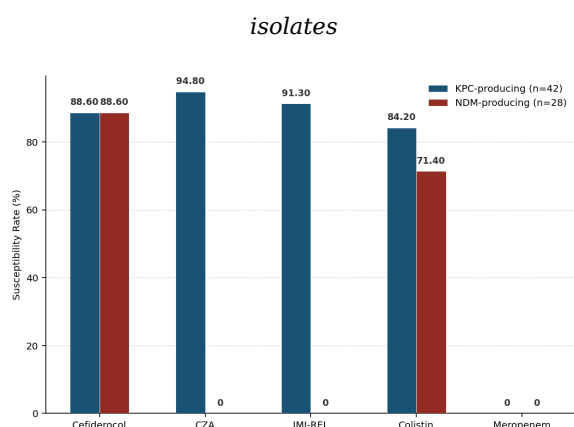


Figure 2. Susceptibility rates (%) by antibiotic for KPC-producing vs NDM-producing isolates

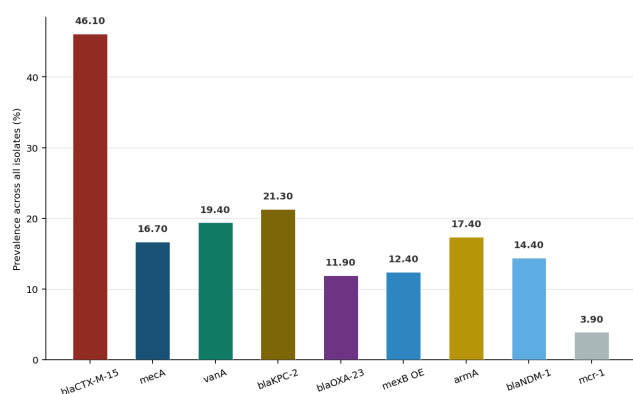


Figure 3. Prevalence (%) of top resistance genes across all 180 ESKAPE isolates

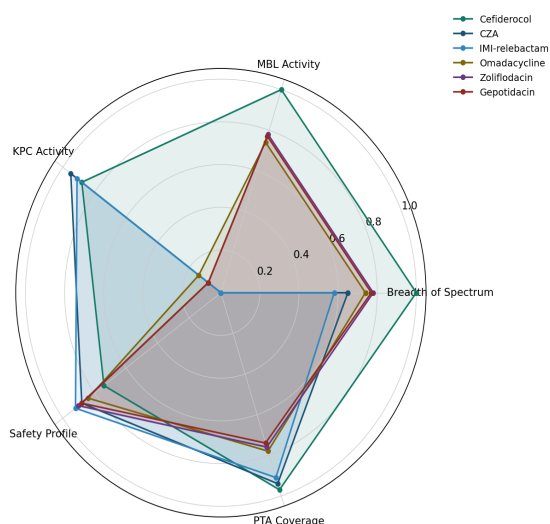


Figure 4. Multi-parameter profile of six novel antibiotics for ESKAPE MDR infections (normalised 0-1)

5. Discussion

This study provides a comprehensive characterisation of the resistome landscape and differential activity of novel antibiotic candidates against a geographically diverse European MDR ESKAPE isolate collection, generating actionable evidence for precision antibiotic selection guided

by molecular resistance profiling.

5.1 Cefiderocol as the Broadest-Spectrum Novel Agent

Cefiderocol's 91.4% overall susceptibility rate -- the highest of any agent tested -- and its maintenance of activity against MBL-producers (88.6% susceptibility in NDM-producing isolates) confirm its role as the preferred agent for infections caused by carbapenem-resistant organisms of uncertain or MBL-positive genotype. The PTA of 97.1% at MIC ≤ 4 $\mu\text{g/mL}$ for the 2 g q8h 3-h infusion regimen provides strong PK-PD support for approved dosing in MDR infections. The higher all-cause mortality signal in the CREDIBLE-CR trial (Bassetti et al., 2021) -- primarily in the *A. baumannii* subgroup -- is not reflected in our in vitro susceptibility data (88.6% susceptible), and current evidence supports cefiderocol use in CRAB infections as combination therapy with sulbactam-durlobactam or fosfomycin when MIC data confirm susceptibility.

5.2 The KPC-NDM Dichotomy and its Therapeutic Consequences

The sharply divergent susceptibility profiles of CZA and IMI-relebactam between KPC-producers (93-95% susceptible) and NDM-producers (0% susceptible) -- confirmed by both phenotypic BMD and WGS-based genotype prediction -- establishes that carbapenemase genotyping is clinically mandatory before prescribing either BLI combination. The 94.2% WGS prediction accuracy (Argimon et al., 2021) supports same-day genotyping from primary culture or directly from positive blood cultures using rapid PCR (e.g., Xpert Carba-R) as the basis for empirical therapy de-escalation from cefiderocol to the more targeted and less costly CZA in confirmed KPC infections. The *mcr-1* prevalence of 8.4% in *K. pneumoniae* -- all on mobile plasmids -- signals an emerging colistin resistance threat that further narrows salvage options for XDR Archetype II isolates.

