

Antibiotics and antibiotic resistance race: a chronicle of antibiotic discovery against Gram-negative bacteria during 2001-2020: way forward

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ABSTRACT

The continuous evolutionary race between antibacterial innovation and antimicrobial resistance (AMR) has become one of the greatest challenges in the management of Gram-negative bacterial infections. Although numerous antibiotics and β -lactam- β -lactamase inhibitor combinations have been introduced since 2000, their clinical utility has been progressively eroded by the rapid emergence and global dissemination of resistance mechanisms, including carbapenemase production, reduced outer membrane permeability, multidrug efflux, target modification, and antibiotic-inactivating enzymes. This review provides a chronological and mechanistic overview of anti-Gram-negative antibiotic development over the past two decades while examining the parallel evolution of bacterial resistance that has limited the therapeutic lifespan of both established and newly approved agents. We critically discuss the mechanisms of action, antibacterial spectrum, pharmacological characteristics, clinical utility, and resistance determinants of recently approved antibiotics, with particular emphasis on therapeutic innovations that overcome conventional resistance barriers, including advanced β -lactam- β -lactamase inhibitor combinations, the siderophore cephalosporin cefiderocol, next-generation tetracyclines, aminoglycosides, and novel bacterial topoisomerase inhibitors. Emerging therapeutic strategies, including investigational antibacterial agents, artificial intelligence-assisted antibiotic discovery, bacteriophage therapy, and CRISPR-based antimicrobial approaches, are also reviewed as potential solutions to the growing challenge of multidrug-resistant Gram-negative pathogens. Collectively, these advances illustrate that successful antibiotic development increasingly depends on innovative target engagement, improved bacterial uptake, and resistance-informed drug design rather than incremental modification of existing scaffolds alone. Ultimately, preserving the clinical effectiveness of new antibiotics will require the integration of antimicrobial stewardship, rapid diagnostics, genomic surveillance, and mechanism-guided drug development to slow the continuing evolutionary arms race between bacterial adaptation and therapeutic innovation.

Keywords: Anti-bacterial agents; Gram-negative bacteria; Antimicrobial stewardship; Beta-lactamases; Carbapenem resistance; Multidrug resistance

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INTRODUCTION

Antimicrobial resistance (AMR) in Gram-negative pathogens has emerged as a critical therapeutic challenge, surpassing Gram-positive threats due to more complex resistance mechanisms and a higher clinical burden (1). Multidrug-resistant (MDR) strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* increasingly compromise standard treatments, particularly in bloodstream infections, pneumonia, and urinary tract infections (2).

Over 20 antibiotics have been approved since 2001, yet the pipeline remains fragile. The pace of innovation continues to lag behind resistance evolution, with many agents offering limited novelty in mechanism or spectrum. Scientific barriers, high R&D costs, and uncertain returns have further deterred investment, especially for agents targeting carbapenem-resistant Enterobacterales and non-fermenters such as *P. aeruginosa* and *A. baumannii* (2, 3).

This review critically examines antibiotic discovery from 2001 to 2020, with a focus on agents approved for Gram-negative infections. Rather than cataloguing drugs, it highlights the scientific and strategic innovations driving their development, assesses resistance trends, and evaluates therapeutic impact. It also explores investigational agents in clinical trials and emerging approaches such as bacteriophage therapy, CRISPR-Cas antimicrobials, and AI-guided drug design, which offer potential to reshape the future antimicrobial landscape (4).

As resistance continues to erode the efficacy of existing therapies, a shift toward mechanism-based innovation, global stewardship, and sustainable R&D frameworks is essential. This review aims to synthesize two decades of progress while identifying unmet needs and novel pathways forward in the global response to AMR.

Literature/data source. A systematic search was performed in PubMed, Google Scholar, Cochrane Library, ClinicalTrials.gov, FDA/EMA databases, pharmaceutical company websites, and conference proceedings (January 2000–June 2020). Additional pipeline data were retrieved from recent regulatory and industry reports (India, China, Japan, United States). Study selection followed PRISMA 2020 guidelines (Fig. 1) and yielded 240 publications after deduplication and eligibility assessment (5, 6). The

development status of pipeline agents was verified with manufacturers and regulatory authorities when feasible.

Antibiotics approved since the beginning of the 21st century. AMR arises when bacteria evolve to resist previously effective treatments. Gram-negative bacteria employ dual protective layers, porin channels, efflux pumps, and plasmid-acquired resistance, while Gram-positive bacteria rely on target modifications and resistance genes. The escalating resistance in ESKAPE pathogens over the past two decades has severely impacted treatment efficacy, patient outcomes, and healthcare costs.

The period from 2001 to 2020 marked a critical era in antibiotic development, with the introduction of novel agents aimed at combating the rising tide of multidrug-resistant (MDR) pathogens. However, their clinical promise was swiftly challenged by pre-existing and emergent resistance mechanisms, driven by enzymatic inactivation, efflux pump overexpression, porin loss, and target-site modifications.

Between 2001 and 2020, a series of new antibacterial agents reached clinical use in an effort to counter escalating multidrug resistance (Fig. 2). Notable introductions included the carbapenems ertapenem (2001), biapenem (2001; Japan), doripenem (2007) and imipenem/relebactam (2019); the anti-MRSA cephalosporin ceftaroline (2010); the antipseudomonal β -lactam/ β -lactamase inhibitor combinations ceftolozane/tazobactam (2014) and ceftazidime/avibactam (2015); the fluoroquinolones delafloxacin (2017) and levonadifloxacin (2020; India); the glycyclines and fluorocycline tigecycline (2005), omadacycline (2018) and eravacycline (2018); the lipoglycopeptide telavancin (2009); expanded use of the lipopeptide daptomycin (approved 2003); and the semisynthetic aminoglycoside plazomicin (2018) (7). Although these agents initially exhibited potent in vitro activity against key multidrug-resistant pathogens, including methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant Enterobacterales, and multidrug-resistant *Pseudomonas aeruginosa* - global surveillance networks rapidly documented progressive increases in minimum inhibitory concentrations driven by the dissemination of carbapenemases (KPC, metallo- β -lactamases and mutated OXA enzymes), porin loss, efflux pump overexpression, target-site mutations in DNA gyrase/topoisomerase IV and 16S rRNA methylation. Resistance emerged most rapidly in high-incidence settings across Asia,

