

# Multidrug-resistant Gram-negative bacterial infections

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Multidrug-resistant Gram-negative bacterial infections cause significant morbidity and mortality globally. These pathogens easily acquire antimicrobial resistance (AMR), further highlighting their clinical significance. Third-generation cephalosporin-resistant and carbapenem-resistant Enterobacterales (eg, *Escherichia coli* and *Klebsiella* spp), multidrug-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter baumannii* are the most problematic and have been identified as priority pathogens. In response, several new diagnostic technologies aimed at rapidly detecting AMR have been developed, including biochemical, molecular, genomic, and proteomic techniques. The last decade has also seen the licensing of multiple antibiotics that have changed the treatment landscape for these challenging infections.

## Introduction

Antimicrobial resistance (AMR) is one of the most crucial public health challenges of the 21st century. In 2021, an estimated 4·71 million deaths were associated with bacterial AMR, with low-income and middle-income countries disproportionately affected.<sup>1,2</sup> Multidrug-resistant Gram-negative bacteria (MDR-GNB) are responsible for much of this threat. In recent decades, these pathogens have become a leading cause of both community and health-care-associated infections,<sup>3</sup> and until recently, the pipeline of new therapeutics for MDR-GNB was almost non-existent. Multiple factors have contributed to the expansion of MDR-GNB resistance, including the misuse and overuse of antibiotics in both human and animal health, the absence of clean water and sanitation, increasing complexity of medical care, and inadequate infection prevention and control.<sup>4,5</sup> On a biological level, these bacteria are incredibly adept at spreading AMR by transfer of mobile genetic elements such as plasmids, for example.<sup>5</sup> Unfortunately, AMR has continued to worsen, with resistance to antibiotics of last resort, such as carbapenems, polymyxins, and even novel  $\beta$ -lactam and  $\beta$ -lactamase inhibitor combinations now being reported,<sup>6–8</sup> which makes the prospect of pan-drug resistance in Gram-negative bacteria increasingly a reality.<sup>9</sup>

The clinical syndromes caused by MDR-GNB infections are the same as those caused by antibiotic-susceptible Gram-negative bacteria. These syndromes include cystitis, complicated urinary tract infection (UTI; eg, pyelonephritis), hospital-acquired or ventilator-associated pneumonia, and intra-abdominal and bloodstream infections.<sup>5,10,11</sup> Risk factors for MDR-GNB infections are consistent across different categories of MDR-GNB and include the presence of comorbidities, previous antibiotic use, previous colonisation with MDR-GNB, previous intensive care unit stay, mechanical ventilation, dialysis, length of hospital stay, and travelling to regions with a high prevalence of MDR-GNB.<sup>12–15</sup> Due to a crucial unmet need, diagnostics have advanced to include the use of multiplex PCR assays, mass spectrometry techniques, and bacterial whole genome sequencing. Similarly, new therapeutics for MDR-GNB have progressed along the development pipeline in the last 5 years and include

novel  $\beta$ -lactam and  $\beta$ -lactamase inhibitor combinations, tetracyclines, aminoglycosides, and novel siderophore-like cephalosporins (eg, cefiderocol).<sup>16</sup> In addition to antibiotic agents, there has also been a push for new treatment approaches such as phage therapy and anti-virulence approaches.<sup>17,18</sup>

## Clinically important mechanisms of AMR

Antibiotics have been developed to target multiple sites of the bacterial cell to impair bacterial growth or cause cell death (figure 1). Gram-negative bacteria are highly capable of developing resistance to antibiotics via a range of mechanisms, including antibiotic modification or degradation (eg,  $\beta$ -lactamase hydrolysis of  $\beta$ -lactam antibiotics), decreasing antibiotic entry into the bacterial cell (eg, loss of porins), altering the target site of the antibiotic (eg, ribosomal alteration), and increasing antibiotic efflux from the bacterial cell (eg, with overexpression of transmembrane efflux pumps; table 1).<sup>19,20,29,33</sup> These mechanisms can arise from mutations in the bacterial chromosome, or from acquisition of new resistance determinants, particularly via plasmids. These mobile genetic elements often carry genes coding for resistance to multiple antibiotic classes.

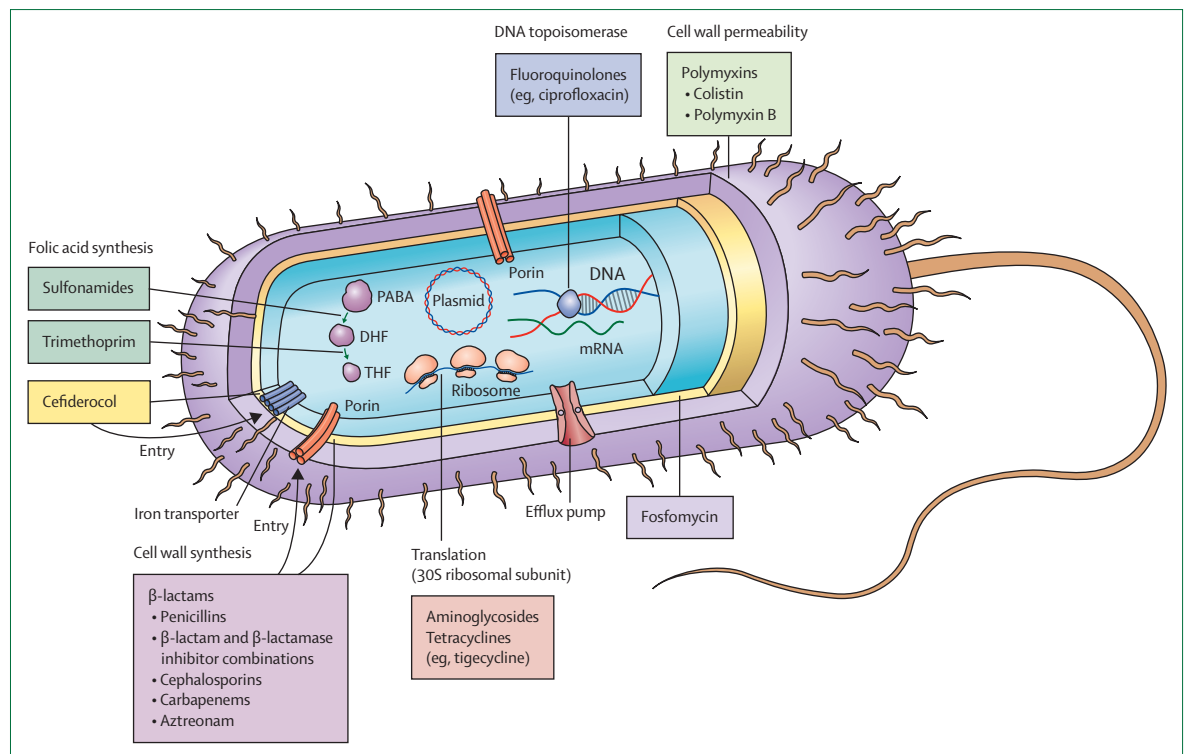
## Definitions of AMR

With this rich repertoire of mechanisms and modes of spread, resistance to multiple classes of antibiotics in Gram-negative bacteria has become a prominent public health problem. In 2012, a standardised international terminology for AMR was developed<sup>34</sup> in which multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antibiotic categories, extensive drug resistance was defined as non-susceptibility to at least one agent in all but one or two antibiotic categories, and pan-drug resistance was defined as non-susceptibility to all agents in all antibiotic categories. More recently, a clinical definition of difficult-to-treat resistance (DTR) for Gram-negative bacteria was developed, defined as treatment-limiting resistance to all first-line agents; that is, all  $\beta$ -lactams, including carbapenems and  $\beta$ -lactamase inhibitor combinations (not including novel combinations), and fluoroquinolones.<sup>35</sup> This definition distinguishes low

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**Figure 1: Mechanisms of action of key antibiotics for the treatment of multidrug-resistant Gram-negative bacterial infections**

