Epidemiology and Mechanisms of Resistance of Extensively Drug Resistant Gram-Negative Bacteria

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Abstract: Antibiotic resistance has increased markedly in gram-negative bacteria over the last two decades, and in many cases has been associated with increased mortality and healthcare costs. The adoption of genotyping and next generation whole genome sequencing of large sets of clinical bacterial isolates has greatly expanded our understanding of how antibiotic resistance develops and transmits among bacteria and between patients. Diverse mechanisms of resistance, including antibiotic degradation, antibiotic target modification, and modulation of permeability through the bacterial membrane have been demonstrated. These fundamental insights into the mechanisms of gram-negative antibiotic resistance have influenced the development of novel antibiotics and treatment practices in highly resistant infections. Here, we review the mechanisms and global epidemiology of antibiotic resistance in some of the most clinically important resistance phenotypes, including carbapenem resistant Enterobacteriaceae, extensively drug resistant (XDR) Pseudomonas aeruginosa, and XDR Acinetobacter baumannii. Understanding the resistance mechanisms and epidemiology of these pathogens is critical for the development of novel antibacterials and for individual treatment decisions, which often involve alternatives to β-lactam antibiotics.

Keywords: gram-negative; antibiotic resistance; carbapenem resistant Enterobacteriaceae; Pseudomonas aeruginosa; Acinetobacter baumannii; extensively drug resistant

1. Introduction

Gram-negative bacteria have a thin peptidoglycan cell wall sandwiched between their inner and outer membranes. This is distinct from the gram-positive bacteria which have a thick peptidoglycan cell wall. Gram-negative bacteria are ubiquitous in nature and cause infection in multiple body sites including the urinary tract, lower respiratory tract, biliary tract, and bloodstream, among others. Due in large part to the selective pressure of antibiotic use, resistance has significantly increased in gram-negative bacteria over the past two decades. This increased resistance has been quite meaningful to patients, clinicians, and the healthcare system generally as antibiotic resistance in gram-negative bacterial infections has been associated with both increased mortality [1–4] and increased healthcare costs [5–8] relative to infections with more susceptible bacterial strains.

The adoption of genotyping and whole genome sequencing of large sets of clinical bacterial isolates has greatly expanded our knowledge of how antibiotic resistance emerges. Bacteria have demonstrated a diverse set of mechanisms for degrading antibiotics, modifying the antibiotic target site, or modulating the influx/efflux of antibiotic into or out of the bacterial cell. Understanding the mechanisms and epidemiology of these resistance mechanisms is critical. In the broadest sense, understanding the mechanisms of antibiotic resistance sheds light on how resistance arises and how it is transmitted between bacteria and to patients. The study of resistance mechanisms has also been important in the pharmaceutical industry, as multiple novel agents have emerged to circumvent known resistance mechanisms. These agents have been generally been developed through
modification of drug classes that have previously been U.S. Food and Drug Administration (FDA)-approved for antibiotic use. Examples include the novel aminoglycoside plazomicin, novel cephalosporin ceftolozane (which was paired with tazobactam), and novel β-lactamase inhibitors such as avibactam, vaborbactam, and relebactam that were combined with existing β-lactam antibiotics to form drugs such as ceftazidime-avibactam, meropenem-vaborbactam, imipenem-relebactam, and aztreonam-avibactam.

Though routine genotyping to determine bacterial resistance mechanisms is not yet commonplace in the clinical setting, such practice can be useful in certain situations. For example, carbapenem resistant Enterobacteriaceae (CRE) containing metallo-β-lactamases (MBL) often have a complex molecular background in which multiple β-lactamases are present [9,10]. Though a single antibiotic may not overcome all resistance mechanisms, knowledge of the particular resistance mechanisms that are present in a bacterial strain can lead to effective directed combination therapy. There is data to suggest that such an approach can be useful in treating MBL-producing CRE [11]. Thus, a thorough understanding of resistance mechanisms in gram-negative bacteria leads to fundamental knowledge of how resistance emerges and transmits, aids in drug discovery, and influences antibiotic therapy in select cases.