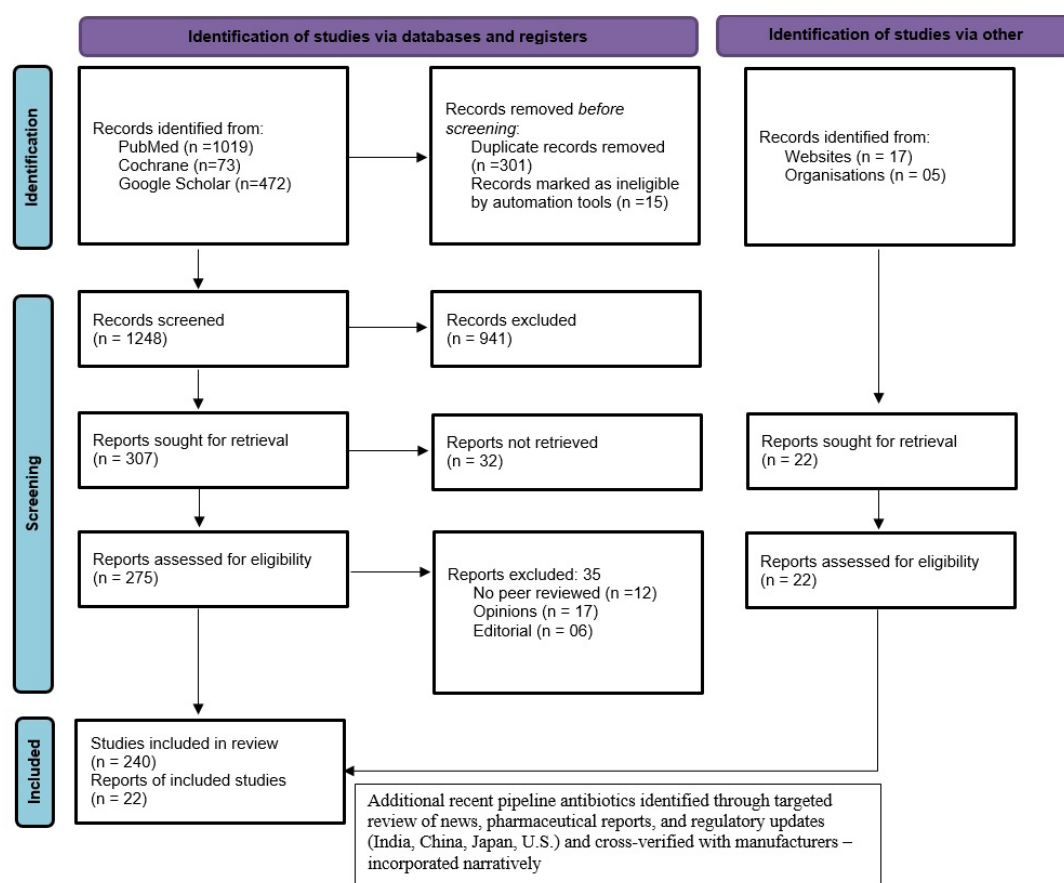


Fig. 1. PRISMA flow diagram for the overview of systematic reviews

South America, India, and Southeast Asia, substantially eroding the clinical lifespan of these compounds and underscoring the persistent challenge of sustaining antibacterial activity in the absence of concomitant resistance-mitigation strategies. Collectively, these agents represented a hopeful renaissance in antibiotic development. Yet despite impressive in vitro data and initial clinical success, resistance rapidly followed, mirroring past experiences. These approved antibiotics were engineered to combat MDR pathogens, including methicillin-resistant *S. aureus* (MRSA), CRE, and MDR *P. aeruginosa*. However, the promise of these therapeutics has been undermined by both pre-existing and emergent resistance mechanisms, ranging from enzymatic inactivation to target-site mutations and reduced drug accumulation.

Resistance mechanisms undermining novel antibiotics. Resistance mechanisms in bacteria, including pre-existing and acquired adaptations, have reduced the effectiveness of recently approved antibiotics (Table 1). These mechanisms typically involve target-site alterations, enzymatic hydrolysis, decreased

permeability, and enhanced efflux. Surveillance data and clinical studies demonstrate the impact of these mechanisms on treatment outcomes in Gram-negative and Gram-positive infections. The following sections summarize resistance profiles based on clinical evidence, including resistance prevalence, therapy-emergent resistance rates, and associated clinical outcomes.

Evolution of novel β -lactamase inhibitors against Gram-negative pathogens. The rapid dissemination of extended-spectrum β -lactamases (ESBLs), AmpC enzymes, and carbapenemases among Gram-negative pathogens during the early 21st century progressively undermined the efficacy of conventional β -lactam antibiotics. In response, antibiotic development shifted from discovering new β -lactam scaffolds toward engineering increasingly erudite BLIs capable of restoring β -lactam activity against MDR Gram-negative bacteria. The evolution of these inhibitors reflects a continuing arms race between antibacterial innovation and bacterial adaptation.