Several classes of antibiotics are available for the treatment of multidrug-resistant Gram-negative bacterial infections. Their mechanisms of action focus on key aspects of bacterial cell function, such as inhibiting cell wall synthesis (ie,  $\beta$ -lactams and fosfomycin) and function (ie, polymyxins), protein synthesis (ie, aminoglycosides and tetracyclines), folic acid synthesis (ie, trimethoprim and sulphonamides), and DNA replication (ie, fluoroquinolones). Because of increasing antimicrobial resistance, novel antibiotics have been developed to overcome some of these mechanisms; for example, by inhibiting antibiotic modification (ie,  $\beta$ -lactamase inhibitors) or by altering entry pathways (ie, cefiderocol, which enters via iron transporters). This figure was created with BioRender.com. DHF=dihydrofolic acid. PABA=para-aminobenzoic acid. THF=tetrahydrofolate.

toxicity first-line agents from agents with higher toxicity and less efficacy, such as aminoglycosides, polymyxins (nephrotoxicity and poor penetration in abdominal and pulmonary sites), and tigecycline (low serum levels and poor lung penetration).<sup>31,36–38</sup>

### Clinically relevant multidrug-resistant Gram-negative pathogens

#### Enterobacterales

Enterobacterales include some of the most common Gram-negative pathogens. These include *Escherichia coli*, *Klebsiella* spp, and *Proteus* spp, which are common commensals of the gastrointestinal tract and often cause urinary tract, intra-abdominal, and bloodstream infections, and *Enterobacter* spp and *Serratia marcescens*, which are more commonly seen within health-care settings. The most clinically relevant resistance mechanisms of Enterobacterales include extended-spectrum  $\beta$ -lactamases (ESBLs) and AmpC  $\beta$ -lactamases, which confer resistance to narrow-spectrum penicillins (eg, benzylpenicillin and amoxycillin) and third-generation cephalosporins (eg, ceftriaxone and ceftazidime), and carbapenemases, which confer resistance to carbapenems (eg, meropenem, imipenem, and ertapenem).<sup>10,11,39</sup> Given

the importance of these antibiotics for the treatment of Enterobacterales infections and increasing reports of resistance, WHO has declared third-generation cephalosporin-resistant and carbapenem-resistant Enterobacterales (CRE) as critical priority pathogens for novel drug development.<sup>3</sup>

ESBLs are a heterogeneous group of enzymes that hydrolyse the  $\beta$ -lactam ring in antibiotics and have a serine in the active site.<sup>40</sup> ESBLs are often located on mobile genetic elements such as plasmids, leading to their rapid horizontal transmission between bacterial pathogens.<sup>19</sup> While conferring resistance to third-generation cephalosporins, bacteria carrying these enzymes remain susceptible to carbapenems. The earliest identified ESBLs were TEM and SHV  $\beta$ -lactamases, with subsequent extensive spread of the CTX-M family of enzymes, particularly plasmid-borne CTX-M-15 associated with the *E coli* sequence type 131 clone.<sup>19,41</sup> ESBLs in Enterobacterales have now become endemic globally in both hospital and community settings, with rates of 5–25% in western Europe, more than 50% in southern and eastern Europe, 30% in Latin America, 11–13% in the USA, 30–80% in Asia (depending on country), and 10–15% for Australia and New Zealand.<sup>40,42–44</sup>

AmpC  $\beta$ -lactamases are most often found in *Enterobacter* spp, *S. marcescens*, *Citrobacter freundii*, *Morganella morganii*, and *Providencia* spp. Despite their resistance to third-generation cephalosporins, these pathogens often remain susceptible to cefepime and carbapenems. AmpC can be distinguished from ESBLs by their resistance to some  $\beta$ -lactamase inhibitors (eg, clavulanic acid) and cephamycins (eg, ceftiofloxacin), to which ESBLs remain susceptible.<sup>45</sup> *ampC* genes are most often located on the bacterial chromosome and their expression can be induced by exposure to various  $\beta$ -lactam antibiotics, including third-generation cephalosporins.<sup>46</sup> The bacteria can initially show in vitro susceptibility to these agents, but then develop resistance if induction ensues.<sup>47</sup> Considerable inducible chromosomal *ampC* expression is most often encountered in the *Enterobacter cloacae* complex, *Klebsiella aerogenes*, and *C. freundii*, whereas *S. marcescens*, *M. morganii*, and *Providencia* spp. have lower levels of inducible *ampC* expression and are therefore less likely to develop third-generation cephalosporin resistance.<sup>48–50</sup> In contrast, plasmid-mediated *ampC* genes are typically detected in *K. pneumoniae*, *E. coli*, and *Salmonella* spp. and confer resistance without requiring induction.<sup>46</sup>

Carbapenemases are enzymes that hydrolyse a broad range of  $\beta$ -lactams, including carbapenems. They can be further classified as carbapenemases that rely on a serine residue in the active site (eg, KPC [Klebsiella pneumoniae carbapenemase], IMI [Imipenem-hydrolysing  $\beta$ -lactamase], and OXA-48 groups) or those that rely on zinc, also known as metallo- $\beta$ -lactamases (eg, NDM [New Delhi metallo- $\beta$ -lactamase], VIM [Verona Integron-encoded metallo- $\beta$ -lactamase], and Imipenemase [IMP]).<sup>19</sup> With the advent of new  $\beta$ -lactamase inhibitors that are enzyme-specific, it has become more important to know the mechanism of carbapenem resistance to help guide optimised antibiotic choice. Carbapenem resistance in Enterobacterales can also result from non-carbapenemase mechanisms, such as ESBL overproduction, porin mutation and loss, and upregulation of efflux pumps.<sup>8</sup> Globally, rates of carbapenem resistance in Enterobacterales remain less than 10%, but there is substantial variation between regions, with more than 50% of *K. pneumoniae* isolates from Greece and Russia being carbapenem resistant.<sup>8,43,51,52</sup> Rates of resistance also differ according to bacterial species, with more carbapenem resistance in *K. pneumoniae*, and a higher proportion being carbapenemase producers.<sup>53</sup> The predominant type of carbapenemase also varies between regions, with KPCs most commonly noted in North America, Asia, and Southern Europe, while NDMs predominate on the Indian subcontinent, the Middle East, and the Balkans, and IMPs in Asia and Australia.<sup>54,55</sup> However, systematic sampling in community and hospital settings is not typically undertaken globally and therefore reported rates could be affected by sampling bias.

