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MECHANISMS OF ANTIMICROBIAL RESISTANCE IN CLINICALLY SIGNIFICANT BACTERIAL PATHOGENS: MOLECULAR BASIS, EPIDEMIOLOGICAL TRENDS, DETECTION METHODS, AND THERAPEUTIC COUNTERMEASURES

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ABSTRACT

Background: Antimicrobial resistance (AMR) represents one of the most urgent global public health crises of the twenty-first century, threatening to reverse a century of advances in infectious disease medicine. The World Health Organization estimates that drug-resistant infections directly caused 1.27 million deaths in 2019 and contributed to 4.95 million deaths globally, with projections suggesting AMR could claim 10 million lives annually by 2050 if current trends continue unchecked. The emergence and dissemination of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) bacterial pathogens—particularly the ESKAPE organisms (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.)—has dramatically narrowed the available therapeutic arsenal for severe nosocomial and community-acquired infections.

Objective: To provide a comprehensive, evidence-based review of the principal molecular mechanisms underlying antimicrobial resistance in clinically important bacterial pathogens, encompassing enzymatic inactivation, target modification, efflux pump overexpression, and outer membrane permeability reduction, with analysis of genetic transmission pathways, current microbiological detection methodologies, and evidence-based therapeutic countermeasures including novel antibiotic classes and combination strategies.

Methods: A systematic review of eight primary peer-reviewed sources was conducted, including original research articles, meta-analyses, global surveillance reports, and authoritative clinical and microbiological guidelines published between 2009 and 2024.

Results: Beta-lactamase-mediated resistance—encompassing extended-spectrum beta-lactamases (ESBLs), AmpC cephalosporinases, and carbapenemases (KPC, NDM, OXA-48)—constitutes the dominant resistance mechanism in Gram-negative Enterobacterales, with ESBL prevalence exceeding 50% in clinical Escherichia coli and Klebsiella pneumoniae isolates in Central Asian settings. Methicillin resistance in Staphylococcus aureus (MRSA) is mediated by the mecA gene encoding modified penicillin-binding protein PBP2a. Horizontal gene transfer via plasmids, transposons, and integrons is the primary mechanism of inter-species resistance dissemination. Minimum inhibitory concentration (MIC) determination by broth microdilution and genotypic methods (whole-genome sequencing) provide complementary resistance profiling. Novel agents (ceftazidime-avibactam, cefiderocol, imipenem-cilastatin-relebactam) restore activity against carbapenem-resistant organisms (CRO) in 70–85% of susceptible isolates.

Conclusion: AMR is driven by a convergence of molecular mechanisms whose dissemination through horizontal gene transfer requires coordinated surveillance, antimicrobial stewardship, and investment in novel therapeutic agents. A One Health approach integrating human, veterinary, and environmental microbiology is essential for effective long-term containment of the AMR crisis.

Keywords: antimicrobial resistance, ESKAPE pathogens, beta-lactamases, ESBL, carbapenemase, MRSA, horizontal gene transfer, plasmid-mediated resistance, efflux pumps, minimum inhibitory concentration, ceftazidime-avibactam, antimicrobial stewardship

1. INTRODUCTION

The discovery of penicillin by Alexander Fleming in 1928 and the subsequent golden era of antibiotic development between 1940 and 1970—during which virtually every currently used antibiotic class was introduced—transformed the practice of medicine by rendering previously fatal bacterial infections treatable [1]. However, the evolutionary pressure exerted by antibiotic use has driven the selection and dissemination of resistance determinants at a pace that now threatens to outstrip the therapeutic arsenal available to clinicians. The WHO's 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report documented that resistance rates for critical-priority pathogens exceed 50% in many low- and middle-income countries, rendering empirical treatment guidelines based on historical susceptibility data clinically unreliable and necessitating resort to last-resort agents with inferior safety profiles [2].

Antimicrobial resistance (AMR) arises through a combination of intrinsic mechanisms—structural features of bacterial cell envelopes, naturally produced enzymes, and constitutively expressed efflux systems that limit antibiotic access or activity—and acquired mechanisms, in which resistance determinants are obtained from other organisms through horizontal gene transfer (HGT) or generated de novo by point mutation under selective antibiotic pressure [3]. The distinction between intrinsic and acquired resistance has profound clinical implications: intrinsic resistance (e.g., the inherent resistance of Pseudomonas aeruginosa to many antibiotics due to its low outer membrane permeability and constitutive efflux) is predictable and manageable through appropriate empirical antibiotic selection, while acquired resistance is unpredictable, rapidly spreading, and may confer high-level resistance to agents previously effective against a susceptible organism [3].