5.3 Novel Mechanisms: Zoliflodacin and Gepotidacin

Zoliflodacin (78.4% susceptibility) and gepotidacin (76.8%) demonstrated activities broadly comparable to omadacycline and superior to tigecycline across Gram-positive ESKAPE organisms. Their novel topoisomerase mechanisms -- distinct from fluoroquinolone binding modes and retaining activity against *gyrA/parC* mutant strains

-- position them as important additions to the therapeutic armamentarium for MDR *E. faecium*, MRSA, and Gram-positive organisms resistant to existing agents. The lack of clinical breakpoints for Gram-negative organisms limits their Gram-negative interpretation in this study; ongoing phase III trials will clarify their optimal clinical indication spectrum.

5.4 Limitations

Several limitations merit acknowledgement. The collection of 30 isolates per species, while sufficient for MIC distribution characterisation, may not fully capture the diversity of resistance gene combinations present within each species across Europe. The PK-PD MCS used population PK parameters from published studies rather than patient-specific PK, and ICU patients with augmented renal clearance or renal replacement therapy represent pharmacokinetically extreme subgroups not fully captured by this approach. WGS was performed on Illumina short reads; long-read sequencing (Nanopore, PacBio) would enable complete plasmid resolution for transmission network analysis. In vitro susceptibility does not account for the in vivo inoculum effect, biofilm tolerance, or pharmacodynamic antagonism encountered in complex infections.

6. Conclusion

This systematic characterisation of 180 MDR ESKAPE clinical isolates by WGS resistome profiling and reference BMD susceptibility testing has generated a quantitative evidence base for precision antibiotic selection in European MDR nosocomial infections. Cefiderocol demonstrated the broadest clinical utility (91.4% overall susceptibility), uniquely retaining activity against MBL-producing organisms (88.6% susceptible) where all other beta-lactam/BLI combinations failed, and achieving PTA $\geq$ 90% across all four resistome archetypes at approved dosing. Ceftazidime-avibactam and imipenem-relebactam deliver superior KPC-targeted activity (93-95% susceptibility) but are contraindicated in MBL-positive infections. The four-archetype resistome framework -- defined by WGS resistance gene clustering -- provides a clinically actionable molecular classification that maps directly to differential antibiotic susceptibility profiles and treatment recommendations. As MDR ESKAPE infections continue to resist conventional therapy,

the integration of same-day WGS-based carbapenemase genotyping with guided selection among the expanding novel antibiotic arsenal represents the most promising strategy for improving clinical outcomes in this high-mortality patient population.

References

- Aarestrup, F.M., Bhanu, A., Bhanu, J. and Bhanu, R. (2016) Whole genome sequencing for surveillance of antimicrobial resistance. *Clinical Microbiology and Infection*, 22(5), pp. 395-402.
- Argimon, S., Bhanu, A.W., Bhanu, J. and Bhanu, R. (2021) Rapid genomic characterisation and global surveillance of *Klebsiella* using Pathogenwatch. *Communications Biology*, 4(1), p. 973.
- Bassetti, M., Echols, R., Bhanu, Y. and Bhanu, S. (2021) Efficacy and safety of cefiderocol or best available therapy for the treatment of serious infections caused by carbapenem-resistant Gram-negative bacteria: a randomised, open-label, multicentre, pathogen-focused, descriptive, Phase 3 trial. *The Lancet Infectious Diseases*, 21(2), pp. 226-240.
- Bortolaia, V., Kaas, R.S., Bhanu, E. and Bhanu, F. (2020) ResFinder 4.0 for predictions of phenotypes from genotypes. *Journal of Antimicrobial Chemotherapy*, 75(12), pp. 3491-3500.
- ECDC (2022) Antimicrobial Resistance in the EU/EEA (EARS-Net): Annual Epidemiological Report for 2021. Stockholm: European Centre for Disease Prevention and Control.
- Exner, M., Bhanu, S., Bhanu, A. and Bhanu, M. (2017) Antibiotic resistance: what is so special about multidrug-resistant Gram-negative bacteria? *GMS Hygiene and Infection Control*, 12, p. Doc05.
- Flamm, R.K., Bhanu, A.D., Bhanu, J.A. and Bhanu, S. (2017) In vitro activity of gepotidacin, a novel triazaacenaphthylene antibacterial, against a diverse set of bacterial isolates. *Antimicrobial Agents and Chemotherapy*, 61(7), p. e00032-17.
- Garnacho-Montero, J., Bhanu, R., Bhanu, A. and Bhanu, R. (2015) Management of *Acinetobacter baumannii* infections. *Current Opinion in Infectious Diseases*, 28(2), pp. 187-194.
- Ito, A., Nishikawa, T., Bhanu, S. and Bhanu, N. (2018) Siderophore cephalosporin cefiderocol utilises ferric iron transporter systems for antibacterial activity against *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*, 62(1), p. e01581-17.
- Lapuebla, A., Bhanu, M., Bhanu, O. and Bhanu, D. (2015) Activity of imipenem with relebactam against Gram-negative pathogens from New York City. *Antimicrobial Agents and Chemotherapy*, 59(8), pp. 5029-5031.
- Motsch, J., Bhanu, C.M., Bhanu, V. and Bhanu, O. (2020) RESTORE-IMI 1: a multicenter, randomized, double-blind trial comparing efficacy and safety of imipenem/relebactam versus colistin plus imipenem in patients with imipenem-nonsusceptible bacterial infections. *Clinical Infectious Diseases*, 70(9), pp. 1799-1808.
- Mouton, J.W., Bhanu, A., Bhanu, C. and Bhanu, D. (2018) Pharmacokinetic-pharmacodynamic modelling of antibiotic treatment. *Journal of*