| Categories                                                                                                                      |                                                                  | Examples                                                                                                                                                               |
|---------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b><math>\beta</math>-lactams<sup>19,20</sup> (eg, piperacillin-tazobactam, cefepime, meropenem, and ceftazidime-avibactam)</b> |                                                                  |                                                                                                                                                                        |
| Antibiotic modification                                                                                                         | $\beta$ -lactamase enzymes                                       | Extended-spectrum $\beta$ -lactamases (SHV, TEM, and CTX-M); carbapenemases (KPC, OXA-48, NDM, IMP, and VIM)                                                           |
| Decreased entry                                                                                                                 | Porin mutations                                                  | <i>Escherichia coli</i> (OmpC and OmpF); <i>Klebsiella pneumoniae</i> (OmpK35 and OmpK36); <i>Pseudomonas aeruginosa</i> (OprD); <i>Acinetobacter baumannii</i> (CarO) |
| Increased efflux                                                                                                                | Efflux pumps                                                     | <i>E. coli</i> (AcrAB-TolC); <i>P. aeruginosa</i> (MexAB-OprM); <i>A. baumannii</i> (AdeABC and AdeJ/K)                                                                |
| <b>Aminoglycosides<sup>21–24</sup> (eg, gentamicin, tobramycin, and amikacin)</b>                                               |                                                                  |                                                                                                                                                                        |
| Antibiotic modification                                                                                                         | Aminoglycoside-modifying enzymes (AMEs)                          | N-acetyltransferases (AAC); O-adenyltransferases (ANT); O-phosphotransferases (APH)                                                                                    |
| Decreased entry                                                                                                                 | Alterations in bacterial cell outer membrane                     | PhoPQ; PmrAB; ParS; CprRS                                                                                                                                              |
| Target alteration                                                                                                               | 16S-ribosomal RNA methyltransferases (RMTs)                      | ArmA; RmtA; RmtB; RmtC; RmtD; RmtE; RmtF; RmtG; NpmA; NpmB; NpmC                                                                                                       |
| Target alteration                                                                                                               | 30S ribosome subunit binding site mutations                      | <i>rps</i> and <i>rplS</i> gene mutations                                                                                                                              |
| <b>Tetracycline derivatives<sup>25–28</sup> (eg, minocycline, tigecycline, and eravacycline)</b>                                |                                                                  |                                                                                                                                                                        |
| Antibiotic modification                                                                                                         | Increased breakdown                                              | Tet(X); Tet(X2); Tet(X3); Tet(X4)                                                                                                                                      |
| Increased efflux                                                                                                                | Major facilitator superfamily (MFS) of transporters              | Tet(A); Tet(B); Tet(C); Tet(D)                                                                                                                                         |
| Increased efflux                                                                                                                | Resistance nodulation division (RND)-type multidrug efflux pumps | AdeABC; TmexCD1-ToprJ1                                                                                                                                                 |
| Target alteration                                                                                                               | Ribosomal protein alteration                                     | RpsJ                                                                                                                                                                   |
| <b>Fluoroquinolones<sup>29,30</sup> (eg, ciprofloxacin)</b>                                                                     |                                                                  |                                                                                                                                                                        |
| Antibiotic modification                                                                                                         | Increased breakdown                                              | AME: AAC(6')-Ib-cr                                                                                                                                                     |
| Increased efflux                                                                                                                | Efflux pumps                                                     | QepA; OqxAB                                                                                                                                                            |
| Target alteration                                                                                                               | Topoisomerase substitution                                       | GyrA; GyrB; ParC; ParE                                                                                                                                                 |
| Target alteration                                                                                                               | DNA gyrase protection                                            | QnrA                                                                                                                                                                   |
| <b>Polymyxins<sup>31</sup> (eg, colistin and polymyxin B)</b>                                                                   |                                                                  |                                                                                                                                                                        |
| Target alteration                                                                                                               | Two component systems                                            | <i>K. pneumoniae</i> (PhoPQ, PmrAB, MgrB, and CrrAB); <i>P. aeruginosa</i> (PhoPQ, PmrAB, ParS, ColRS, and CprRS); <i>A. baumannii</i> (PmrAB)                         |
| Target alteration                                                                                                               | Mobile (plasmid-mediated) colistin resistance enzymes            | Encoded by <i>mcr</i> genes                                                                                                                                            |
| <b>Fosfomycin<sup>32</sup></b>                                                                                                  |                                                                  |                                                                                                                                                                        |
| Antibiotic modification                                                                                                         | Fosfomycin modifying enzymes                                     | Encoded by <i>fos</i> genes                                                                                                                                            |
| Decreased entry                                                                                                                 | Alterations in fosfomycin transporters                           | GlpT; UhpT; CyaA; PtsI                                                                                                                                                 |

**Table 1: Mechanisms of resistance to key classes of antibiotics used for the treatment of multidrug-resistant Gram-negative bacterial infections**

### *Pseudomonas aeruginosa*

*P. aeruginosa* is a common nosocomial pathogen that can readily develop resistance to multiple antibiotics. While resistance in Enterobacterales is driven by acquired  $\beta$ -lactamases, resistance in *P. aeruginosa* is often due to the presence of efflux pumps that actively remove multiple antibiotics (eg, MexAB-OprM), and mutations of porins (eg, OprD) that decrease permeability to

antibiotics.<sup>56</sup> These factors act in concert with overexpression of *Pseudomonas*-derived cephalosporinase (PDC), a chromosomal AmpC-type  $\beta$ -lactamase. In addition to these intrinsic mechanisms, *P aeruginosa* can also acquire resistance mechanisms via plasmids, such as  $\beta$ -lactamase genes (including carbapenemase genes—eg, *bla*<sub>VIM</sub> and *bla*<sub>KPC</sub>) and quinolone resistance.<sup>57,58</sup> Global rates of carbapenem resistance in *P aeruginosa* generally range from 10–20% and MDR rates from 5–30%, depending on the region and the site of infection.<sup>8,43,57,59,60</sup> Due to the novelty of the DTR definition, few epidemiological data are available that document DTR prevalence in *P aeruginosa* globally, with 2·3% and 16·9% representing the proportion of *P aeruginosa* that have DTR reported in the multicentre US study of Gram-negative bacteria bloodstream isolates that proposed the definition of DTR and in the global ATLAS surveillance network, respectively.<sup>35,61</sup>

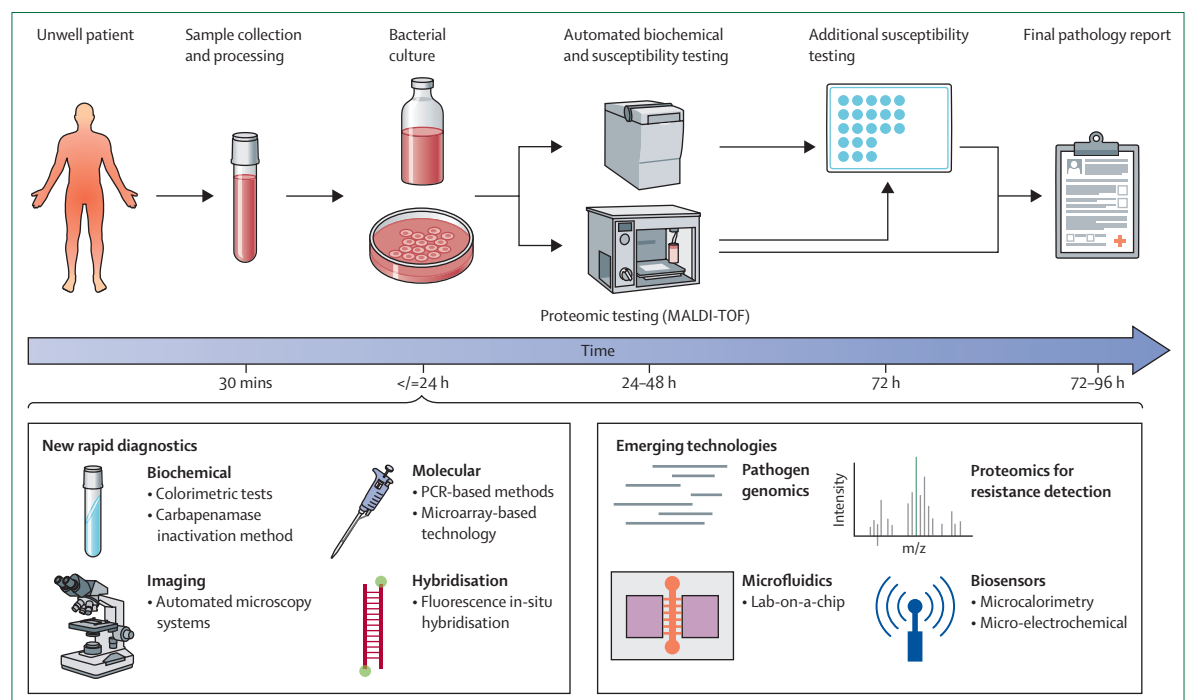
#### *Acinetobacter baumannii*

Similar to *P aeruginosa*, AMR in *Acinetobacter baumannii* results from multiple coexisting mechanisms including porin mutations and the production of multiple  $\beta$ -lactamases, aminoglycoside-modifying enzymes, and efflux pumps.<sup>62,63</sup> While these mechanisms often act together, the most concerning development leading to

the rise of carbapenem resistance has been the acquisition of carbapenemases, predominately the OXA-type (eg, OXA-23) and metallo- $\beta$ -lactamases (eg, IMP, NDM, and VIM).<sup>62</sup> In a recent global survey, 91% of carbapenem resistant isolates carried a carbapenemase gene, with *bla*<sub>OXA-23</sub> accounting for 88%.<sup>64</sup> It is possible for multiple carbapenemases to be acquired by the same bacterium, both in *A baumannii* and other Gram-negative pathogens. Rates of carbapenem resistance in *A baumannii* are greater than 30% globally, with many regions, such as southern and eastern Europe reaching greater than 50%, and parts of Asia greater than 80%.<sup>8,43,65,66</sup>