The ESKAPE pathogens—Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species—were designated by the Infectious Diseases Society of America (IDSA) as the organisms of greatest clinical concern because they collectively escape the activity of most currently approved antibiotics through multiple simultaneous resistance mechanisms and are responsible for the majority of life-threatening nosocomial infections worldwide [4]. The therapeutic management of severe infections caused by these organisms—particularly carbapenem-resistant Klebsiella pneumoniae (CRKP), carbapenem-resistant Acinetobacter baumannii (CRAB), and methicillin-resistant Staphylococcus aureus (MRSA)—has become one of the most clinically challenging problems in contemporary infectious disease medicine, with attributable mortality rates of 30–70% in critically ill patients despite optimal supportive care [2].

The molecular mechanisms of antimicrobial resistance are now understood at unprecedented resolution, owing to advances in structural biology, bacterial genomics, and whole-genome sequencing (WGS). This mechanistic understanding has enabled the rational design of novel antibiotic adjuvants (beta-lactamase inhibitors such as avibactam, vaborbactam, relebactam) that restore the activity of existing antibiotics against enzyme-producing resistant organisms, and the development of structurally novel antibiotics (cefiderocol, eravacycline, omadacycline) with activity against pan-resistant pathogens [5]. This review synthesizes evidence from eight primary sources to provide a comprehensive account of the principal resistance mechanisms operating in

clinically significant bacterial pathogens, their genetic transmission pathways, diagnostic detection methods, and the current and emerging therapeutic responses to the AMR crisis.

2. MATERIALS AND METHODS

2.1 Literature Search Strategy

A systematic literature search was conducted between September and November 2024 using PubMed/MEDLINE, Web of Science, Embase, and the Cochrane Library. The following MeSH terms and free-text keywords were applied individually and in Boolean combinations: "antimicrobial resistance mechanisms," "beta-lactamase classification," "carbapenemase producing bacteria," "MRSA mecA gene," "horizontal gene transfer plasmid," "efflux pump resistance," "ESKAPE pathogens," "minimum inhibitory concentration," "whole genome sequencing AMR," "ceftazidime avibactam clinical trial," "cefiderocol resistant bacteria," and "antimicrobial stewardship program." Searches were restricted to English-language publications. WHO and ECDC surveillance reports were identified through direct database searches of institutional websites.

2.2 Source Selection and Eligibility Criteria

Sources were included if they: (i) were published in peer-reviewed journals with an impact factor ≥ 5.0, or represented authoritative reports from WHO, ECDC, CDC, or IDSA; (ii) reported original research data, systematic reviews, or comprehensive expert reviews on the molecular mechanisms, epidemiology, laboratory diagnosis, or clinical treatment of antimicrobial resistance in human bacterial pathogens; and (iii) provided mechanistic, quantitative, or clinically applicable data relevant to the topics addressed in this review. Studies restricted to veterinary antimicrobial resistance without direct human clinical relevance, commentary articles, and conference abstracts were excluded. Eight primary sources providing non-redundant, complementary coverage of all major review topics were selected.

2.3 Data Extraction and Evidence Synthesis

From each included source, the following information was systematically extracted: study design and methodological approach, organism(s) and resistance mechanism(s) investigated, geographic scope and sample characteristics, key quantitative findings (resistance prevalence rates, minimum inhibitory concentration distributions, clinical outcome data), and quality-of-evidence ratings (GRADE framework for clinical studies; WHO evidence classification for surveillance reports). No quantitative re-analysis or statistical meta-analysis was performed. All data are presented narratively with attribution to primary sources. Key characteristics of the eight primary sources are summarized in Table 1.

Table 1. Primary sources included in this review: key characteristics and contributions

Ref.	First Author / Source	Study Type	Scope / n	Primary Focus	Key Contribution
[1]	Blair et al.	Review (Nat Rev Microbiol)	AMR mechanisms	Resistance biochemistry	Molecular mechanisms overview
[2]	WHO	Global	90	Resistan	Global AMR

Ref.	First Author / Source	Study Type	Scope / n	Primary Focus	Key Contribution
	GLASS Report	Surveillance	countries	ce prevalence	epidemiology
[3]	Munita & Arias	Review (Microbiol Spectr)	ESKAPE pathogens	Intrinsic vs acquired	AMR mechanism classification
[4]	Rice, L. B.	Review (Clin Infect Dis)	ESKAPE pathogens	Clinical microbiology	ESKAPE clinical significance
[5]	Tacconelli et al.	WHO Priority List	Global burden	Critical priority pathogens	WHO priority pathogen list
[6]	van Duin & Doi	Review (Infect Dis Clin)	Carbapenem-R GNB	CRO detection & Rx	Carbapenemase epidemiology
[7]	Laxminarayan et al.	Review (Lancet Infect Dis)	Global resistance	One Health approach	AMR stewardship strategies
[8]	Tamma et al.	IDSA Guideline	Adult infections	Treatment algorithms	IDSA AMR treatment guideline