In this review we aim to describe the primary mechanisms of resistance and global epidemiology for the most significant antibiotic resistant gram-negative pathogens in the clinical setting: Carbapenem resistant Enterobacteriaceae, extensively drug resistant (XDR) Pseudomonas aeruginosa, and XDR Acinetobacter baumannii. These three pathogens are labeled by the World Health Organization a “critical” threat [12]. Understanding the resistance mechanisms and epidemiology of these pathogens is important, as their treatment often requires therapy with alternatives to β-lactam antibiotics, such as the polymyxins.

2. Mechanisms and Epidemiology of Antibiotic Resistance in Carbapenem Resistant Enterobacteriaceae (CRE)

2.1. Overview of Mechanisms of Resistance in CRE

There are three primary mechanisms by which Enterobacteriaceae employ resistance to carbapenems: (1) enzymatic degradation through carbapenemase production, (2) expression of efflux pumps, and (3) decreased outer membrane permeability via porin mutations [13]. These mechanisms are discussed in detail in Sections 2.3 through 2.8 and illustrated in Figure 1. Carbapenemase expression is a particularly common mechanism of resistance. Carbapenemases and β-lactamases more generally are classified according to their molecular structure with the ambler classification system (Table 1). Carbapenem resistance may emerge through expression of β-lactamases in ambler class A, B, and D, and these enzymes will be discussed more fully in Sections 2.3 through 2.5.

![Figure 1. Mechanisms of carbapenem resistance in Enterobacteriaceae.](image-url)


### Table 1. Ambler classification of β-lactamases.

| Ambler Class | β-Lactamases | Active Site Agent | Examples | Substrates                      |
|--------------|--------------|-------------------|----------|---------------------------------|
| A            | Penicillinas | Serine            | PSE      | Penicillins                     |
|              |              |                   | TEM, SHV, CTX-M, VEB, PER, GES, KPC, SME, IMI/NMC-A | 3rd generation cephalosporins, All β-lactams |
| B            | Metallo-β-lactamases | Zinc          | IMP, VIM, NDM, SPM, GIM | All β-lactams, except monobactams |
| C            | Cephalosporinases | Serine         | AmpC     | Cephamycins, 3rd generation cephalosporins |
| D            | Oxacillinas  | Serine            | OXA      | All β-lactams, though class D enzymes have highly variable spectra of activity |

Abbreviations: CTX-M, active against cefotaxime (CTX) and isolated in Munich (-M); GES, Guiana extended spectrum; GIM, German imipenemase; IMP, active on imipenem; KPC, Klebsiella pneumoniae carbapenemase; NDM, New Delhi metallo-β-lactamase; NMC, not metalloenzyme carbapenemase; OXA, oxacillinase; PER, Pseudomonas aeruginosa RNL-1; PSE, Pseudomonas specific enzyme; SHV, sulfhydrl reagent variable; SME, Serratia marcescens enzyme; SPM, Sao Paulo metallo-β-lactamase; VEB, Vietnamese extended-spectrum β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase.

2.2. Risk Factors for CRE Infection

CRE are a global threat, though, as detailed below, there is great geographic variability in the prevalence and mechanisms of carbapenem resistance. For example, in Europe, CRE infections are endemic in Italy and Greece and sporadic in much of Scandinavia [14]. Significant intranational or regional variability may be present as well. For example, in the U.S., CRE are endemic primarily in the northeast and Great Lakes regions [15]. Therefore, travel to endemic areas is an important risk factor [16]. Additional risk factors generally involve those that increase exposure to antibiotic resistant pathogens such as immune suppression, advanced age, intensive care unit admission, mechanical ventilation, solid organ or hematopoietic transplantation, or prolonged hospital stay [3,17]. Prior antibiotic use is clearly a risk factor as well [18].