#### Recent advances in diagnostics

Rapid diagnosis of AMR helps guide treatment and has been shown to improve timeliness and appropriateness of antibiotic use, and in some cases, patient outcomes.<sup>67–71</sup> Traditional laboratory techniques for antibiotic susceptibility testing require culture of an organism with subsequent quantitative (eg, broth dilution tests or antibiotic gradient methods) or qualitative (eg, disk diffusion) phenotypic methods for detecting resistance.<sup>72</sup> Despite some of these techniques being automated in commercial instruments,<sup>73</sup> turn-around times for susceptibility testing results can vary from 18 h to 48 h



**Figure 2: Diagnostic testing of multidrug-resistant Gram-negative bacteria**

Diagnosis of multidrug-resistant Gram-negative bacterial infections involves both species identification and antibiotic susceptibility testing. Although clinical microbiology laboratories have undergone automation of these processes and have seen the introduction of proteomic methods, such as MALDI-TOF, delays in turnaround time remain and might affect patient outcomes. New biochemical, molecular (eg, PCR), automated microscopy, and hybridisation-based rapid diagnostics now allow faster detection of antimicrobial resistance, including by testing clinical samples directly. Emerging technologies are seeking to improve both the speed of testing and provide more detailed information regarding the underlying mechanisms of antimicrobial resistance. This figure was created with BioRender.com. MALDI-TOF=matrix-assisted laser desorption/ionisation time-of-flight.

depending on sample type and use of rapid phenotypic methods (figure 2).<sup>73–75</sup> More prolonged testing times can be seen for MDR-GNB, where additional tests for non-first-line antibiotics are often needed. Moreover, in most cases, these phenotypic tests do not provide information regarding the underlying mechanism of resistance, which increasingly helps guide antibiotic therapy and infection prevention and control interventions.

### Current rapid diagnostics

There are multiple biochemical assays aimed at rapid identification of AMR in MDR-GNB, with a particular focus on carbapenemase detection.<sup>76,77</sup> These include CarbaNP (an assay developed to detect the presence of carbapenemase production), the related Blue Carba and Beta Carba assays,<sup>78–80</sup> and the carbapenemase inactivation method.<sup>76,81</sup> Similar tests have also been developed for the detection of colistin and cefiderocol resistance.<sup>82,83</sup>

Molecular methods are being extensively used for AMR detection in clinical microbiology laboratories and have high sensitivity and rapid turnaround time (typically the same day to detect a broad category—eg, genes encoding KPCs).<sup>8,76,84,85</sup> PCR-based methods that rely on nucleic acid amplification focus on acquired resistance genes as targets, including ESBL (eg, CTX-M) and carbapenemase genes (eg, KPC and NDM). While purely PCR-based assays are restricted in the number of targets, microarray technology can use hundreds of DNA probes that hybridise to DNA targets, which allows for the inclusion of numerous bacterial identification and resistance gene targets that can directly inform therapy (also known as theranostics). Several commercial multiplexed PCR and microarray panels are currently in use in clinical microbiology laboratories,<sup>77,84</sup> and data on their clinical effects are being reported. A recent prospective multicentre study noted that PCR testing for the *bla*<sub>KPC</sub> carbapenemase gene was associated with faster administration of effective antibiotic therapy (median 24 h vs 50 h) and decreased 14-day (16% vs 37%) and 30-day (24% vs 47%) mortality in patients with CRE bacteraemia.<sup>67</sup> Several other single-centre studies showed similar findings following the introduction of rapid diagnostics.<sup>86,87</sup> In addition, two systematic reviews focused on the use of rapid diagnostics in bloodstream infections (not MDR-GNB infections specifically) have been performed. The first review found improvement in timeliness of therapy when coupled with antimicrobial stewardship advice, and an association with mortality reduction.<sup>68</sup> The second noted that rapid diagnostics have the potential to improve timeliness of targeted therapy and possibly improve other patient outcomes, but found there was low overall strength of evidence of effectiveness.<sup>69</sup>

Advances in microscopy have also played an important role in improving diagnosis, particularly through use of automated techniques that enable earlier detection of bacterial growth and high-throughput screening.<sup>88,89</sup>

Automated microscopy techniques are being integrated with other diagnostic methods (eg, molecular diagnostics and fluorescence-based methods) and can be analysed using artificial intelligence.<sup>73,90,91</sup> Other approaches currently available but not in widespread use include fluorescence in-situ hybridisation, which has been adapted for rapid bacterial identification and detection of specific AMR genes,<sup>77</sup> T2 magnetic resonance-based biosensing for detection of several Gram-negative pathogens and resistance genes directly from blood, and the use of volatile organic compounds to establish antibiotic susceptibility.<sup>92–95</sup>

### Emerging technologies

Several emerging technologies for rapid detection of AMR are undergoing development, including microfluidics, biosensor technologies, immune assays, and proteomic and genomic approaches. We will focus on proteomic and genomic assays but refer the reader to a comprehensive review for a more extensive discussion.<sup>77</sup> Matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry is a commercially available proteomic technology that is routinely used in many clinical microbiology laboratories for rapid organism identification.<sup>96</sup> Rapid species identification can guide initial antibiotic choices, especially if a species with considerable rates of AMR is identified. In addition to this primary use, there has also been growing interest in using MALDI-TOF for AMR detection, including carbapenem resistance, but it has not reached widespread clinical use.<sup>97–99</sup>

The use of pathogen genomics for diagnostic purposes has also garnered increasing interest due to rapid decreases in cost and increases in accuracy of the technologies on offer. These efforts have included whole genome sequencing to characterise individual pathogens,<sup>100,101</sup> and metagenomic approaches to detect multiple pathogens and resistance determinants directly from clinical samples.<sup>102,103</sup> Several studies have shown good correlation in MDR-GNB between genotypic and phenotypic antibiotic susceptibility testing in research settings.<sup>104–106</sup> While phenotypic antibiotic susceptibility testing remains the gold standard, it is often time consuming, suffers from low reproducibility, and for some antibiotics (eg, polymyxins) might be difficult to perform outside of a reference laboratory, thus making genomic testing an attractive addition.<sup>107</sup> However, considerable logistical, technical, and regulatory barriers remain, such as access to sequencing instruments and computational resources, the need for bioinformatics expertise to analyse and interpret sequencing data, and the need for more data from diverse pathogens on the relationship between genotype and phenotype. As a result, pathogen genomics to diagnose AMR in clinical settings remains in development but considerable efforts are underway to facilitate access to this technology.<sup>100,108,109</sup>

| <div><div>Active</div><div>Variable</div><div>Not recommended</div></div> |                                                                                                                 | Enterobacterales                                                |                                                    |                                                 |                                                      |                                            | Lactose non-fermenting organisms                           |                                                     |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|----------------------------------------------------|-------------------------------------------------|------------------------------------------------------|--------------------------------------------|------------------------------------------------------------|-----------------------------------------------------|
|                                                                           | Typical dosing regimen for serious infections <sup>11,110,111</sup>                                             | Extended-spectrum $\beta$ -lactamase-producing Enterobacterales | AmpC $\beta$ -lactamase-producing Enterobacterales | Ambler class A carbapenemases (eg, KPC and IMI) | Metallo- $\beta$ -lactamases (eg, NDM, VIM, and IMP) | Ambler class D carbapenemases (eg, OXA-48) | Difficult-to-treat resistant <i>Pseudomonas aeruginosa</i> | Carbapenem-resistant <i>Acinetobacter baumannii</i> |
| <b><math>\beta</math>-lactam</b>                                          |                                                                                                                 |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Ceftolozane-tazobactam                                                    | 3 g IV every 8 h, infused over 3 h                                                                              |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Ceftazidime-avibactam                                                     | 2.5 g IV every 8 h, infused over 3 h                                                                            |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Meropenem-vaborbactam                                                     | 4 g IV every 8 h, infused over 3 h                                                                              |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Imipenem-relebactam                                                       | 1.25 g IV every 6 h, infused over 30 min                                                                        |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Cefiderocol                                                               | 2 g IV every 8 h, infused over 3 h                                                                              |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Ceftazidime-avibactam and aztreonam                                       | Ceftazidime-avibactam: 2.5 g IV every 8 h, infused over 3 h plus aztreonam: 2 g IV every 8 h, infused over 3 h* |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Aztreonam-avibactam                                                       | 2 g/0.67 g loading dose then 1.5 g/0.5 g every 6 h, infused over 3 h                                            |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Cefepime-enmetazobactam                                                   | 2 g/0.5 g every 8 h, infused over 4 h                                                                           |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Sulbactam-durlobactam†                                                    | 1 g of each drug IV every 6 h, infused over 3 h†                                                                |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| <b>Tetracycline derivative</b>                                            |                                                                                                                 |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |
| Eravacycline                                                              | 1 mg per kg IV every 12 h                                                                                       |                                                                 |                                                    |                                                 |                                                      |                                            |                                                            |                                                     |

**Figure 3: Summary of new antibiotic agents for the treatment of multidrug-resistant Gram-negative bacterial infections**

Green=antibiotic is reliably active in vitro and guideline recommended. Yellow=variable activity; antibiotic might be effective if in vitro susceptibility is shown, clinical data might be lacking to support routine use. Red=not recommended, likely absence of in vitro susceptibility. IV=intravenously. \*Administered simultaneously via Y-site administration. †Administration was studied in combination with imipenem-cilastatin.