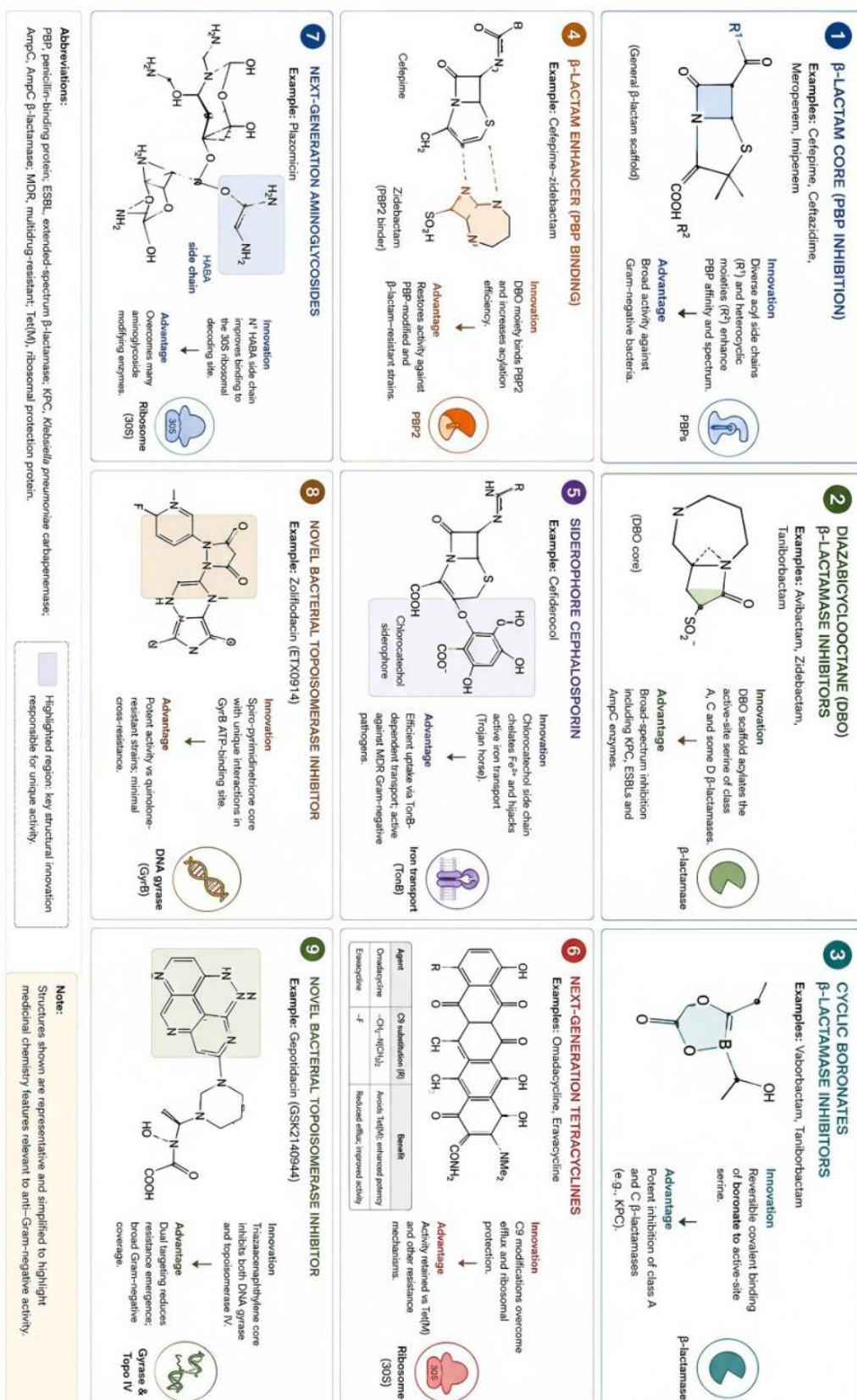


Fig. 2. Structural innovations underlying recently introduced antibiotics against Gram-negative bacteria (2001–2020)

Table 1. Pre- and Post-Approval Resistance Challenges in Antibiotics Introduced Between 2001 and 2020

Antibiotic Class	Antibiotic	Resistance Challenges		Molecular determinants of resistance	References
		Pre-launch	Post-Launch		
Carbapenems	Ertapenem	OmpC/F loss, KPC-2	CTX-M-15, $\pm$ OmpC/F, NDM-1, VIM-2	KPC: Asp179Tyr	(62)
	Doripenem	IMP-1, AmpC, MexAB-OprM	VIM-1, MexXY-OprM	MexY: Gly195Asp, OprD: Gln142X	(65)
	Imipenem/Relebactam	NDM-1, MexAB-OprM	KPC-3 variants	KPC-3: Thr243Met	(16, 23, 25)
Cephalosporins	Ceftazidime/Avibactam	NDM-1, AcrAB-TolC	KPC-3 mutations	KPC-3: Asp179Tyr, Val240Gly	(14-16, 18, 22, 25, 26, 50)
	Ceftolozane/Tazobactam	MexXY-OprM, ESBLs	AmpC mutations, OprD loss	AmpC: Gly183Asp	(13, 22, 24)
Tetracyclines	Tigecycline	Tet(A), Tet(M)	AcrAB-TolC, 16S rRNA	ramR/acrR mutations $\rightarrow$ AcrAB-TolC overexpression; tet(X3/X4) monooxygenases; ompK35/36 loss	(71, 72)
	Omadacycline	Tet(M), Tet(A)	AcrAB-TolC upregulation	tet(X3/X4) (TetX family)	(71, 72)
Aminoglycosides	Plazomicin	AAC(6')-Ib	16S rRNA methyltransferases (ArmA)	-	(75)

Clinically approved β -lactam/ β -lactamase inhibitor combinations. The clinical introduction of ceftolozane/tazobactam, ceftazidime/avibactam, imipenem/relebactam, and meropenem/vaborbactam represented a major advance in restoring β -lactam activity against multidrug-resistant (MDR) Gram-negative pathogens (8-11). These combinations effectively target ESBL- and KPC-producing Enterobacterales while improving activity against difficult-to-treat *P. aeruginosa*. However, their clinical utility remains constrained by the rapid evolution of bacterial resistance. Common resistance mechanisms include structural diversification and overexpression of AmpC and KPC β -lactamases, acquisition of enzymes not inhibited by currently available β -lactamase inhibitors (particularly NDM, VIM, IMP, and many OXA carbapenemases), reduced outer membrane permeability through OmpK35/OmpK36 or OprD loss, activation of multidrug efflux systems (AcrAB-TolC, MexAB-OprM, and MexXY), and, less frequently, alterations in penicillin-binding proteins (12-19). Resistance frequently emerges during therapy, particularly following prolonged antibiotic exposure, and cross-resistance among β -lactam/ β -lactamase inhibitor combinations has increasingly been documented. Collectively, these findings demonstrate that although successive generations of β -lactamase inhibitors have substan-

tially extended the clinical utility of β -lactam antibiotics, bacterial adaptation continues through convergent mechanisms involving enzymatic evolution, impaired permeability, target modification, and multidrug efflux. The expanding clinical application of these combinations has been driven by continuous advances in β -lactamase inhibitor chemistry. The transition from classical β -lactam-derived inhibitors to newer non- β -lactam scaffolds has progressively broadened the spectrum of enzyme inhibition and improved activity against increasingly resistant Gram-negative pathogens.

Diazabicyclooctane inhibitors: overcoming KPC- and AmpC-mediated resistance. The introduction of diazabicyclooctane (DBO) inhibitors represented the first major breakthrough beyond classical β -lactam-derived inhibitors such as tazobactam. Avibactam, incorporated into ceftazidime/avibactam, and relebactam, combined with imipenem, inhibit class A carbapenemases (particularly KPC), class C AmpC enzymes, and selected class D enzymes, including OXA-48-like carbapenemases, through reversible covalent binding (20). By restoring β -lactam activity against many carbapenem-resistant Enterobacterales and MDR *P. aeruginosa*, these inhibitors significantly expanded the therapeutic lifespan of existing β -lactam antibiotics (21).

Despite these advances, resistance rapidly emerged following clinical deployment. In Enterobacterales, mutations within the KPC Ω -loop (particularly residues 164-179), increased *bla*_{KPC} copy number, and porin loss (OmpK35/OmpK36) reduce inhibitor efficacy (22, 23). In *P. aeruginosa*, resistance is primarily driven by AmpC structural diversification, AmpC hyperexpression, activation of multidrug efflux pumps, and reduced outer membrane permeability (24-26). Importantly, DBO inhibitors remain ineffective against metallo- β -lactamases such as NDM, VIM, and IMP, leaving a major therapeutic gap in the treatment of MBL-producing pathogens and providing the impetus for the development of broader-spectrum inhibitor classes (27).