2.3. Class A Carbapenemases

Class A carbapenemases (Table 1) require serine at their active site and are able to hydrolyze a broad spectrum of beta-lactams, including penicillins, cephalosporins, and aztreonam [19]. The class A carbapenemases consist primarily of the Klebsiella pneumoniae carbapenemase (KPC), Serratia marcescens enzyme (SME), and imipenemase/non-metallo-carbapenemase-A (IMI/NMC-A). In the U.S., KPC is the most commonly encountered carbapenemase in clinical practice [15]. Several recently FDA-approved antibiotics, including ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-relebactam, contain novel β-lactamase inhibitors that inhibit most KPC enzymes [20–22]. The gene coding for KPC, blacrc, is located on transferrable plasmids flanked by transposable elements, permitting seamless transference between bacteria of different species [15,23]. In contrast to KPC, the SME and the IMI/NMC-A enzymes are chromosomally encoded [24]. SME has only been found in small sub-population of Serratia marcescens, [24] and IMI/NMC-A enzymes have only been identified sporadically in Enterobacter cloacae complex isolates [25].

The KPC family of enzymes has the most global distribution of all carbapenemases associated with the Enterobacteriaceae [15]. The KPC enzyme was first reported in a K. pneumoniae clinical isolate in North Carolina, USA, in 1996 [26]. This discovery was soon followed by reports of KPC-producing Enterobacteriaceae along the East coast of the United States in the following five years [19,27–31]. As of 2017, KPC containing Enterobacteriaceae have been reported in every state in the U.S. [32], and 23 KPC subtypes have been submitted to Genbank (KPC-2–KPC-24; https://externalwebapps.lahey.org/studies/). The incidence of CRE in the U.S. varies highly with
geography. For example, in a recent survey from the Centers for Disease Control and Prevention (CDC) involving 7 U.S. institutions, the annual incidence of CRE ranged from 0.35–4.80 annual incident CRE cases/100,000 population [33]. In this study, 47% of the CRE isolates contained a carbapenemase, and all were from the KPC family. Other studies have found the KPC enzyme to be more prevalent, however. For example, in a large study involving 121 cases of CRE bacteremia from 8 medical centers in the New York/New Jersey area, a KPC enzyme was detected in 98 (81%) of cases [34]. KPC-producing Enterobacteriaceae are particularly common in the northeastern and upper Midwest regions of the U.S. [15].

KPC was first identified in Europe in 2005 when a patient who had previously been treated at a medical center in New York returned to France [35]. Since that time, KPC-producing Enterobacteriaceae have been identified with increasing frequency across Europe and the Mediterranean. KPC-producing Klebsiella pneumoniae are now reported to be endemic in Italy and Greece [14]. Studies from these countries have revealed high overall rates of CRE infections that are in large part driven by KPC-producing organisms (80–99% KPC) [36,37].

Emergence of KPC-producing Enterobacteriaceae has also been described with increasing frequency in South America, Central America, the Middle East, and Asia. In Latin America, a study of 255 patients with Enterobacteriaceae bacteremia in 11 hospitals from 7 countries (Argentina, Colombia, Ecuador, Guatemala, Mexico, Peru, Venezuela) revealed that 21% of cases were from carbapenemase-producing bacteria. The majority of these (83%) were KPC-producing Enterobacteriaceae. In Israel, a national intervention to curb CRE infections, driven primarily by KPC-producing bacteria, led to a decrease in CRE acquisitions/100,000 patient days from 55.5 in 2008–4.8 in 2012 [38]. In Asia, KPC-producing CRE are particularly prevalent in China. A study of 109 carbapenem resistant K. pneumoniae bacteremia isolates at a China teaching hospital revealed that 71% were KPC-producers [39]. There is little data on CRE epidemiology in Africa.

Molecular epidemiology studies indicate that the global spread of KPC-producing Enterobacteriaceae is in large part due to the clonal expansion of K. pneumoniae sequence type (ST) 258 [40–42]. ST258 has been shown to consist of two genetic clades with distinct capsule polysaccharide gene regions [43,44]. Interestingly, ST258 clade I is more associated with subtype KPC-2, while ST258 is more associated with subtype KPC-3 [45]. Given the clinical importance of ST258, multiple groups are developing immunotherapeutics directed against K. pneumoniae ST258 [46,47]. However, there is significant regional variability in the molecular epidemiology of KPC-producing K. pneumoniae, as a survey of 111 KPC-producing K. pneumoniae isolates from throughout Spain in 2012–2014 did not identify a single ST258 strain [48]. Interestingly, this study found that single-locus variants of ST258, including ST11 (associated with KPC-2) and ST512 (associated with KPC-3), as well as the unrelated ST101 (associated with KPC-2), were the most prevalent KPC-producing K. pneumoniae.