## Treatment

In the last decade, several new antibiotics with activity against MDR-GNBs have reached clinical practice (figure 3). Numerous agents remain in development and several non-pharmacological approaches such as phage and microbiome-based therapies are being investigated. With the advent of newer and more targeted therapies, it has become increasingly important to have a framework of therapy for MDR-GNB that takes into account four key factors: (1) the site of infection (eg, urinary tract, lung, or blood), (2) the severity of infection, (3) the bacterial pathogen causing the infection (eg, Enterobacterales, *Pseudomonas*, *Acinetobacter*), and (4) the likely resistance mechanisms involved (eg, ESBL, AmpC, KPC, or NDM). Internationally recognised treatment guidelines now categorise recommendations based on these four factors (figure 4). There is some divergence in recommendations because of differences in MDR-GNB definitions, regional availability of antibiotics, and varied interpretations of the literature

due to the lack of comparative effectiveness trials.<sup>112</sup> In addition, guidelines from several other societies are available.<sup>110,111,113–115</sup>

## Established antibiotics for MDR-GNB

Until the newly licensed antibiotics became available (figure 3), options for the treatment of MDR-GNB infections were chiefly restricted to older and sometimes more toxic antibiotics. Antibiotics such as trimethoprim-sulfamethoxazole, quinolones, or nitrofurantoin remain useful for treatment of some MDR-GNB infections (eg, UTIs) if in vitro susceptibility is shown (figure 4).

$\beta$ -lactam agents also continue to be cornerstones of therapy. Carbapenems (eg, meropenem, imipenem, and ertapenem) are broad spectrum  $\beta$ -lactam agents that are recommended for use against serious infections caused by third-generation cephalosporin-resistant Enterobacterales infections, irrespective of the resistance mechanism (figure 4).<sup>10,11,110,113,114</sup> Of note, ertapenem can be hydrolysed by ESBLs, making it necessary to confirm

| IDSA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | ESCMID<br>3GCephR-E                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Comments                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><b>ESBL-E cystitis:</b> preferred treatments are nitrofurantoin and trimethoprim-sulfamethoxazole. Alternative treatments include ciprofloxacin, levofloxacin, and carbapenems. Single-dose aminoglycoside or oral fosfomycin (for <i>Escherichia coli</i> only)</p> <p><b>ESBL-E pyelonephritis or complicated UTIs:</b> trimethoprim-sulfamethoxazole, ciprofloxacin, and levofloxacin are preferred. Ertapenem, meropenem, or imipenem if there is toxicity to trimethoprim and sulfamethoxazole or fluoroquinolones. Alternative treatments include aminoglycoside for a full treatment course</p> <p><b>All other ESBL-E infections (including serious infections):</b> meropenem, imipenem, or ertapenem</p> <p><b>AmpC infections:</b> ceftipime for organisms at moderate risk of considerable AmpC production (<i>Enterobacter cloacae</i> complex, <i>Klebsiella aerogenes</i>, <i>Citrobacter freundii</i>) that test susceptible or susceptible dose dependent</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | <p><b>Bloodstream infection and severe infection:</b> carbapenem (imipenem or meropenem)</p> <p><b>Bloodstream infection with no septic shock:</b> ertapenem can be considered instead of meropenem or imipenem</p> <p><b>Low-risk non-severe infection:</b> piperacillin-tazobactam, amoxicillin-clavulanic acid or quinolones</p> <p><b>Complicated UTIs:</b> aminoglycosides (for short durations) or intravenous fosfomycin</p>                                                                                                                                                                                     | <ul style="list-style-type: none"> <li>ESCMID guidelines use only a categorisation of 3GCephR-E and do not make specific recommendations regarding the mechanism of third-generation cephalosporin resistance</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| CRE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | CRE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <p><b>Cystitis:</b> preferred treatments are nitrofurantoin, trimethoprim-sulfamethoxazole, ciprofloxacin, or levofloxacin. Alternative treatments include a single dose of an aminoglycoside, oral fosfomycin (for <i>E. coli</i> only), colistin, ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, or ceftiderocol</p> <p><b>Pyelonephritis or complicated UTIs:</b> preferred treatments are trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, or ceftiderocol. Alternative treatments are aminoglycosides</p> <p><b>Ertapenem-resistant, meropenem-susceptible:</b> Standard-infusion meropenem or imipenem for cystitis. Extended-infusion meropenem or extended-infusion imipenem for other indications</p> <p><b>All other infections (including serious infections):</b> Treatment depends on carbapenemase testing: (1) no carbapenemase production or carbapenemase testing unavailable: ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam are preferred treatments. However, choice depends on local epidemiology. If previous MBL (metallo-<math>\beta</math>-lactamase) or suggestive epidemiology, use the recommendations for MBL producers. (2) KPC producers: meropenem-vaborbactam, ceftazidime-avibactam, and imipenem-relebactam. Ceftiderocol is an alternative. (3) MBL (eg, NDM, VIM, and IMP) producers: ceftazidime-avibactam and aztreonam combination therapy, or ceftiderocol. (4) OXA-48-like producers: ceftazidime-avibactam is preferred and ceftiderocol is an alternative.</p> | <p><b>Severe infection:</b> meropenem-vaborbactam or ceftazidime-avibactam if active in vitro</p> <p><b>Non-severe infection:</b> use of an old antibiotic (eg, aminoglycosides or tigecycline), chosen from among the in vitro active agents on an individual basis and according to the source of infection</p> <p><b>Complicated UTI:</b> aminoglycosides over tigecycline</p> <p><b>MBL and/or resistant to other antibiotics:</b> Ceftiderocol. Ceftazidime-avibactam and aztreonam combination therapy is an alternative</p>                                                                                      | <ul style="list-style-type: none"> <li>In CRE infection, carbapenemase testing can be crucial to informing optimal treatment decisions and is encouraged by the IDSA</li> <li>Ceftiderocol has in vitro activity against most CRE infections (regardless of carbapenemase presence), but clinical data are currently scarce. ESCMID recommends ceftiderocol as a first-line agent for MBL infection</li> <li>Older agents such as aminoglycosides, polymyxins, and tigecycline are no longer recommended as first-line options. These agents remain alternative agents if in vitro susceptibility is shown and there is non-susceptibility to first-line therapies. These agents are recommended by the ESCMID to be used in combination therapy with more than one drug active in vitro</li> <li>Tetracycline antibiotics (eg, tigecycline and eravacycline) can be considered as alternative agents for CRE infection outside the blood and urinary tract, regardless of carbapenemase presence</li> <li>Co-formulated aztreonam-avibactam can be used instead of ceftazidime-avibactam and aztreonam combination therapy, if available</li> </ul> |
| DTR-P aeruginosa                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | DTR-P aeruginosa                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <p><b>Cystitis:</b> preferred treatments are ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam, or ceftiderocol. Alternative treatments include a single dose of tobramycin or amikacin</p> <p><b>Pyelonephritis or complicated UTIs:</b> ceftolozane-tazobactam, ceftazidime-avibactam, imipenem-relebactam, or ceftiderocol. Alternative treatments are once-daily tobramycin or amikacin.</p> <p><b>All other infections (including serious infections):</b> preferred treatments are ceftolozane-tazobactam, ceftazidime-avibactam, and imipenem-relebactam. Alternatively treat with ceftiderocol</p> <p><b>MBL (eg, NDM, VIM, or IMP) producers:</b> ceftiderocol</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <p><b>Severe infection:</b> ceftolozane-tazobactam if active in vitro. Currently insufficient evidence for use of ceftiderocol, imipenem-relebactam or ceftazidime-avibactam</p>                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| CRAB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | CRAB                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <p>Sulbactam-durlobactam in combination with a carbapenem (ie, imipenem-cilastatin or meropenem). Alternative treatments are high-dose ampicillin-sulbactam in combination with at least one other agent (ie, polymyxin B, minocycline, tigecycline, or ceftiderocol), if sulbactam-durlobactam is not available.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | <p><b>Hospital-acquired pneumonia or ventilator-associated pneumonia with susceptibility to sulbactam:</b> ampicillin-sulbactam</p> <p><b>CRAB resistant to sulbactam:</b> a polymyxin or high dose tigecycline</p> <p><b>Severe and high-risk infections:</b> combination therapy including two in vitro active antibiotics among the available antibiotics (polymyxin, aminoglycoside, tigecycline, or sulbactam combinations)</p> <p><b>Meropenem with a minimum inhibitory concentration of 8 mg per L or less:</b> consider carbapenem combination therapy using high dose extended infusion carbapenem dosing</p> | <ul style="list-style-type: none"> <li>Durlobactam does not inhibit MBLs and therefore, other treatment options are required if an MBL is present</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