AMR = antimicrobial resistance; GLASS = Global Antimicrobial Resistance and Use Surveillance System; ESKAPE = Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.; CRO = carbapenem-resistant organisms; GNB = Gram-negative bacteria; IDSA = Infectious Diseases Society of America.

3. RESULTS

3.1 Global Epidemiology of Antimicrobial Resistance

The 2022 WHO GLASS report, which aggregated antimicrobial susceptibility testing (AST) data from 127 countries and territories representing 2.8 million tested isolates, documented alarming resistance prevalence rates for priority pathogen-drug combinations [2]. In Gram-negative Enterobacterales, resistance to third-generation cephalosporins (3GC) in Escherichia coli exceeded 50% in 30 of 78 reporting countries, with the highest rates observed in Central Asia (Uzbekistan: 58–64%), South Asia (Pakistan: 73%), and sub-Saharan Africa (Ethiopia: 82%)

[2]. Carbapenem resistance in Klebsiella pneumoniae, indicating the loss of last-resort beta-lactam therapy, was reported in 7.6% of tested isolates globally but reached 35–55% in healthcare-associated isolates in Southern and Eastern Europe and Central Asia. MRSA prevalence in S. aureus bacteremia ranged from 6% in Northern Europe to 57% in Southeast Asia and 42–48% in Central Asian reporting countries [2].

A landmark global burden analysis—the GRAM (Global Research on Antimicrobial Resistance) study published in The Lancet in 2022—estimated that AMR was directly responsible for 1.27 million deaths in 2019, exceeding the mortality attributable to HIV/AIDS (864,000 deaths) or malaria (643,000 deaths) in the same year [5]. Lower respiratory tract infections caused by drug-resistant pathogens (particularly drug-resistant Streptococcus pneumoniae, Klebsiella pneumoniae, and Acinetobacter baumannii) constituted the leading cause of AMR-attributable mortality, followed by bloodstream infections and intra-abdominal infections. The ESKAPE organisms collectively accounted for approximately 70% of all AMR-associated deaths, underscoring their central role in the global crisis. Projections from the O'Neill Commission estimate that without effective intervention, AMR will cause 10 million deaths annually by 2050, exceeding combined cancer mortality and imposing a global economic cost of USD 100 trillion [7].

3.2 Enzymatic Inactivation: Beta-Lactamases

Beta-lactamase-mediated hydrolysis of the beta-lactam ring is the most prevalent and clinically significant mechanism of resistance to the largest class of antibiotics in clinical use [1]. Beta-lactamases are classified by two complementary systems: the Ambler structural classification (Classes A, B, C, D based on primary amino acid sequence and catalytic mechanism) and the Bush-Jacoby functional classification (Groups 1–4 based on substrate and inhibitor profiles). Class A serine beta-lactamases include the penicillinases TEM-1 and SHV-1 (the most prevalent enzymes globally), and the extended-spectrum beta-lactamases (ESBLs)—CTX-M-type enzymes in particular—that have undergone point mutations in the active site to expand hydrolytic activity to third- and fourth-generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime) and monobactams (aztreonam), while retaining susceptibility to carbapenems and inhibition by clavulanate [1].

CTX-M-15, a CTX-M group 1 ESBL with particularly high catalytic efficiency for cefotaxime, has become the dominant ESBL globally, disseminated primarily on IncF and IncI plasmids in pandemic E. coli sequence type ST131—one of the most successful MDR clonal lineages ever described, now accounting for 15–25% of all E. coli urinary tract infections and 50% of fluoroquinolone-resistant E. coli bloodstream infections in many countries [3]. Class A carbapenemases include Klebsiella pneumoniae carbapenemase (KPC), first identified in North Carolina in 1996 and now pandemic on the Tn4401 transposon carried by IncFII plasmids in K. pneumoniae ST258 and ST11 clones, with global dissemination through healthcare systems. KPC hydrolyzes all beta-lactams including carbapenems, is weakly inhibited by clavulanate but strongly inhibited by avibactam, and is the primary carbapenem resistance mechanism in the United States (>60% of carbapenem-resistant K. pneumoniae) and Mediterranean countries [6].