The first SME-producing Serratia marcescens was found in England from a clinical isolate collected in 1982 [49] and has since been infrequently identified across the U.S. and the United Kingdom [50]. The IMI/NMC-A enzymes appear to be related to SME-1, with 70% amino acid identity [51], and have been detected in Enterobacter cloacae complex isolates in France, Argentina, and the U.S. [52].

2.4. Class B Carbapenemases

The Ambler Class B carbapenemases, referred to as metallo-β-lactamases (MBLs), utilize zinc as an essential cofactor in cleaving the β-lactam ring [53]. The class B MBLs hydrolyze all β-lactams, save for the monobactams (i.e., aztreonam) [11,54]. MBLs are not hydrolyzed by β-lactamase inhibitors that are commonly encountered in the clinical setting such as clavulanate, tazobactam, or avibactam, and instead are inhibited by metal chelating agents such as ethylenediaminetetraacetic acid (EDTA) which are not available for clinical use [53]. However, pipeline agents, including the novel cephalosporin cefiderocol and the monobactam-β-lactamase inhibitor combination aztreonam-avibactam, have demonstrated good in vitro efficacy against MBL-containing CRE [55,56]. The class B MBLs can be further categorized based on whether they are encoded by transferrable elements versus chromosomes. The notable transmissible MBLs in Enterobacteriaceae are the Verona integron-
encoded metallo-β-lactamase (VIM), IMP (for ‘active on imipenem’), and New Delhi metallo-β-lactamase (NDM) enzymes.

A substantial burden of class B carbapenemase-producing Enterobacteriaceae is in Asia. For example, the carbapenemase NDM is endemic in India, Pakistan, and Bangladesh [57]. It is named for New Delhi where it was first isolated from a Swedish patient infected with K. pneumoniae who had previously received health care in New Delhi, India, in 2008 [58]. Studies from India and Pakistan have demonstrated that 8–12% of all Enterobacteriaceae harbor an NDM enzyme, with little detection of non-NDM carbapenemases [59–61]. Worryingly, studies from these countries have also revealed that NDM-containing Enterobacteriaceae often contained additional β-lactamas (such as the CTX-M [so named for its ‘activity against cefotaxime’ and isolation from Munich]) that confers the extended-spectrum β-lactamase (ESBL) phenotype [62] and are commonly isolated from environmental samples such as tap water and sewage effluent [63]. Furthermore, these environmental samples revealed spread of blanDM1 (i.e., the gene for NDM-1) to novel bacterial species including Shigella boydii and Vibrio cholerae [63]. NDM-producing Enterobacteriaceae have also been identified with increasing frequency in the UK as well as across Europe, with notable interregional spread in Denmark, Romania, and Poland [14]. NDM-producing bacteria are less common in Canada, South America, and the U.S. [15]. As of 2017, 35 US states have reported NDM-producing Enterobacteriaceae with a cumulative incidence of 379 cases [32]. The VIM carbapenemase is the most frequently reported MBL worldwide, though is relatively rare in Enterobacteriaceae [54,64]. VIM-producing Enterobacteriaceae are largely found in Italy, Greece, Spain, and Hungary [65].

2.5. Class D Carbapenemases

The class D serine-carbapenemases consist of the OXA β-lactamases, some of which are able to hydrolyze carbapenems. The most commonly encountered class D β-lactamase in Enterobacteriaceae are the OXA-48-like enzymes, which weakly hydrolyze carbapenems and typically spare the expanded spectrum cephalosporins [66]. OXA-48-like producing Enterobacteriaceae were first identified in Turkey in 2001 [67] and have reached endemic levels in Turkey and in Malta based on assessments by national experts [14]. Recent studies from Turkey showed that 69–92% of carbapenemases were OXA-48 [68,69]. OXA-48-like-producing Enterobacteriaceae have spread to neighboring countries in Europe, with high levels of interregional spread in Belgium, France, Romania, and Spain [14]. OXA-48-like producing Enterobacteriaceae are uncommon in the U.S. [32].