**Figure 4: Summary of IDSA and ESCMID guidelines for the treatment of multidrug-resistant Gram-negative bacterial infections**

3GCephR-E=third-generation cephalosporin-resistant Enterobacterales. AmpC-E= $\beta$ -lactamase producing Enterobacterales. CRAB=carbapenem-resistant *Acinetobacter baumannii*. CRE=carbapenem-resistant Enterobacterales. DTR-P aeruginosa=difficult-to-treat resistant *Pseudomonas aeruginosa*. ESBL-E=extended-spectrum  $\beta$ -lactamase-producing Enterobacterales. ESCMID=European Society for Clinical Microbiology and Infectious Diseases. IDSA=Infectious Diseases Society of America. MBL=metallo- $\beta$ -lactamase. UTI=urinary tract infection.

ertapenem susceptibility before treatment. Meropenem can still be considered for ertapenem-resistant, meropenem-susceptible organisms.<sup>11</sup> The MERINO trial showed that definitive treatment with meropenem for ceftriaxone-resistant *E coli* and *K pneumoniae* resulted in reduced mortality compared with piperacillin–tazobactam.<sup>116</sup> Reanalysis of the original MERINO data using reference antibiotic susceptibility testing methods indicated that this difference is no longer statistically significant.<sup>117</sup> Further studies are underway to validate these findings (eg, the PETERPEN study, NCT03671967). Based on numerous observational studies, there is a potential role for established  $\beta$ -lactam and  $\beta$ -lactamase inhibitors (eg, piperacillin–tazobactam) in non-serious third-generation cephalosporin-resistant Enterobacterales infections,<sup>10</sup> or as a continuation of therapy if clinical improvement is seen.<sup>11</sup>

For Enterobacterales that produce AmpC (eg, *Enterobacter* spp.), cefepime can be considered.<sup>118,119</sup> While the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines recommend against cefepime use for third-generation cephalosporin-resistant Enterobacterales due to a paucity of data,<sup>10</sup> the Infectious Diseases Society of America (IDSA) guidelines state that cefepime use could be appropriate for AmpC-producing Enterobacterales at moderate risk of considerable AmpC production (eg, *E cloacae* complex, *K aerogenes*, and *C freundii*).<sup>11</sup>

Aminoglycosides remain a treatment option for many MDR-GNB and might have a particular role in UTIs. Aminoglycosides are currently recommended as alternative treatments for UTIs due to third-generation cephalosporin-resistant Enterobacterales, CRE, and DTR-*P aeruginosa* (figure 4), when susceptible. These agents carry risks of nephrotoxicity and ototoxicity, restricting long term use. Polymyxins (including colistin and polymyxin B) are cationic compounds that interact with lipopolysaccharides of the outer membrane of Gram-negative bacteria to cause increased permeability and cell death.<sup>31</sup> These agents were previously first-line CRE therapy, but have considerable toxic effects (eg, nephrotoxicity) and have largely been replaced by novel  $\beta$ -lactam and  $\beta$ -lactamase inhibitor combinations, such as ceftazidime–avibactam, which have been associated with a mortality benefit in CRE treatment and fewer toxic events.<sup>120–122</sup> As part of combination regimens, polymyxins remain a treatment option for serious carbapenem-resistant *A baumannii* (CRAB) infections (figure 4) and might have a role in MDR-GNB infections that are not susceptible to any other antibiotic class.

Tetracycline derivatives, such as minocycline and tigecycline, remain a therapeutic option in combination regimens for treatment of CRAB and CRE infections, but are ineffective against *P aeruginosa*.<sup>25,38</sup> After intravenous administration, tigecycline has rapid tissue distribution leading to poor concentrations in the serum and urine so caution is recommended for treating infection at these

sites. Tigecycline also has poor lung penetration leading to concerns regarding its use as a treatment for ventilator-associated pneumonia.<sup>38</sup> Tigecycline is more suited to the treatment of intra-abdominal and skin and soft tissue infections. High doses of tigecycline (ie, 200 mg intravenous loading dose then 100 mg daily) are recommended for use in CRAB and CRE infections.<sup>11</sup>

Fosfomycin is available in both oral and intravenous formulations,<sup>32</sup> and is an alternative treatment for uncomplicated UTI caused by ESBL-*E coli* or CRE-*E coli*.<sup>11</sup> Several Gram-negative organisms (including *K pneumoniae*) have intrinsic resistance to fosfomycin due to the presence of *fosA* genes, which might lead to clinical failure.<sup>123,124</sup> In a recent multicentre randomised controlled trial, intravenous fosfomycin did not show non-inferiority to  $\beta$ -lactam therapy for ESBL *E coli* bloodstream infection from a urinary source, however this was due to discontinuation because of side-effects in the fosfomycin group, with clinical cure being similar for both groups.<sup>125</sup>

Sulbactam is an irreversible competitive  $\beta$ -lactamase inhibitor with activity against *A baumannii* (including CRAB) via saturation of penicillin-binding proteins,<sup>63</sup> which is unique to sulbactam and is not shown by other  $\beta$ -lactamase inhibitors. High dose sulbactam in combination with at least one other antibiotic is recommended as a treatment option for CRAB infections (figure 4).<sup>11,126</sup> Of note, sulbactam is typically formulated as ampicillin–sulbactam.

### New antibiotics for MDR-GNB

The arrival of multiple newer antibiotics has changed the treatment landscape for MDR-GNB infections. Several new  $\beta$ -lactam and  $\beta$ -lactamase inhibitor combinations have been useful additions for treatment. Ceftolozane–tazobactam pairs a novel fifth-generation cephalosporin with an established  $\beta$ -lactamase inhibitor and has a role in therapy for carbapenem-resistant and DTR-*P aeruginosa* due to ceftolozane's improved affinity for penicillin-binding proteins that render it less affected by porin mutations or efflux pumps.<sup>127</sup> In surveillance studies, 66–98% of all *P aeruginosa* isolates were susceptible to ceftolozane–tazobactam, including 63–95% of MDR *P aeruginosa* isolates.<sup>127–130</sup> As a result, ceftolozane–tazobactam is broadly accepted as first-line therapy for serious DTR-*P aeruginosa* infections if active in vitro. Ceftolozane–tazobactam activity against ESBL-Enterobacterales is mixed, with high rates of susceptibility for ESBL *E coli* (66–100%) but more restricted activity against ESBL *K pneumoniae* (42–84%).<sup>127,130</sup>

Other new combinations include ceftazidime–avibactam, meropenem–vaborbactam, and imipenem–relebactam. These agents are now first-line treatments for CRE infection and their activity varies depending on carbapenemase class. These combinations are active against most organisms producing Ambler class A carbapenemases (eg, KPC), but only ceftazidime–avibactam

has reliable activity against those with Ambler class D carbapenemases such as OXA-48. Observational data suggest that meropenem–vaborbactam might have a lower likelihood of resistance emergence for KPC-producing bacteria.<sup>131</sup> None of these agents alone are active against organisms producing metallo- $\beta$ -lactamases (Ambler class B carbapenemases—eg, NDM, VIM, and IMP).<sup>132–134</sup> In this scenario, treatment with a combination of ceftazidime–avibactam and aztreonam is recommended by both the ESCMID and IDSA guidelines.