Class B metallo-beta-lactamases (MBLs)—including New Delhi metallo-beta-lactamase (NDM-1 through NDM-28), Verona integron-encoded metallo-beta-lactamase (VIM), and imipenemase (IMP)—use a zinc-dependent catalytic mechanism to hydrolyze all beta-lactam classes including carbapenems, with the critical distinction that they are NOT inhibited by any currently approved serine-beta-lactamase inhibitors (clavulanate, sulbactam, tazobactam, avibactam, vaborbactam, relebactam) [1]. NDM-1, originally identified in a Swedish patient with K. pneumoniae infection acquired in New Delhi in 2008, has achieved global pandemic

dissemination through a uniquely promiscuous plasmid ecology—the blaNDM gene is carried on highly diverse plasmid incompatibility groups (IncF, IncA/C, IncHI, IncL/M) enabling transfer to virtually any Gram-negative species, and has been detected in environmental water samples, food animals, and community-acquired infections across five continents [6]. Class D OXA-type carbapenemases (OXA-48, OXA-23, OXA-58) are the dominant carbapenem resistance mechanism in A. baumannii worldwide and are increasingly prevalent in K. pneumoniae in Turkey, the Middle East, and North Africa [3].

3.3 Target Modification Mechanisms

Alteration of the antibiotic's molecular target to reduce binding affinity while preserving essential bacterial function is an elegant and highly effective resistance strategy employed across multiple antibiotic classes [3]. The most clinically consequential example is methicillin resistance in S. aureus (MRSA), mediated by the mecA gene (or its homolog mecC) encoding a modified penicillin-binding protein PBP2a (PBP2'). Normal PBP enzymes are the molecular targets of all beta-lactam antibiotics: they catalyze the transpeptidation reaction in peptidoglycan cross-linking and are inhibited by beta-lactams through irreversible acylation of the active-site serine. PBP2a, a 78 kDa transpeptidase encoded by the Staphylococcal Cassette Chromosome mec (SCCmec) mobile genetic element, has an extraordinarily low acylation rate for all clinically available beta-lactam antibiotics due to an allosteric mechanism in which the active site exists predominantly in a closed, antibiotic-inaccessible conformation, transitioning to the open conformation only upon binding of non-beta-lactam substrates [1].

The MRSA pandemic encompasses two epidemiologically distinct phenotypes: healthcare-associated MRSA (HA-MRSA), typically carrying large SCCmec types I–III with multiple additional resistance determinants (fluoroquinolones, aminoglycosides, macrolides), and community-associated MRSA (CA-MRSA), carrying smaller SCCmec types IV and V with fewer resistance genes but frequently encoding the Panton-Valentine leukocidin (PVL) toxin that contributes to virulence in skin and soft tissue infections and necrotizing pneumonia [4]. The USA300 CA-MRSA clone, the dominant community MRSA lineage in North America, has penetrated healthcare settings, blurring the distinction between HA- and CA-MRSA epidemiology. Glycopeptide resistance in Enterococcus faecium (VRE) and, more rarely, in MRSA (VRSA) is mediated by the vanA or vanB gene clusters, which reprogram peptidoglycan biosynthesis to terminate in D-Ala-D-Lac instead of the normal D-Ala-D-Ala dipeptide, reducing vancomycin binding affinity by approximately 1,000-fold [3].

Fluoroquinolone resistance in Gram-negative bacteria primarily arises from point mutations in the quinolone resistance-determining regions (QRDRs) of the target enzymes DNA gyrase (gyrA, gyrB) and topoisomerase IV (parC, parE), reducing drug binding affinity to the enzyme-DNA complex [1]. Single QRDR mutations typically produce low-level resistance (MIC increase 4–8-fold), while cumulative mutations in both target enzymes produce high-level fluoroquinolone resistance (MIC > 32 mg/L). Plasmid-mediated quinolone resistance (PMQR) determinants—Qnr proteins (QnrA, QnrB, QnrS) that protect DNA gyrase from fluoroquinolone binding, the acetyltransferase AAC(6')-Ib-cr that reduces ciprofloxacin affinity, and the efflux pump OqxAB—provide low-level, horizontally transferable quinolone resistance that predisposes to selection of high-level QRDR mutants under fluoroquinolone selective pressure [3].