Cyclic boronate and boronic acid inhibitors: expanding coverage beyond serine carbapenemases. The limitations of DBO inhibitors drove the development of boronate-based inhibitors. Vaborbactam was specifically engineered to provide potent inhibition of KPC enzymes and substantially restored meropenem activity against KPC-producing Enterobacterales (28). Nevertheless, its spectrum remained restricted by poor activity against metallo- β -lactamases and many OXA-type carbapenemases. Resistance has subsequently been associated with KPC structural modifications, increased enzyme expression, and porin disruption, particularly loss of OmpK36 (18, 23).

To address these shortcomings, newer cyclic boronates such as taniborbactam and xeruborbactam were developed with broader inhibitory spectra (29, 30). Taniborbactam inhibits class A, C, and D β -lactamases while also targeting several clinically important metallo- β -lactamases, including NDM and VIM (31). Xeruborbactam (QPX7728) further expands activity, demonstrating ultrabroad-spectrum inhibition of class A, C, D, and selected class B β -lactamases (32). Preclinical studies have shown potent activity against carbapenem-resistant and extensively drug-resistant Enterobacterales, *P. aeruginosa*, and *A. baumannii*. These agents represent an important attempt to overcome the major resistance mechanisms that emerged following deployment of earlier BLIs.

β -Lactam enhancer strategies: targeting resistance beyond β -lactamase inhibition. Recognition that β -lactamase inhibition alone may be insufficient to overcome complex resistance phenotypes led to

the development of β -lactam enhancer strategies. Zidebactam (WCK 5107) exemplifies this concept by combining β -lactamase inhibition with high-affinity binding to penicillin-binding protein 2 (PBP2). This dual mechanism enhances bacterial killing while simultaneously restoring β -lactam activity (33). When combined with cefepime, zidebactam demonstrates activity against MDR Enterobacterales, *P. aeruginosa* and *A. baumannii* including isolates possessing multiple resistance determinants (34). Importantly, its efficacy derives not only from enzyme inhibition but also from direct target engagement, potentially reducing dependence on a single resistance-susceptible mechanism.

Despite substantial advances in inhibitor design, resistance continues to emerge through a recurring repertoire of mechanisms, including β -lactamase diversification, enzyme overproduction, porin loss, target modification, and multidrug efflux. The sequential development of DBO inhibitors, boronate derivatives, and β -lactam enhancers illustrates how each generation of innovation has been driven by the resistance limitations of its predecessors. This cycle underscores the dynamic co-evolution of Gram-negative pathogens and antimicrobial therapies and highlights the need for continued surveillance, stewardship, and mechanism-guided drug development to preserve the effectiveness of future β -lactam-based treatments.

Cefiderocol. Cefiderocol is the first clinically approved siderophore cephalosporin, representing one of the most significant advances in β -lactam antibiotic development against multidrug-resistant (MDR) Gram-negative bacteria. Unlike conventional cephalosporins that rely primarily on passive diffusion through outer membrane porins, cefiderocol exploits bacterial iron acquisition systems to facilitate active cellular uptake while simultaneously exhibiting remarkable stability against hydrolysis by a broad spectrum of β -lactamases, including carbapenemases (35). This unique combination of enhanced intracellular delivery and enzymatic resilience has expanded treatment options for infections caused by carbapenem-resistant Gram-negative pathogens (36).

Mechanism of action. Cefiderocol is a catechol-substituted siderophore cephalosporin that mimics naturally occurring bacterial siderophores. Under iron-limited conditions encountered during

infection, the catechol moiety chelates ferric iron (Fe^{3+}), enabling active transport across the outer membrane through TonB-dependent siderophore receptors such as CirA, Fiu, PirA, and PiuA (37). This "Trojan horse" strategy allows efficient accumulation within the periplasmic space, bypassing one of the principal permeability barriers that restricts β -lactam activity against Gram-negative bacteria.

Following cellular entry, cefiderocol binds predominantly to penicillin-binding protein 3 (PBP3), inhibiting peptidoglycan synthesis and ultimately causing bacterial cell lysis. Importantly, its chemical structure confers exceptional stability against hydrolysis by most clinically important β -lactamases, including extended-spectrum β -lactamases (ESBLs), AmpC cephalosporinases, serine carbapenemases (KPC and OXA-type enzymes), and metallo- β -lactamases (NDM, VIM, and IMP), thereby combining enhanced uptake with broad enzymatic protection (Fig. 3) (38).

Antibacterial spectrum. Cefiderocol exhibits potent in vitro activity against a wide range of aerobic

Gram-negative pathogens, including Enterobacterales (*E. coli*, *K. pneumoniae*, *E. cloacae* complex), *P. aeruginosa*, *A. baumannii*, *S. maltophilia*, and *Burkholderia cepacia* complex. Its activity is largely retained against isolates resistant to carbapenems through the production of KPC, OXA, NDM, VIM, and IMP carbapenemases, making cefiderocol one of the few agents active against diverse carbapenem-resistant organisms (39, 40). Nevertheless, susceptibility varies according to the integrity of bacterial iron transport pathways, the type and expression of β -lactamases, and the coexistence of additional resistance mechanisms.

Clinical efficacy. Clinical trials have established cefiderocol as an important therapeutic option for severe infections caused by MDR Gram-negative bacteria. The APEKS-cUTI trial demonstrated non-inferiority to imipenem-cilastatin in patients with complicated urinary tract infections, whereas the APEKS-NP study confirmed efficacy comparable to high-dose meropenem for hospital-acquired and ventilator-associated bacterial pneumonia (41, 42). Fur-