Aztreonam is a monobactam  $\beta$ -lactam with stability against metallo- $\beta$ -lactamases, while avibactam (given as ceftazidime–avibactam) inhibits the serine  $\beta$ -lactamases that can breakdown aztreonam and are often found concurrently in metallo- $\beta$ -lactamase-producing organisms. Co-formulated aztreonam–avibactam has been approved for use by the European Medicines Agency (EMA) and is expected for US Food and Drug Administration (FDA) submission. Ceftazidime–avibactam and imipenem–relebactam are also active against MDR-*P. aeruginosa*, with susceptibility rates ranging from 68–89% for ceftazidime–avibactam and 59–60% for imipenem–relebactam.<sup>130</sup> There is a difference in recommendations regarding their use for treatment of DTR-*P. aeruginosa* with a conditional recommendation from the IDSA, but not the ESCMID. Cefepime–enmetazobactam is a novel combination with activity against ESBL (eg, CTX-M, SHV, and TEM), AmpC, and OXA-48-producing Enterobacterales.<sup>135</sup> This combination was approved for use by the EMA and FDA in 2024, following a phase 3 trial that showed non-inferiority against piperacillin–tazobactam for treatment of complicated UTI and pyelonephritis.<sup>136</sup>

Sulbactam–durlobactam is one of the newest combination agents and was specifically developed to target CRAB infections. Sulbactam has direct activity against *A. baumannii* via attachment of penicillin-binding proteins, while durlobactam inhibits Ambler class A, C, and D  $\beta$ -lactamases (including OXA carbapenemases). A phase 3 trial showed non-inferiority to colistin with a statistically significantly lower incidence of nephrotoxicity leading to FDA approval for treatment of hospital-acquired and ventilator-associated pneumonia.<sup>137</sup> Clinical data and availability are restricted, but given the paucity of alternative therapies, sulbactam–durlobactam is an emerging treatment option for CRAB infections and was recommended (in combination with a carbapenem) as first-line therapy by the IDSA. Notably, in the registration trial, sulbactam–durlobactam was used in combination with imipenem–cilastatin but the role of adjunctive carbapenem therapy in clinical use remains to be established.<sup>137,138</sup>

Durlobactam is not active against metallo- $\beta$ -lactamases (eg, NDM) and alternative regimens should be considered for treatment of metallo- $\beta$ -lactamase-producing CRAB infections. Due to the high prevalence of CRAB in some countries and the lack of availability of

sulbactam–durlobactam, less well-studied combinations that aim to have a similar effect are being explored. Similar to durlobactam, avibactam inhibits Ambler class A, C, and D  $\beta$ -lactamases.<sup>130</sup> Combining avibactam with sulbactam has therefore been suggested as a potential treatment for CRAB, but clinical data are absent.<sup>139,140</sup>

Cefiderocol is a siderophore cephalosporin with a novel mechanism of action. By binding to iron, cefiderocol more easily enters bacterial cells via iron transporters, affording it protection from  $\beta$ -lactamases (including carbapenemases), porin mutations, and efflux pumps.<sup>141</sup> As a result, cefiderocol has broad in vitro activity against most MDR-GNB, with susceptibility rates of 97% for CRE (including all carbapenemases), 97% for carbapenem-resistant *P. aeruginosa*, and 95% for CRAB.<sup>142</sup> However, metallo- $\beta$ -lactamase-producing Enterobacterales and *A. baumannii* show high rates of non-susceptibility (24·9% [95% CI 16·6–35·5%] and 40·9% [95% CI 34·5–55·4%], respectively), with NDM-producers being most problematic (38·8% [95% CI 22·6–58·0%] and 44·7% [95% CI 34·5–55·4%], respectively).<sup>143</sup> CREDIBLE-CR, a randomised multicentre phase 3 trial, compared cefiderocol with the best available therapy in patients with carbapenem-resistant Gram-negative infections and noted similar clinical and microbiology efficacy, but a numerically higher number of deaths in the cefiderocol group, driven by patients with *Acinetobacter* spp. infections.<sup>144</sup> For CRAB infections, IDSA guidelines recommend that cefiderocol should be restricted to infections refractory to or where there is intolerance to other antibiotics, while the ESCMID guidelines conditionally recommend against its use. Real-world clinical data are now emerging, with a retrospective observational study of cefiderocol use in Italy showing 30-day mortality of 37% and identifying cefiderocol resistance in 28% of tested isolates.<sup>145</sup>

Beyond  $\beta$ -lactams, new tetracycline derivatives have reached clinical use. Eravacycline has good activity against MDR-GNB and received both FDA and EMA approval. Eravacycline has the same mechanism of action as previous tetracycline agents and has the same rapid tissue distribution as tigecycline, but is less affected by common tetracycline resistance mechanisms such as efflux.<sup>146</sup> Eravacycline has a similar spectrum of activity as tigecycline, including activity against ESBLs, CRE, and CRAB, but not *P. aeruginosa*, with minimum inhibitory concentrations being two-fold to four-fold lower against CRE than tigecycline.<sup>147</sup>

### Agents in development

Several gaps in our pharmacological armamentarium for treating MDR-GNB exist. Apart from eravacycline, the described new agents require intravenous administration highlighting the need for oral alternatives. Oral carbapenems, such as tebipenem and sulopenem, are highly active against ESBL-producing Enterobacterales (ESBL-E) and have undergone phase 3 trials. In Japan,

tebipenem has been approved for clinical use.<sup>148–151</sup> These agents could have a role for treatment of complicated UTIs and pyelonephritis caused by ESBL-E. Pivmecillinam has extensive activity against ESBL-E with more than 80% isolates being susceptible to treatment.<sup>152</sup> After longstanding use in Europe, pivmecillinam has now been approved for use by the FDA in uncomplicated UTIs. Gepotidacin is a new oral type IIA topoisomerase inhibitor with activity against ESBL-E and fluoroquinolone-resistant isolates.<sup>153</sup> Two phase 3 trials (EAGLE-2 and EAGLE-3) compared gepotidacin with nitrofurantoin for treatment of uncomplicated UTI in women and were terminated early due to meeting the combined primary efficacy endpoint favouring gepotidacin (clinical and microbiological resolution at the test-of-cure visit).<sup>154,155</sup>

Several MDR-GNB pathogens still have few treatment options, including metallo- $\beta$ -lactamase-producing CRE, DTR-*P aeruginosa*, and CRAB. Cefepime–taniborbactam is a promising novel  $\beta$ -lactam and  $\beta$ -lactamase inhibitor combination with activity against these groups (except IMP carbapenemases).<sup>156</sup> A phase 3 trial showed superiority of cefepime–taniborbactam to meropenem for treatment of complicated UTIs and has led to its submission for FDA approval.<sup>157</sup> Cefepime–zidebactam and meropenem–xeruborbactam have a similar spectrum of in vitro activity and are undergoing evaluation.<sup>158,159</sup>

### Non-antibiotic approaches

Although the availability of new antibiotics for MDR-GNB infections is promising, almost all agents discussed belong to pre-existing antibiotic classes. Clinical infections with organisms resistant to these agents have already been described,<sup>6,160,161</sup> highlighting the need to develop alternative or adjunctive non-antibiotic treatments. Phage therapy is an example and consists of administering naturally occurring viruses (bacteriophages or “phages”) to kill bacterial pathogens.<sup>17</sup> Phages have high specificity for a pathogen and therefore neither contribute to further emergence of AMR nor affect the surrounding microbiota.<sup>162</sup> Phage therapy might also increase antibiotic susceptibility,<sup>163</sup> leading to interest in its use in conjunction with antibiotics for potential synergistic effects and to prevent the emergence of resistance.<sup>164</sup> Clinical data for MDR-GNB treatment are largely restricted to case reports and case series with therapy tested against the most common MDR-GNB pathogens, including ESBL-E, CRE, MDR *P aeruginosa* infections, and CRAB.<sup>162,165–167</sup> Multiple clinical trials are ongoing (NCT05453578, NCT05498363, and NCT04596319). In addition, phages produce phage-derived peptides (eg, endolysins) that are proteins that target the bacterial cell wall to cause lysis. While this process forms part of phage infection strategies, phage-derived peptides have been generated as recombinant proteins and experimentally evaluated as anti-infective treatments (eg, in *A baumannii* infections).<sup>168,169</sup>