2.6. Efflux Pumps

While enzymatic degradation of antibiotic by carbapenemases is the primary mechanism of carbapenem non-susceptibility in Enterobacteriaceae, additional mechanisms, such as efflux pumps and porin mutations, may also play a role (Figure 1). Efflux pumps belonging to the resistance-nodulation-division (RND) family are clinically significant mechanisms of resistance in gram-negative bacteria. One such pump is the AcrAB-ToIC pump found in Enterobacteriaceae. It is tripartite complex that spans the inner membrane, the periplasm, and the outer membrane in order to expel antibiotics from the cell. The AcrAB-ToIC pump may confer resistance to other antibiotics as well, such as other β-lactam antibiotics, macrolides, tetracycline, and fluoroquinolones, among others [70]. The extent to which the upregulation of efflux pumps influences the global epidemiology of carbapenem resistant Enterobacteriaceae (CRE) is not clear.

2.7. Porin Mutations

Mutations in porins such as OmpK35 and OmpK36 alone do not generally result in carbapenem resistance, though can achieve such when present in AmpC or CTX-M producing Enterobacteriaceae [71–74] (Figure 1). CTX-M and AmpC enzymes possess low levels of carbapenem hydrolytic activity. In some cases, the combination of high β-lactamase expression and decreased porin expression creates an “antibiotic trapping phenomenon” whereby the carbapenem is “trapped” by the β-lactamase through irreversible binding, but not degraded [71]. In addition, the presence of ompK36
porin gene mutations in KPC-producing *K. pneumoniae* strains has been associated with high-level carbapenem resistance and attenuated responses to carbapenem–colistin therapy [75–77]. The contribution of porin mutations in the global epidemiology of CRE is not well understood, though in the U.S. a multicenter study revealed *ompK35* and *ompK36* porin mutations in 84% and 34% of carbapenem-resistant *K. pneumoniae*, respectively [34].

### 2.8. Colistin Resistance

Unfortunately, *Enterobacteriaceae* strains which are resistant to carbapenems frequently acquire resistance to other classes of antibiotics, including aminoglycosides, tetracyclines, trimethoprim-sulfonamides, and fluoroquinolones [78]. Infections with such highly resistant *Enterobacteriaceae* commonly require therapy with a polymyxin, though even this is potentially complicated by the emergence of polymyxin resistance. Colistin is a polycationic peptide that displaces cations in the lipopolysaccharide (LPS) component of bacterial outer membrane, leading to disruption of the outer membrane and cell death [79]. Colistin resistance can emerge through multiple mechanisms including increased capsule production (which decreases binding of colistin to its target LPS), loss of LPS, and modification of LPS [80]. Unfortunately, colistin resistance can be transmitted between bacteria by way of the plasmid-encoded *mcr-1*, which modifies the antibiotic target site and decreases polymyxin binding [81].

### 3. Mechanisms and Epidemiology of Antibiotic Resistance in XDR *P. aeruginosa*

#### 3.1. Overview of Mechanisms of Resistance in XDR *P. aeruginosa*

*P. aeruginosa* is intrinsically resistant to multiple antibiotics including rifampin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and many β-lactams [82]. Low membrane permeability and efflux pump expression are partly responsible for this intrinsic resistance [82]. Additional resistance mechanisms may be present and drive highly antibiotic resistant phenotypes such as XDR *P. aeruginosa*, which is defined as susceptible to only one or two classes of antipseudomonal antimicrobials [83,84]. There are four general mechanisms by which *P. aeruginosa* generate resistance to antipseudomonal antimicrobials: (1) efflux pumps, (2) porin expression, (3) antibiotic target mutations, and (4) drug-inactivating enzymes [85] (Figure 2). It is the combination of these mechanisms which results in the XDR phenotype. Mechanisms of antibiotic resistance in *P. aeruginosa* are discussed in detail in sections 3.3–3.6.