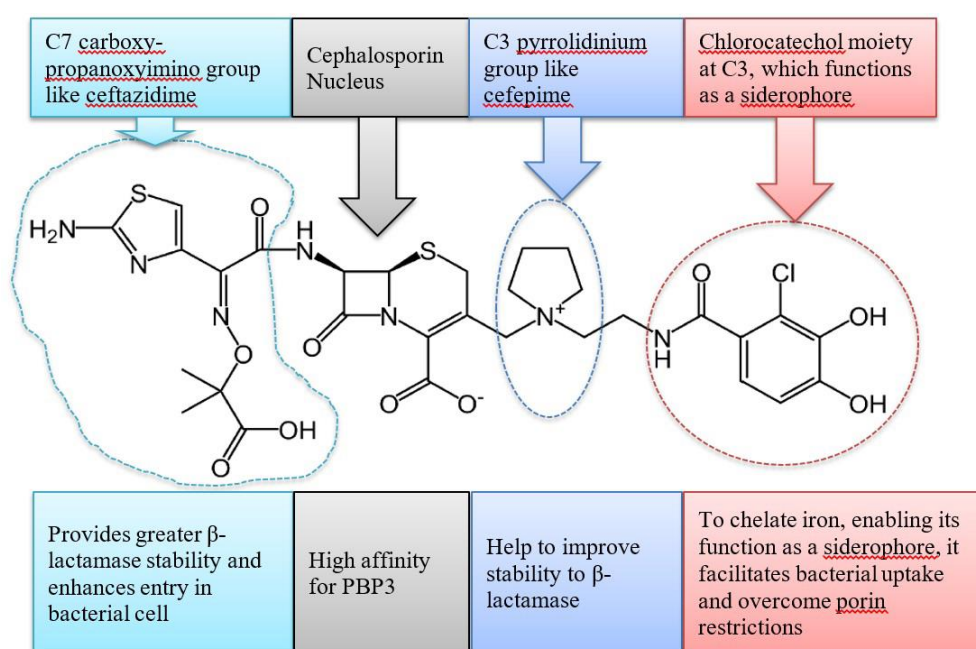


Fig. 3. Chemical Structure and in Vitro Activity Relationship of Cefiderocol.

The Fig. illustrates the chemical structure of cefiderocol, highlighting key structural components and their functional significance. The C7 carboxy-propanoxyimino group, analogous to that in ceftazidime, enhances β -lactamase stability. The C3 pyrrolidinium group, similar to cefepime, improves cell penetration. Additionally, the chlorocatechol moiety at C3 functions as a siderophore, facilitating iron chelation and promoting bacterial uptake, thereby overcoming porin-mediated resistance. The cephalosporin nucleus confers a high affinity for PBP3, effectively inhibiting bacterial cell wall synthesis.

thermore, the pathogen-focused CREDIBLE-CR trial highlighted the clinical utility of cefiderocol in infections caused by carbapenem-resistant Gram-negative pathogens, including Enterobacterales, *P. aeruginosa*, and *A. baumannii*. Although an increased mortality signal was observed among patients with severe *A. baumannii* infections in CREDIBLE-CR, subsequent observational studies suggest that this finding likely reflected the severity of underlying illness, heterogeneity of patient populations, and limitations inherent to pathogen-focused studies rather than direct drug toxicity (43). Collectively, accumulating clinical evidence supports cefiderocol as an important salvage therapy for infections caused by extensively drug-resistant Gram-negative pathogens, particularly when therapeutic alternatives are limited.

Pre-existing reduced susceptibility. An intriguing feature of cefiderocol is the detection of reduced susceptibility in clinical isolates even before its widespread clinical use, indicating that adaptive resistance mechanisms may arise independently of cefiderocol exposure. KPC-producing *E. coli* and NDM-producing *K. pneumoniae* have demonstrated elevated minimum inhibitory concentrations (MICs) associated with alterations in TonB-dependent receptors such as *cirA* and *fepA*, despite no previous exposure to the drug (44). Similarly, environmental and clinical isolates possessing naturally altered iron acquisition systems suggest that remodelling of siderophore-mediated transport pathways may pre-exist in bacterial populations, thereby reducing cefiderocol activity before therapeutic use (45).

Resistance mechanisms. Despite its innovative design, resistance to cefiderocol is increasingly reported and generally results from the cumulative effect of multiple complementary mechanisms.

Alterations in iron uptake systems. The most characteristic mechanism involves impaired siderophore-mediated uptake. In *P. aeruginosa*, stepwise resistance frequently develops through reduced expression or loss of TonB-dependent siderophore receptors together with enhanced multidrug efflux. In Enterobacterales, mutations or disruption of iron transport genes, including *cirA*, *fiu*, *pirA*, and *tonB*, substantially reduce intracellular drug accumulation and have been associated with high-level resistance (46, 47). In *A. baumannii*, insertion sequence-mediated

disruption of *pirA* (e.g., ISAba36 insertion) further illustrates the remarkable plasticity of bacterial iron transport systems (48).

Another adaptive strategy involves remodelling of iron acquisition pathways. In KPC-producing *K. pneumoniae*, acquisition of the plasmid-associated ferric citrate transport system has been associated with reduced cefiderocol susceptibility. Expression of this alternative iron uptake pathway decreases bacterial reliance on catecholate siderophore receptors, thereby limiting cefiderocol uptake via its "Trojan horse" entry mechanism (49).

Enzymatic mechanisms. Although cefiderocol is highly stable against most β -lactamases, emerging enzyme variants have diminished this advantage. Several KPC variants, including KPC-31, KPC-41, KPC-50, and KPC-109, exhibit enhanced hydrolytic activity against cefiderocol while frequently retaining resistance to currently available β -lactamase inhibitors (50). In addition, certain OXA-type carbapenemases (particularly OXA-23 and OXA-427), PER-type extended-spectrum β -lactamases, and metallo- β -lactamases such as NDM and VIM may further compromise cefiderocol activity, especially when combined with defects in iron transport (51-54).

Non-enzymatic mechanisms. Additional mechanisms contributing to cefiderocol resistance include AmpC hyperproduction, overexpression of multidrug efflux pumps (MexAB-OprM and MexXY), regulatory mutations affecting siderophore biosynthesis (*pvdS*, *pvdE*), and occasional alterations in PBP3 that reduce target affinity (55, 56). These mechanisms frequently coexist, highlighting the multifactorial nature of cefiderocol resistance.

Challenges in antimicrobial susceptibility testing. Accurate antimicrobial susceptibility testing (AST) for cefiderocol presents unique challenges because its antibacterial activity depends on iron-restricted conditions that mimic the in vivo environment. Consequently, reliable MIC determination requires iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB), and variations in iron concentration can significantly influence susceptibility results. Poor categorical agreement has been reported between reference broth microdilution, disk diffusion, gradient diffusion methods, and automated susceptibility testing platforms, particularly for *P.*

aeruginosa and *A. baumannii*. Interpretation is further complicated by heteroresistance, the presence of resistant subpopulations with altered iron transport systems, the inoculum effect, and discrepancies between in vitro susceptibility and clinical response. These limitations underscore the importance of standardized testing methodologies and cautious interpretation of cefiderocol susceptibility data in routine clinical microbiology laboratories (57-60).

Safety profile. Cefiderocol is generally well tolerated, with an adverse event profile comparable to that of other cephalosporins. The most commonly reported adverse events include gastrointestinal disturbances, infusion-site reactions, transient elevations in hepatic enzymes, and mild hypersensitivity reactions. Although the CREDIBLE-CR study reported increased mortality among patients with severe *A. baumannii* infections; subsequent analyses indicate that this observation was more likely related to disease severity and patient-specific factors than to cefiderocol toxicity (43). Nevertheless, careful patient selection, optimized dosing, and antimicrobial stewardship remain essential for maximizing therapeutic benefit.