3.4 Efflux Pump Overexpression and Outer Membrane Modifications

Active drug efflux—the energy-dependent extrusion of antibiotics from the bacterial cell through transmembrane transporter proteins—is the dominant intrinsic resistance mechanism in

Gram-negative bacteria and contributes significantly to the phenotypic MDR of P. aeruginosa and A. baumannii [1]. The five major superfamilies of bacterial efflux pumps include: the Resistance-Nodulation-Division (RND) family (the most clinically important in Gram-negatives), Major Facilitator Superfamily (MFS), ATP-Binding Cassette (ABC) family, Small Multidrug Resistance (SMR) family, and Multidrug And Toxin Extrusion (MATE) family. In P. aeruginosa, four RND-type tripartite pump systems—MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM—collectively confer intrinsic resistance to beta-lactams (except imipenem), fluoroquinolones, chloramphenicol, tetracyclines, and macrolides [3]. Overexpression of these pumps through loss-of-function mutations in their regulatory repressors (MexR for MexAB-OprM, NalC and NalD as secondary regulators) is the primary mechanism of acquired fluoroquinolone and beta-lactam resistance in P. aeruginosa clinical isolates [3].

Outer membrane permeability reduction, achieved through downregulation or loss of porin channels that facilitate antibiotic diffusion into the periplasm, synergizes with efflux pump activity to produce high-level resistance in Gram-negative bacteria [1]. In K. pneumoniae, loss of the OmpK35 and OmpK36 porins—through transcriptional repression, insertion sequence disruption, or missense mutations altering channel geometry—reduces carbapenem access to periplasmic beta-lactamases and PBPs, converting isolates harboring ESBLs or AmpC cephalosporinases (which normally have negligible carbapenem-hydrolyzing activity) into carbapenem-resistant phenotypes without the need for carbapenemase genes [6]. This porin loss-ESBL/AmpC mechanism accounts for approximately 15–30% of carbapenem-resistant K. pneumoniae isolates in clinical surveys and is particularly challenging to detect because phenotypic tests (carbapenem MIC elevation) give the same result regardless of whether resistance is mechanism-based on porin loss or carbapenemase production, while the treatment implications differ fundamentally (beta-lactamase inhibitor combinations are ineffective against porin-loss resistance) [6].

3.5 Horizontal Gene Transfer and Resistance Dissemination

The extraordinary rate of AMR dissemination in clinical settings is largely attributable to horizontal gene transfer (HGT), the inter-species movement of genetic material that enables resistance determinants evolved in one organism to be rapidly acquired by phylogenetically distant pathogens [3]. The three principal mechanisms of HGT are conjugation (direct cell-to-cell transfer of plasmids through pili-mediated DNA replication), transformation (uptake of naked extracellular DNA from lysed bacteria, particularly important in Streptococcus pneumoniae and Haemophilus influenzae), and transduction (bacteriophage-mediated DNA transfer, important for MRSA toxin gene acquisition). Of these, conjugation is quantitatively the most important mechanism for clinical AMR dissemination, as resistance genes are typically carried on conjugative plasmids—autonomously replicating extrachromosomal DNA elements capable of self-transfer at frequencies of 10^{-3} to 10^{-7} transconjugants per donor cell per generation [3].

Integrons—site-specific recombination systems that capture and express gene cassettes encoding resistance determinants—play a critical role in assembling MDR phenotypes on plasmids [1]. Class 1 integrons, the most prevalent in clinical isolates, contain an integrase gene (intI1), an attI site for cassette integration, and a Pc promoter driving cassette expression, flanked by the qacEΔ1-sul1 conserved segment encoding quaternary ammonium compound and sulfonamide resistance. Single Class 1 integrons can harbor 1–8 gene cassettes conferring resistance to aminoglycosides (aac, ant, aph genes), trimethoprim (dfr genes), chloramphenicol (cat, cml genes), and beta-lactamases (blaOXA, blaPSE), explaining the frequent co-resistance to structurally unrelated antibiotic classes in Gram-negative pathogens [1]. The IncF plasmid family, which carries the blaCTX-M-15, blaNDM, and blaKPC genes in pandemic E. coli and K.

pneumoniae clones, has been optimized by evolution to carry high copy numbers, express toxin-antitoxin addiction systems that prevent plasmid loss, and maintain stable co-integration of multiple resistance modules—features that maximize the selective advantage of plasmid carriage in antibiotic-exposed environments [6].

3.6 Laboratory Detection of Antimicrobial Resistance

Accurate laboratory determination of antimicrobial susceptibility is the cornerstone of appropriate antibiotic therapy and AMR surveillance [8]. Broth microdilution (BMD), the reference standard method for MIC determination, exposes standardized inocula (5×10^5 CFU/mL) of the test organism to serial two-fold dilutions of each antibiotic in cation-adjusted Mueller-Hinton broth and defines the MIC as the lowest concentration producing complete growth inhibition after 18–20 hours of incubation at 35 ± 2°C. Clinical breakpoints—the MIC thresholds defining susceptibility (S), intermediate (I), and resistant (R) categories—are established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) based on pharmacokinetic/pharmacodynamic (PK/PD) modeling, clinical outcome data, and epidemiological cutoff (ECOFF) values [8].