![Figure 2. Mechanisms of antibiotic resistance in *P. aeruginosa*.](image)
3.2. Prevalence of and Risk Factors for XDR P. aeruginosa

Study of the epidemiology of XDR P. aeruginosa is complicated by a lack of global surveillance data and the diverse mechanisms by which P. aeruginosa develops resistance. The global emergence of XDR P. aeruginosa is due to the accumulation of multiple unrelated resistance mechanisms [85]. In the U.S., the international network for optimal resistance monitoring (INFORM) group evaluated P. aeruginosa isolates across 79 U.S. medical centers and found that 9% of all isolates exhibited the XDR phenotype. A similar or higher prevalence of the XDR phenotype among clinical P. aeruginosa isolates has been found in epidemiological studies from Spain (11%) [86], Thailand (22%) [84], Greece (25%) [87], and Iran (33%) [88]. In some patient populations, the XDR phenotype may occur at an alarmingly high rate, as in P. aeruginosa from burn patients in a single center study in Iran (87%) [89] or solid organ transplant patients from a single center study in Spain (63%) [90]. Risk factors for acquisition of XDR P. aeruginosa infections, relative to more susceptible P. aeruginosa infections, include receipt of total parenteral nutrition [84], prior fluoroquinolone use [84,87], prior carbapenem use [84], hematological malignancy [87], mechanical ventilation [91], and Acute Physiology and Chronic Health Evaluation II (APACHE-II) score [91].

3.3. Efflux Pumps

Efflux pumps contribute to antibiotic resistance of multiple drug classes (Figure 2). For example, mutations leading to upregulation of efflux pumps such as the MexA-MexB-OprM system can lead to increased resistance to anti-pseudomonal β-lactams, fluoroquinolones, and aminoglycosides [92–94]. When upregulation of efflux pump expression is present in combination with targeted outer membrane mutations, additional resistance may be achieved [95]. In vitro overexpression of mexAB-oprM is readily achieved under selection pressure from antibiotic exposure, and often stems from mutations in the transcription factors that regulate mexAB-oprM expression [96,97]. The global epidemiology of mexAB-oprM expression and its influence on the P. aeruginosa XDR phenotype is not well understood.

3.4. Porin Mutations

OprD is a porin protein which allows passive uptake of basic amino acids across the outer membrane, as well as uptake of carbapenems [93]. Loss of OprD confers resistance to imipenem and reduced susceptibility to meropenem [93] (Figure 2). When the loss of OprD is combined with upregulation of MexA-MexB-OprM, resistance to meropenem, imipenem, ceftazidime, ureidopenicillins, carboxypenicillins, quinolones, tetracycline, and chloramphenicol is achieved [93]. OprD mutation is particularly associated with the “high risk” P. aeruginosa clone ST75 (see section 3.6. below).

3.5. Modification of Antibiotic Target Site

While fluoroquinolone resistance is commonly driven by changes in efflux pump expression (Section 3.3), mutations in the fluoroquinolone targets parC and gyrA have been identified in P. aeruginosa strains which confer resistance to fluoroquinolones [98]. These genes code for topoisomerase IV and DNA gyrase, respectively, and are critical in bacterial DNA replication [99,100]. Aminoglycosides exert their antibacterial effect through binding and inhibiting the bacterial 16S rRNA, and resistance to aminoglycosides in P. aeruginosa may emerge through modification of the antibiotic target 16S rRNA. For example, 16S rRNA methylases RmtA, RmtD, and ArmA have been described in P. aeruginosa and may confer resistance to all clinically useful aminoglycosides [101–103]. Colistin resistance in P. aeruginosa may emerge through mutation of multiple genes such as pmrAB and phoPQ, among others, with the final common pathway being the modification of the LPS target of colistin [104].