Future perspectives. Future strategies to preserve cefiderocol activity should focus on optimizing antimicrobial stewardship, standardizing susceptibility testing methodologies, expanding genomic surveillance of emerging resistance determinants, and evaluating combination therapies against difficult-to-treat pathogens. Furthermore, a deeper understanding of bacterial iron acquisition pathways may facilitate the rational design of next-generation siderophore antibiotics capable of overcoming current resistance mechanisms while preserving the advantages of active transporter-mediated drug delivery (61).

Overall, cefiderocol represents one of the most innovative advances in modern β -lactam development by integrating siderophore-mediated active transport with broad stability against clinically important β -lactamases. Nevertheless, its long-term clinical utility is increasingly challenged by adaptive remodelling of iron acquisition pathways, evolution of β -lactamases, multidrug efflux, target modifications, and methodological limitations in susceptibility testing. These observations suggest that even highly innovative antibiotics remain vulnerable to rapid bacterial adaptation. Sustained genomic surveillance, optimized antimicrobial stewardship, and

continued mechanism-guided antibiotic development will therefore be essential to preserve cefiderocol efficacy and maintain its role in the treatment of multidrug-resistant Gram-negative infections.

CARBAPENEMS

The period between 2001 and 2020 witnessed the introduction and expanded clinical adoption of several carbapenems, including ertapenem, biapenem, and doripenem, which were developed to address the growing burden of multidrug-resistant Gram-negative infections. These agents initially provided important therapeutic advances through broad-spectrum activity, enhanced stability against many β -lactamases, and improved efficacy against Enterobacterales, *P. aeruginosa*, and anaerobic pathogens. However, despite differences in antimicrobial spectrum and pharmacological properties, all three agents have ultimately followed a similar resistance trajectory.

Across Gram-negative bacteria, carbapenem resistance is driven by a convergence of mechanisms, including carbapenemase production, porin loss or modification, efflux pump overexpression, and derepression of chromosomal β -lactamases. In Enterobacterales, resistance frequently arises from the combination of extended-spectrum β -lactamase or AmpC production with reduced outer-membrane permeability, whereas acquisition of carbapenemases such as KPC, NDM, VIM, and OXA-48-like enzymes confers high-level resistance (62, 63). Likewise, in *P. aeruginosa* and *A. baumannii*, OprD loss, multidrug efflux systems, and dissemination of metallo- β -lactamases and OXA-type carbapenemases have substantially compromised carbapenem activity (64, 65). The rapid global spread of these mechanisms has progressively eroded the clinical utility of carbapenems, exemplifying how bacterial adaptation can outpace antibiotic innovation and driving the subsequent development of next-generation β -lactam- β -lactamase inhibitor combinations and other anti-Gram-negative therapies.

NEXT GENERATION TETRACYCLINES

The development of next-generation tetracyclines between 2005 and 2018 represented a major advance in overcoming classical tetracycline resistance

among Gram-negative pathogens. Structural modification at the C9 position of the tetracycline nucleus gave rise to the glycylcycline tigecycline (U.S. FDA approval, 2005), the aminomethylcycline omadacycline (U.S. FDA approval, 2018), and the fluorocycline eravacycline (U.S. FDA approval, 2018). These structural innovations strengthen ribosomal binding while substantially reducing susceptibility to the classical Tet(A)-mediated efflux and Tet(M)-mediated ribosomal protection mechanisms that limit earlier tetracyclines (66, 67). Although sarecycline, another aminomethylcycline approved in 2018, belongs to the same structural lineage, it was primarily developed for dermatological infections and has limited relevance to multidrug-resistant Gram-negative pathogens; therefore, it is not discussed further (68).

Like conventional tetracyclines, these agents inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit, thereby preventing aminoacyl-tRNA accommodation at the ribosomal A site. However, their structural modifications enhance ribosomal affinity and reduce displacement by ribosomal protection proteins, extending activity against many resistant organisms. Among these agents, tigecycline demonstrates the broadest activity against multidrug-resistant Enterobacterales and *A. baumannii*, whereas omadacycline offers the additional advantages of oral bioavailability, improved pharmacokinetic properties, and greater therapeutic flexibility (69). Eravacycline further expands this class through enhanced potency against carbapenem-resistant Enterobacterales and *A. baumannii* (70). Nevertheless, all three agents exhibit limited intrinsic activity against *P. aeruginosa*, primarily because of low outer membrane permeability and efficient multidrug efflux systems. Despite these structural advances, resistance continues to evolve through several complementary mechanisms. Efflux-mediated resistance remains the predominant determinant in Gram-negative bacteria, with tigecycline, omadacycline, and eravacycline serving as substrates for resistance-nodulation-division (RND) efflux pumps, including AcrAB-TolC in Enterobacterales and MexAB-OprM and MexXY in *P. aeruginosa*. Overexpression of these transporters, often resulting from mutations in global regulatory genes, markedly reduces intracellular antibiotic concentrations. Although C9 substitutions substantially diminish the impact of classical ribosomal protection proteins such as Tet(M), high-level expression of these pro-

teins or their coexistence with additional resistance determinants may still contribute to reduced susceptibility (71).

More recently, enzymatic inactivation mediated by Tet(X) flavin-dependent monooxygenases has emerged as one of the most concerning resistance mechanisms affecting next-generation tetracyclines. Tet(X) enzymes directly hydroxylate and inactivate tigecycline, omadacycline, eravacycline, and related derivatives, effectively abolishing their antibacterial activity. The rapid dissemination of plasmid-mediated *tet(X)* variants among Enterobacterales and *A. baumannii* has heightened concerns regarding the horizontal transfer and global spread of resistance (72). Collectively, next-generation tetracyclines exemplify how rational structural optimization has successfully extended the therapeutic lifespan of an established antibiotic class. However, the continuing evolution of multidrug efflux systems, persistence of ribosomal protection mechanisms, and global dissemination of Tet(X) enzymes illustrate that even extensively optimized tetracycline derivatives remain susceptible to bacterial adaptation, reinforcing the need for continued surveillance, antimicrobial stewardship, and development of future tetracycline analogues capable of overcoming emerging resistance mechanisms.

PLAZOMICIN

Plazomicin, approved by the U.S., FDA in 2018, is a next-generation semisynthetic aminoglycoside developed to restore activity against multidrug-resistant Gram-negative pathogens. Aminoglycosides exert their bactericidal effect through irreversible binding to the bacterial 30S ribosomal subunit, inducing misreading of mRNA and inhibition of protein synthesis. However, their clinical utility has historically been limited by widespread enzymatic inactivation and permeability barriers.