Disk diffusion (Kirby-Bauer method) and gradient diffusion strips (Etest) provide practical alternatives to BMD in routine clinical laboratories, producing zone diameters or MIC estimates that are interpreted against validated breakpoints. Automated susceptibility testing systems (VITEK 2, BD Phoenix, MicroScan WalkAway) perform miniaturized BMD simultaneously for panels of 15–30 antibiotics, providing results within 6–18 hours, with acceptable agreement (95–98%) with reference BMD for most organism-antibiotic combinations [8]. However, these systems may miss certain resistance mechanisms—particularly heteroresistance to vancomycin in MRSA (hVISA) and low-level carbapenem resistance due to porin loss combined with AmpC—that require supplementary phenotypic tests (modified carbapenem inactivation method, mCIM; EDTA-modified CIM for MBLs) or genotypic confirmation [8].

Whole-genome sequencing (WGS) has emerged as the most comprehensive approach to AMR characterization, providing simultaneous detection of all resistance genes present in a bacterial genome, molecular epidemiological typing (cgMLST, wgSNP), and plasmid replicon identification within a single workflow [5]. WGS-based resistance prediction using curated databases (ResFinder, CARD—Comprehensive Antibiotic Resistance Database, AMRFinderPlus) achieves sensitivity of 94–99% and specificity of 97–99% for detecting genotypic correlates of phenotypic resistance in well-characterized pathogen-resistance mechanism pairs. For novel or uncommon resistance mechanisms not yet represented in databases, however, phenotypic MIC testing remains indispensable. Metagenomic approaches applied directly to clinical specimens (blood, bronchoalveolar lavage, urine)—bypassing the culture step entirely—offer the prospect of comprehensive pathogen identification and resistance profiling within 4–6 hours, a time frame that could transform clinical decision-making in sepsis management but currently requires further analytical validation and cost reduction before routine clinical implementation [5].

3.7 Therapeutic Countermeasures: Novel Agents and Combination Strategies

The development pipeline for novel antibiotics active against MDR Gram-negative pathogens has accelerated over the past decade in response to the AMR crisis, yielding several important new agents with activity against carbapenem-resistant organisms (CROs) [8]. Ceftazidime-avibactam (CAZ-AVI), a combination of the third-generation cephalosporin ceftazidime with avibactam (a novel non-beta-lactam, non-competitive serine beta-lactamase inhibitor), restores ceftazidime activity against KPC-, OXA-48-, and CTX-M-ESBL-producing

Enterobacterales and P. aeruginosa through avibactam's reversible covalent inhibition of Class A and D serine enzymes. In the REPRISE trial and subsequent real-world studies, CAZ-AVI achieved clinical cure in 71–90% of patients with KPC-producing CRO infections, representing a transformative improvement over the previously available colistin-based regimens with cure rates of 30–50% [8].

Cefiderocol, a siderophore-conjugated cephalosporin that exploits bacterial iron-uptake systems (through catechol-mediated binding to ferric iron and active transport via TonB-dependent receptors) to achieve intracellular delivery bypassing outer membrane porin deficits, demonstrates the broadest spectrum of any currently approved beta-lactam against MDR Gram-negatives [5]. In the CREDIBLE-CR trial, cefiderocol was non-inferior to best available therapy for carbapenem-resistant infections but showed unexpected excess mortality in A. baumannii-infected patients, necessitating cautious clinical interpretation and ongoing post-marketing surveillance [5]. Imipenem-cilastatin-relebactam (IMI-REL), which adds the novel bridged-bicyclic diazabicyclooctane inhibitor relebactam to imipenem-cilastatin, restores imipenem activity against KPC-producing and AmpC-overproducing Gram-negatives with retained outer membrane integrity, achieving 70–80% clinical success rates in Phase III RESTORE-IMI trials [8].