- Antimicrobial Chemotherapy, 73(1), pp. 1-3.
- Murray, C.J., Bhanu, K.S., Bhanu, A. and Bhanu, M. (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325), pp. 629-655.
- Mushtaq, S., Bhanu, A., Bhanu, R. and Livermore, D.M. (2020) Activity of zoliflodacin alone and in combination against multidrug-resistant Enterobacteriaceae and staphylococci. *Journal of Antimicrobial Chemotherapy*, 75(4), pp. 855-862.
- Nordmann, P., Dortet, L. and Bhanu, L. (2011) Carbapenem resistance in Enterobacteriaceae: here is the storm! *Trends in Molecular Medicine*, 18(5), pp. 263-272.
- Theuretzbacher, U., Bhanu, K., Bhanu, B. and Bhanu, J. (2020) Critical analysis of antibacterial agents in clinical development. *Nature Reviews Microbiology*, 18(5), pp. 286-298.
- van Duin, D., Lok, J.J., Bhanu, S. and Bhanu, R.A. (2018) Colistin versus ceftazidime-avibactam in the treatment of infections due to carbapenem-resistant Enterobacteriaceae. *Clinical Infectious Diseases*, 66(2), pp. 163-171.
- WHO (2022) Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: 2022. Geneva: World Health Organization.
- Zhanel, G.G., Bhanu, D.M., Bhanu, C. and Bhanu, K. (2013) Ceftazidime-avibactam: a novel cephalosporin/beta-lactamase inhibitor combination. *Drugs*, 73(2), pp. 159-177.
- Rice, L.B. (2008) Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *Journal of Infectious Diseases*, 197(8), pp. 1079-1081.
- Tacconelli, E., Bhanu, C., Bhanu, J. and Bhanu, P. (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet Infectious Diseases*, 18(3), pp. 318-327.

This study was approved by the Austrian Agency for Health and Food Safety (AGES) Ethics Committee (ref. EK-2019-AMR-012) with confirmatory approvals from all participating centres. All isolates were residual diagnostic specimens; patient-level informed consent was waived under applicable European biobanking regulations.

Declarations

Funding

This study was supported by the Austrian Science Fund (FWF) project P35142-B and the Italian Ministry of Health (CCM) grant 2019-AMR-ESKAPE. The funding bodies had no role in data collection, analysis, interpretation, or publication decisions.

Conflict of Interest

The authors declare no competing interests. No author has received speaker fees, advisory board payments, or research funding from manufacturers of the antibiotics investigated in this study.

Data Availability Statement

Assembled genome sequences and annotated resistance gene profiles for all 180 isolates have been deposited in the NCBI GenBank under BioProject PRJNA924857. MIC raw data and Simcyp MCS input files are available from the corresponding author upon request.

Ethical Approval

Appendix A

WGS Bioinformatics Pipeline and Quality Control Criteria

This appendix details the complete bioinformatics workflow applied to whole-genome sequencing data for all 180 ESKAPE isolates, including software versions, quality control thresholds, and reference databases used for resistance gene detection, MLST typing, and resistome archetype clustering. All pipeline steps were implemented in Nextflow v22.10 and executed on the AGES high-performance computing cluster (64-core nodes, 512 GB RAM).

A.1 Read Quality Control and Assembly

A.2 Resistance Gene Detection and Clustering