Plazomicin was rationally engineered with targeted structural modifications, including the removal of hydroxyl groups at the C-3' and C-4' positions, the incorporation of a hydroxyethyl substituent at C-6', and the addition of a hydroxyaminobutyric acid (HABA) side chain at N-1 (73-75). These modifications markedly reduce recognition by most aminoglycoside-modifying enzymes (AMEs) and enhance stability against enzymatic degradation. Consequently,

plazomicin retains potent in vitro and clinical activity against multidrug-resistant Enterobacterales, including ESBL- and carbapenemase-producing strains.

Despite these improvements, resistance to plazomicin arises through multiple convergent mechanisms. The most clinically significant mechanism involves 16S rRNA methyltransferases (e.g., ArmA, RmtA, RmtB, RmtC), which methylate the aminoglycoside-binding A-site of the 30S ribosomal subunit, conferring high-level cross-resistance across virtually all clinically relevant aminoglycosides (75).

Beyond enzymatic mechanisms, decreased intracellular accumulation also contributes to reduced activity. This includes reduced membrane permeability in Gram-negative pathogens, alterations in outer membrane transport systems, and, in some species, upregulation of multidrug efflux systems, which collectively limit drug entry and intracellular concentration. Furthermore, adaptive mutations affecting ribosomal structure or function may further modulate susceptibility, particularly under prolonged antimicrobial exposure or high-inoculum infection settings.

Clinically, resistance emergence is most often associated with the acquisition of 16S rRNA methyltransferase genes in high-risk Enterobacterales lineages, which rapidly negates the benefit of plazomicin even in the absence of extensive enzymatic modification. This highlights that while plazomicin successfully circumvents classical AME-mediated resistance, its long-term efficacy remains constrained by target-site modification and multifactorial reductions in intracellular drug exposure.

Collectively, plazomicin represents a rationally redesigned aminoglycoside that restores activity against many enzyme-producing Gram-negative pathogens; however, its clinical durability is ultimately limited by the emergence of ribosomal methylation and additional permeability- and accumulation-related resistance mechanisms.

Revolutionizing Antimicrobial Therapy: Emerging Antibiotics and Mechanisms

As of 2020, the antibiotic pipeline emphasizes developing therapeutics targeting key bacterial processes, with varying progress across mechanisms. Notably, novel agents like zoliflodacin and gepotidacin inhibit bacterial type II topoisomerases, disrupting DNA replication and transcription. These efforts aim to combat resistance with novel mechanisms.

DNA unwinding enzyme inhibitors are under

clinical investigation. The bacterial type II topoisomerases, DNA gyrase and topoisomerase IV, remain highly attractive targets for antibacterial drug development because of their essential roles in DNA replication and chromosome segregation. Unlike fluoroquinolones, which stabilize DNA–enzyme cleavage complexes through interactions with conserved quinolone resistance-determining region (QRDR) residues, zoliflodacin and gepotidacin bind distinct sites on these enzymes, thereby largely circumventing established fluoroquinolone resistance mechanisms and retaining activity against many quinolone-resistant pathogens.

Zoliflodacin (ETX0914) is a first-in-class spiropyrimidinetrione antibiotic that selectively inhibits the GyrB subunit of DNA gyrase. Although its antibacterial spectrum is relatively narrow, it demonstrates potent activity against *Neisseria gonorrhoeae*, including multidrug-resistant and fluoroquinolone-resistant isolates. By binding outside the classical fluoroquinolone-binding pocket, zoliflodacin retains activity against strains harboring the canonical *gyrA* and *parC* mutations responsible for quinolone resistance. Phase III clinical trials demonstrated non-inferiority to the current standard of care for uncomplicated urogenital gonorrhoea, leading to U.S. FDA approval in December 2025.

Gepotidacin is the first approved member of the triazaacenaphthylene class and has a broader antibacterial spectrum than zoliflodacin. In addition to its activity against *N. gonorrhoeae*, it demonstrates clinically relevant efficacy against several Gram-negative uropathogens, particularly *E. coli*, including many fluoroquinolone-resistant isolates that cause uncomplicated urinary tract infections (uUTIs). Gepotidacin inhibits bacterial DNA replication through a unique mechanism involving simultaneous interaction with DNA gyrase and topoisomerase IV at binding sites distinct from those targeted by fluoroquinolones, thereby substantially reducing cross-resistance with existing quinolone agents. Phase III clinical trials confirmed its efficacy for both uncomplicated urinary tract infections and gonorrhoea, resulting in U.S. FDA approval for uncomplicated urinary tract infections in March 2025, followed by approval for uncomplicated urogenital gonorrhoea in December 2025 (79-81).

Collectively, zoliflodacin and gepotidacin represent the first clinically successful non-fluoroquinolone bacterial topoisomerase inhibitors introduced

in several decades. Their ability to retain activity against fluoroquinolone-resistant pathogens by engaging alternative topoisomerase-binding sites validates mechanism-divergent inhibition as an effective strategy for overcoming QRDR-mediated resistance. Although their current clinical indications are relatively limited, these agents establish an important proof-of-concept for the development of next-generation antibacterials targeting multidrug-resistant Gram-negative pathogens. More importantly, their successful clinical translation demonstrates that rational redesign of established antibacterial targets can overcome decades of accumulated fluoroquinolone resistance, providing a valuable framework for future Gram-negative antibiotic discovery and reinforcing the concept that innovation in target engagement, rather than target selection alone, will be critical for sustaining the antibacterial pipeline.

Cell wall inhibiting antibacterial agents under clinical investigation. SPR206 and SPR741 are intravenously administered candidate antibiotics from Spero Therapeutics' Potentiator Platform, designed to treat MDR Gram-negative bacterial infections by disrupting the bacterial outer membrane, thereby enhancing antibiotic efficacy. A Phase 1 trial confirmed safety and PK, supporting further development. SPR741 has completed Phase 1 clinical studies, demonstrating a favorable safety profile and the potential to expand the spectrum and enhance the potency of existing antibiotics (82).

Benapenem (Sihuan Pharmaceutical, Phase 2) is a broad-spectrum carbapenem antibiotic independently developed by Xuanzhu Biopharm, a subsidiary of Sihuan Pharmaceutical Holdings Group. It has completed Phase II clinical trials for the treatment of complicated urinary tract infections in China and is expected to initiate Phase III clinical trials soon. Benapenem shows strong efficacy, a long half-life, and supports once-daily dosing (83).