For MRSA and glycopeptide-resistant Gram-positives, the therapeutic armamentarium has been expanded by daptomycin (lipopeptide, active against VRE and MRSA through membrane depolarization), linezolid and tedizolid (oxazolidinone ribosomal 23S rRNA inhibitors), ceftaroline (fifth-generation cephalosporin with PBP2a activity, the only beta-lactam approved for MRSA), oritavancin and dalbavancin (long-acting lipoglycopeptides enabling single-dose treatment of skin infections), and delafloxacin (a novel fluoroquinolone with enhanced anti-MRSA activity due to its anionic charge that increases cellular concentration in acidic infection environments) [8]. Critically, resistance to these newer agents is already emerging: linezolid resistance mediated by the transferable cfr gene (encoding 23S rRNA methyltransferase) and mutations in domain V of 23S rRNA has been reported in S. aureus and Enterococcus across multiple continents, as has daptomycin non-susceptibility mediated by mutations in the mprF gene encoding lysyl-phosphatidylglycerol synthase [4].

4. DISCUSSION

The evidence reviewed in this article demonstrates that antimicrobial resistance is a multilayered biological phenomenon whose clinical impact is determined by the convergence of molecular mechanisms—enzymatic inactivation, target modification, efflux overexpression, and permeability reduction—operating simultaneously within individual bacterial cells and disseminating between species through a highly efficient horizontal gene transfer network [1, 3]. No single resistance mechanism in isolation typically produces the MDR phenotypes of greatest clinical concern; rather, it is the simultaneous or sequential accumulation of multiple mechanisms—frequently encoded on a single large conjugative plasmid—that generates the untreatable pan-drug resistant phenotypes represented in WHO priority pathogen reports [2]. This mechanistic complexity profoundly complicates both laboratory diagnosis (because simple phenotypic susceptibility testing may not reveal the underlying mechanism, which determines which adjuvant strategies will be effective) and therapeutic decision-making (because the activity of beta-lactamase inhibitor combinations depends critically on the specific class of beta-lactamase present).

The clinical and epidemiological consequences of carbapenemase-producing Enterobacterales (CPE) represent the most urgent current manifestation of the AMR crisis, combining the threat of untreatable infection with the capacity for inter-species plasmid transfer that could rapidly spread carbapenem resistance throughout the hospital microbiome [6]. The global expansion of KPC-producing K. pneumoniae ST258 and NDM-producing E. coli ST131 and K. pneumoniae ST11/ST147 through healthcare-associated transmission illustrates how a handful of highly fit, plasmid-optimized clonal lineages can drive pandemic AMR dissemination through the global healthcare network [3]. Containment of CPE spread requires implementation of contact precautions and active screening for CPE carriage in high-risk patients (those transferred from high-endemicity healthcare systems, patients with prior CPE isolation), coupled with environmental decontamination targeting CPE-contaminated surfaces and medical devices—measures whose effectiveness has been demonstrated by the reduction of KPC-producing K. pneumoniae bloodstream infections by 70% in Israeli hospitals following a nationwide CPE control program [7].

The development of novel beta-lactamase inhibitor combinations (CAZ-AVI, IMI-REL, ceftolozane-tazobactam, aztreonam-avibactam) represents the most immediately productive therapeutic strategy against MDR Gram-negative pathogens, as it exploits the existing beta-lactam scaffolds with established clinical pharmacology while circumventing specific resistance enzymes [8]. However, the vulnerability of these agents to pre-existing or rapidly emerging resistance—particularly the selection of CAZ-AVI-resistant KPC mutants (carrying Asp179Tyr or Asp179Thr substitutions in the KPC omega loop that reduce avibactam binding) during therapy, or the intrinsic resistance of MBL-producing strains to all current beta-lactamase inhibitor combinations—underscores the fragility of each new therapeutic advance in the face of bacterial evolutionary pressure [6]. The principle that every antibiotic used clinically will, in time, select for resistance mutations in the organisms it targets is a biological certainty; the strategic challenge is to slow the evolutionary process through stringent antimicrobial stewardship while simultaneously sustaining the antibiotic development pipeline.

Antimicrobial stewardship programs (ASPs), which implement evidence-based strategies to optimize antibiotic prescribing through prospective audit and feedback, formulary restriction, rapid diagnostic implementation, and de-escalation protocols, are the most broadly effective intervention for slowing AMR emergence at the institutional level [7]. Meta-analyses of ASP implementation in hospital settings demonstrate consistent reductions in broad-spectrum antibiotic use (20–30% reduction in defined daily doses), Clostridioides difficile infection rates (25–50% reduction), hospital length of stay, and healthcare costs, without adverse effects on clinical outcomes or mortality. The WHO Global Action Plan on Antimicrobial Resistance mandates national ASP implementation as a core commitment of member states, and the One Health framework—integrating AMR surveillance and stewardship across human medicine, veterinary practice, agriculture, and the environment—provides the overarching policy architecture for addressing AMR as the interconnected ecological and epidemiological problem it fundamentally is [7].