Microbiota-based therapies (eg, faecal microbiota transplantation) are another non-antibiotic therapy being investigated for decolonisation of MDR-GNB before the development of active infection. Most studies have been case series and have focused on ESBL-E and CRE, with decolonisation rates varying substantially.<sup>170–172</sup> A single randomised controlled trial did not show a significant difference in ESBL-E or CRE colonisation when non-absorbable antibiotics were administered with faecal microbiota transplantation, but was terminated early due to poor enrolment and numerous trials are ongoing.<sup>173</sup>

Antivirulence therapies are also a potential alternative to antibiotics as these treatments target virulence factors rather than trying to kill or inhibit the growth of pathogens. This approach might cause less selection pressure compared with antibiotics, thus preventing further emergence of AMR.<sup>174</sup> Some antivirulence strategies include targeting bacterial adhesion and colonisation, preventing biofilm formation, interference with bacterial toxins, inhibition of specialised secretion systems, and regulation of virulence gene expression.<sup>175,176</sup> Although promising, these therapies remain investigational and have not undergone extensive clinical trials in Gram-negative pathogens.

Several therapies that enlist the immune system against MDR-GNB pathogens also form important antibiotic alternatives.<sup>177</sup> Antibody treatments typically target virulence factors and have also been combined with small-molecule therapies (eg, antibiotics) to form antibody–drug conjugates, which simultaneously target the pathogen and engage multiple components of the immune system.<sup>177,178</sup> Vaccines for several common MDR-GNB pathogens are in development, including an extra-intestinal pathogenic *E coli* vaccine in phase 3, an enterotoxigenic *E coli* vaccine in phase 2, and a *K pneumoniae* vaccine in a phase 1 and 2 trial.<sup>179</sup> However, vaccines for other important pathogens (eg, *P aeruginosa* and *A baumannii*) are either in preclinical development or are inactive due to several hurdles such as the need for broad coverage among diverse bacterial strains within a species.<sup>180</sup> Mirroring the recent advances in cancer treatment, immunotherapy that targets host immune response (eg, checkpoint inhibition, cytokine therapies, and cellular therapies) rather than the pathogen itself is also in preclinical development for several MDR-GNB pathogens.<sup>177,181</sup>

Antibiotic adjuvants are compounds that enhance the effectiveness of antibiotics and help overcome bacterial resistance.<sup>182</sup> While  $\beta$ -lactam and  $\beta$ -lactamase inhibitor combinations have been the most prominent examples, several other classes are in development. Efflux pump inhibitors prevent bacteria from expelling antibiotics, thus increasing their intracellular concentration and activity. Membrane permeabilisers assist antibiotics in penetrating bacterial cells, especially the outer membrane of Gram-negative bacteria. Some compounds (eg, NV716)

can act as both efflux pump inhibitors and membrane permeabilisers and also remain in preclinical development.<sup>183</sup>

## Knowledge gaps in treatment

### The role of combination therapy

Before the availability of novel MDR-GNB antibiotics, combination therapy of multiple agents with in vitro efficacy was recommended for CRE, DTR-*P aeruginosa*, and CRAB infections. Clinical data of the efficacy and safety of newer agents have led to acceptance of use of novel  $\beta$ -lactam and  $\beta$ -lactamase inhibitors as monotherapy for most CRE infections and serious DTR-*P aeruginosa* infections where in vitro activity is shown.<sup>10,11,110,113</sup> However, there is a paucity of data regarding the role of newer agents in treating metallo- $\beta$ -lactamase-producing CRE and CRAB, either as single agents or in combination therapy. While cefiderocol has activity against these organisms,<sup>184</sup> further data from clinical trials are needed.

### Duration of therapy for MDR-GNB infections

There have been multiple efforts to shorten the duration of therapy for treatment of bacterial infection but studies have not specifically focused on MDR-GNB.<sup>185</sup> In studies where there was a considerable proportion of MDR-GNB, shorter courses were non-inferior.<sup>186–189</sup> A retrospective observational study of CRE bacteraemia showed similar odds of recurrent bacteraemia or death within 30 days in patients receiving short courses (7–10 days) as in those receiving long courses of active therapy (14–21 days; odds ratio 1.21 [95% CI 0.55–2.31]).<sup>190</sup> Of the guidelines reviewed, only two made specific recommendations regarding duration of therapy,<sup>110,113</sup> while others made no recommendations or stated that therapy for resistant organisms should not differ to that of susceptible organisms with factors such as clinical response, achievement of source control, and the host's immunological status playing an important role in determining duration of therapy.<sup>10,11</sup> Several clinical trials on the duration of therapy with inclusion of MDR-GNB are ongoing (NCT05124977, NCT05124977, NCT05210387, and NCT03005145). Use of biomarkers (eg, procalcitonin) for personalised therapy duration has also been proposed.<sup>191</sup>

## Conclusion

Infections caused by MDR-GNB will continue to be a considerable global health problem. We find ourselves at an exciting crossroads with several new diagnostic and antibiotic treatments becoming available. We must define the role of these newer diagnostics and antibiotics in clinical care, in turn allowing us to move towards a more precision-guided approach to treatment of MDR-GNB infections. Our increased ability to rapidly detect underlying resistance mechanisms offers an important opportunity to guide and maximise the utility of novel antibiotics for MDR-GNB infections, which increasingly

target specific mechanisms. Early detection of AMR with improved diagnostics also plays a key role in preventing the spread of MDR-GNB by allowing earlier institution of infection prevention and control measures.

Despite these advances, several key challenges remain in improving outcomes for patients with MDR-GNB infections. Many low-income and middle-income countries lack laboratory infrastructure and many of the new diagnostics discussed in this Review are not commonly used outside of reference laboratories,<sup>192</sup> even in resource-rich settings. Similarly, access to new antibiotics is highly restricted. In many regions, these treatments are simply unavailable, and even in resource-rich settings, ensuring access might require innovations in the economics of antibiotic use.<sup>16</sup> Subscription-based rather than use-based models for restricted antibiotics, as recently introduced in the UK,<sup>193</sup> are one such innovation, but ongoing incentives for obtaining regulatory approval and ensuring availability of new treatments post-approval are also needed.<sup>194</sup>

With these new diagnostics and treatments, we need robust clinical trial data to guide management decisions. Clinical trials in MDR-GNB infections struggle to enrol sufficient numbers of patients, making it difficult to obtain meaningful clinical efficacy data that compare new treatments not only to current best-available therapies, but also in head-to-head trials.<sup>195</sup> Furthermore, defining the optimal duration of therapy and identifying meaningful outcomes to study remains challenging. Several novel clinical trial designs specifically address these shortcomings, including Bayesian adaptive randomisation, Sequential Multiple Assignment Randomized Trial-Comparing Personalized Antibiotic Strategies, Desirability of Outcome Rankings, and the DURATIONS design.<sup>196,197</sup>

Lastly, we need to think beyond antibiotics to avoid the Sisyphean cycle of AMR. While non-antibiotic therapies for MDR-GNB infections hold promise, most are at preclinical stages and require substantial development. Preserving new and established treatments with antimicrobial stewardship is therefore an urgent priority, while infection prevention and control remains a cornerstone of stopping further spread of MDR-GNB. Despite the desperate challenges posed by MDR-GNB infections, we now have many more tools in our armamentarium, giving reason for hope that we can better address this crucially important global health issue.

### Contributors

NM and AYP contributed to the conceptualisation, literature review, manuscript drafting, and critical revision of the study. A-CU contributed to the conceptualisation, manuscript drafting, and critical revision.

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