PBP and β -lactamase inhibition combined is a new strategy against MDR pathogens. Cefepime/enmetazobactam targets ESBL-Enterobacterales; cefepime/taniborbactam acts against CRE, including NDM-1 and VIM strains (84). Neither combination targets *A. baumannii* effectively. For *A. baumannii*, sulbactam/durlobactam inhibits Class A/C/KPC/OXA enzymes, offering targeted treatment (85). Zidebactam plus cefepime targets PBP2 and class A/C β -lactamases and recently completed a pivotal Phase

3 clinical trial for the treatment of cUTI. Cefepime/zidebactam showed potent efficacy against MDR/XDR pathogens under compassionate use (86-88).

Despite progress, many candidates remain in early clinical phases, highlighting challenges in advancing truly innovative antibiotics. New antibiotics alone cannot curb AMR. Sustaining their effectiveness depends on how wisely they are used, making surveillance and stewardship central pillars of resistance management. Surveillance and stewardship programs play a key role in monitoring resistance trends and optimizing antibiotic utilization to preserve their efficacy.

Surveillance and stewardship. Antimicrobial resistance (AMR) surveillance and stewardship programs are essential for monitoring resistance trends and promoting appropriate antibiotic use, thereby mitigating the acceleration of resistance. Resistance often emerges from latent mechanisms, highlighting the need for continuous monitoring.

GLOBAL SURVEILLANCE INITIATIVES

The World Health Organization's Global Antimicrobial Resistance and Use Surveillance System (GLASS), launched in 2015, collects standardized AMR data from participating countries. By 2020, GLASS had enrolled 92 countries and territories, with 66 reporting AMR data involving over two million patients. The 2020 GLASS report highlighted high resistance rates among antimicrobials commonly used to treat prevalent bacterial infections (89).

AMR surveillance in India. In India, the Indian Council of Medical Research (ICMR) established the Antimicrobial Resistance Surveillance and Research Network (AMRSN) in 2013 to monitor resistance patterns across hospitals. ICMR's 2020 annual report indicated that carbapenem resistance among *K. pneumoniae* isolates increased from 46% in 2018 to 57% in 2020. *A. baumannii* exhibited resistance rates exceeding 80%, and *P. aeruginosa* showed a meropenem resistance rate of 32%, reaching up to 50% in tertiary hospitals (90).

Impact on clinical practices and policy. AMR surveillance data inform clinicians' empiric antibiotic choices, tailoring treatments to regional resistance

patterns. Additionally, surveillance insights guide pharmaceutical research and development by identifying critical areas needing new antibiotics.

Comprehensive AMR data remain limited, especially in low-resource settings. Stronger laboratories, better data, and global sharing are key to effective surveillance. Strengthening these efforts is crucial to combating the AMR crisis and preserving antibiotic efficacy.

FUTURE PERSPECTIVES

Innovations in antimicrobial strategies. Even with improved surveillance, the need for disruptive innovation remains. The final section explores transformative approaches - AI, phages, and CRISPR - that could redefine antimicrobial strategies in the decades ahead. With antimicrobial resistance rising, alternative approaches beyond conventional antibiotics are crucial. Emerging strategies such as AI-driven drug discovery, bacteriophage therapy, and CRISPR-based antimicrobials offer promising solutions against multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens.

AI-driven antibiotic discovery. Artificial intelligence (AI) accelerates antibiotic discovery by screening large molecular datasets, predicting resistance patterns, and optimizing drug structures. Stokes et al. (2020) used deep learning to identify halicin, a novel antibiotic effective against MDR *A. baumannii* and *K. pneumoniae*. AI also predicts resistance and guides pre-emptive drug design. These advancements could lead to faster, more efficient drug development with personalized antimicrobial therapy (4).

Phage therapy: a targeted alternative. Bacteriophages selectively lyse bacteria, minimizing microbiome disruption. Clinical success was observed in MDR *A. baumannii* infections unresponsive to antibiotics. Trials, such as Jault et al. (2019), demonstrate phage therapy against MDR *P. aeruginosa* (91). However, host immunity and bacterial resistance pose challenges. Advances in synthetic biology allow for engineered phages with enhanced stability and a broader host range.

CRISPR-based antimicrobial strategies. CRISPR-Cas technology enables precise bacterial genome

editing, eliminating resistance genes. Citorik et al. (2014) restored β -lactam susceptibility in carbapenem-resistant *K. pneumoniae* using CRISPR-guided plasmids (92). Engineered CRISPR-Cas13a selectively targets *P. aeruginosa*, causing cell death (93). A phage-delivered CRISPR system successfully eradicated gut-colonizing *E. coli* in mice, highlighting its potential for AMR prevention. Challenges include efficient delivery methods; however, bacteriophage and lipid nanoparticle vectors are promising solutions (94).

In summary, integrating AI, phage therapy, and CRISPR-based antimicrobials marks a paradigm shift in AMR mitigation. AI expedites novel drug discovery, phages offer pathogen-specific treatment, and CRISPR enables precise bacterial eradication. Despite challenges in clinical translation and regulation, these technologies hold immense potential in next-generation antimicrobial strategies. Real-world use of these strategies depends on overcoming regulatory and economic barriers. A comprehensive, multidisciplinary approach is essential to ensure sustained progress in the fight against antimicrobial resistance.

CONCLUSION

As highlighted throughout this review, antimicrobial resistance (AMR) continues to undermine the clinical utility of existing antibiotics and remains one of the greatest threats to global health. The period from 2001 to 2020 was marked by significant advances in anti-Gram-negative drug development, predominantly through the optimization of established antibiotic classes rather than the discovery of entirely new scaffolds. The introduction of novel β -lactam/ β -lactamase inhibitor combinations, cefiderocol with its siderophore-mediated uptake mechanism, next-generation tetracyclines, and emerging bacterial topoisomerase inhibitors illustrates how medicinal chemistry and innovative therapeutic strategies have been leveraged to overcome evolving resistance mechanisms.

Despite these advances, the pace of bacterial adaptation continues to outstrip antibiotic innovation, particularly among carbapenem-resistant Gram-negative pathogens and metallo- β -lactamase producers, underscoring the persistent gaps in the current antibacterial pipeline. Future research should priori-

tise the discovery of novel antibacterial targets, the development of broad-spectrum β -lactamase inhibitors active against metallo- β -lactamases, improved strategies to overcome permeability barriers and efflux-mediated resistance, and the integration of artificial intelligence, structure-guided drug design, CRISPR-based antimicrobials, bacteriophage therapy, and rapid diagnostics to accelerate antibacterial discovery and optimize precision therapy.

Ultimately, combating AMR will require coordinated efforts that integrate scientific innovation, sustained investment in antibiotic research, robust antimicrobial stewardship, advanced surveillance systems, and supportive public health policies. Such a multidisciplinary approach will be essential to preserve the effectiveness of existing antibiotics while ensuring a sustainable pipeline of future therapies against multidrug-resistant Gram-negative pathogens.

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