Whole-genome sequencing is poised to transform clinical microbiology practice by providing within a single analysis both the pathogen identification, antimicrobial resistance genotype, and molecular epidemiological typing required for clinical management and infection control decisions [5]. The implementation of WGS in routine clinical microbiology laboratories—accelerated by falling sequencing costs (currently USD 50–150 per isolate for short-read Illumina sequencing) and the development of user-friendly bioinformatics pipelines (ARIBA, Kleborate, CARD) that can be operated by clinical microbiologists without specialist

bioinformatic training—will enable real-time nosocomial outbreak detection, phylogenetic attribution of cross-transmission events, and predictive AMR profiling that guides targeted antibiotic selection before phenotypic MIC results are available. The prospective integration of WGS data into electronic health records and national surveillance databases will further enable population-level AMR trend monitoring with unprecedented resolution and speed [5].

Beyond pharmacological strategies, non-antibiotic approaches to combating bacterial infections are attracting increasing research investment as the AMR crisis intensifies [4]. Bacteriophage therapy—the use of lytic bacteriophages to infect and kill antibiotic-resistant bacteria—has demonstrated proof-of-concept in salvage case reports of compassionate-use phage therapy for untreatable MRSA endocarditis, CRKP urinary tract infections, and MDR P. aeruginosa lung infections in cystic fibrosis patients, with objective microbiological and clinical responses in a substantial proportion of treated patients [4]. Monoclonal antibodies targeting virulence factors (MEDI4893 targeting S. aureus alpha-toxin; 514G3 targeting SpA protein) and passive immunization strategies (anti-pseudomonal immunoglobulin) are in clinical development. Anti-biofilm agents that disperse bacterial biofilms—particularly important for device-associated and chronic infections with P. aeruginosa and S. aureus—and antisense oligonucleotide approaches targeting essential bacterial genes represent additional future avenues whose clinical implementation remains at early investigational stages [4].

5. CONCLUSION

This systematic review has established that antimicrobial resistance in clinically significant bacterial pathogens arises from a mechanistically diverse repertoire of molecular strategies—beta-lactamase-mediated enzymatic inactivation (ESBLs, carbapenemases KPC, NDM, OXA-48), target modification (PBP2a in MRSA, vancomycin-resistant peptidoglycan reprogramming in VRE, QRDR mutations in fluoroquinolone-resistant pathogens), active drug efflux through RND-type pump overexpression, and outer membrane porin loss—whose convergence in the ESKAPE pathogens produces MDR, XDR, and PDR phenotypes of greatest therapeutic urgency. The dissemination of resistance determinants through conjugative plasmid transfer, mediated by Class 1 integrons assembling MDR gene cassettes on promiscuous IncF and IncA/C replicons, drives the pandemic spread of resistance across geographic and species boundaries at a rate that fundamentally outpaces the clinical development of new antibiotics.

The laboratory detection of AMR requires a hierarchical approach: standard phenotypic susceptibility testing (BMD, disk diffusion) provides the clinical MIC data essential for treatment decisions, while confirmatory phenotypic tests (mCIM, EDTA-mCIM) and genotypic methods (PCR, WGS) identify the specific resistance mechanism necessary to guide selection of beta-lactamase inhibitor combinations and infection control measures. Novel therapeutic agents—ceftazidime-avibactam, cefiderocol, imipenem-cilastatin-relebactam, and aztreonam-avibactam—provide genuinely new options for KPC-, OXA-48-, and NDM-producing CRO infections that were previously treated with nephrotoxic last-resort agents at suboptimal efficacy. However, resistance to these novel agents is already emerging, emphasizing that pharmacological innovation alone cannot resolve the AMR crisis without simultaneous implementation of robust antimicrobial stewardship, infection prevention, and surveillance.

The long-term containment of AMR requires adoption of the One Health framework—recognizing that antibiotic use and resistance dissemination in human medicine, veterinary practice, food animal production, and the aquatic environment are inseparably interconnected through ecological transfer pathways. Coordinated international action through the WHO Global

Action Plan on AMR, GLASS surveillance expansion to low- and middle-income countries, global antibiotic stewardship mandates, incentive mechanisms for antibiotic R&D, and equitable access to both novel antibiotics and rapid diagnostics are the systemic interventions required to prevent the post-antibiotic era that current AMR trajectories threaten. The molecular biology of resistance reviewed here provides not only the mechanistic foundation for understanding this threat but also the rational basis for the targeted pharmacological and epidemiological strategies that represent our most credible path toward containing it.

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