3.6. Antibiotic Degradation
Upregulation or acquisition via horizontal transfer of drug-inactivating enzymes contribute to antimicrobial resistance in *Pseudomonas*. *P. aeruginosa* intrinsically possess class C AmpC enzymes, which are chromosomally encoded cephalosporinases (Table 1). Mutations causing increased expression of AmpC enzymes, or mutations which enable hyper-inducible AmpC production, result in resistance to penicillins, monobactams, and cephems [73]. Additionally, horizontal acquisition of a wide variety of β-lactamases from classes A, B, and D may result in a wide spectrum of resistance. Some of these β-lactamases are associated with particular “high risk” *P. aeruginosa* clones that have a wide global distribution (Section 3.7 below). Acquisition of narrow spectrum β-lactamases such as PSE (*Pseudomonas* specific enzyme)-1, PSE-4, and some OXA-type enzymes enable resistance to the antipseudomonal penicillins and cefoperazone but do not have activity against monobactams, carbapenems, or antipseudomonal cephems (Table 1) [82]. Acquisition of broad-spectrum β-lactamases such as PER (*Pseudomonas aeruginosa* RNL-1)-1, VEB (Vietnamese extended-spectrum β-lactamase)-1, GES (Guiana extended spectrum)-1, GES-2, and some OXA-type enzymes confer resistance to anti-pseudomonal penicillins, anti-pseudomonal cephems, and monobactams, but not carbapenems [82]. Finally, acquisition of MBLs including IMP, SPM (Sao Paulo metallo-β-lactamase), GIM (Germany imipenemase), and VIM enzymes can confer resistance to all antipseudomonal β-lactams, save for the monobactams [52,82,105].

Aminoglycoside modifying enzymes can similarly be acquired via horizontal gene transfer. The aminoglycoside modifying enzymes include enzymes which may phosphorylate, adenylate, and acetylate the aminoglycoside, thereby decreasing its affinity to bind the ribosomal subunit [85]. The most frequent aminoglycoside modifying enzyme in *P. aeruginosa* is AAC(6′)-II which mediates resistance to tobramycin, gentamicin, and netilmicin, and ANT(2″)-I, which mediates resistance to gentamicin and tobramycin [106]. The novel aminoglycoside plazomicin retains activity against these aminoglycoside-modifying enzymes through structural modification of the antibiotic [107].

### 3.7. Clonal Structure of XDR *P. aeruginosa*

*P. aeruginosa* demonstrates significant genomic variability, though isolates with the XDR phenotype display a more clonal structure [86,108,109]. For example, in one study, 90% of all XDR *P. aeruginosa* isolates belonged to one of three “high risk” clones: ST11, ST175, or ST235 [109]. Of these, ST235 has the most global distribution and has been detected on five continents [110]. ST11 is also globally distributed, with the exception of Oceania, while ST175 is uncommon outside Europe [110].

Even among *P. aeruginosa* clinical isolates within a single high-risk clone, the resistance mechanisms driving the XDR phenotype are remarkably diverse. In ST235, for example, 39 different β-lactamases from classes A, B, and D have been identified [110]. Class A enzymes such as the ESBLs PER-1 and GES enzymes are particularly widespread [111–113]. Class B carbapenemases are more common among ST235 isolates, and VIM-1 is the most geographically widespread [85,111]. Of concern is the discovery of ST235 *P. aeruginosa* isolates containing the KPC-2 enzyme in Colombia [111,114].

*P. aeruginosa* ST11 also contains an array of β-lactamases, albeit fewer than those belonging to ST235 [110]. The most widespread β-lactamase in ST11 isolates is the class B enzyme VIM-2 [86,111,114,115], though class A enzymes including the ESBLs, GES, and VEB enzymes may be detected as well [111,116]. As with ST235, *Klebsiella pneumoniae* carbapenemase-2 (KPC-2) containing ST111 have been detected in Colombia [115].

*P. aeruginosa* ST175, in contrast to ST235 and ST11, often generates the XDR phenotype through OprD porin activation, AmpC hyperproduction, and efflux pump overexpression [86]. As with ST235 and ST111, however, the VIM-2 enzyme is commonly present [117,118].

### 4. Mechanisms and Epidemiology of Antibiotic Resistance in XDR *A. baumannii*

#### 4.1. Mechanisms of Resistance in XDR *A. baumannii*

*A. baumannii* is a nosocomial and opportunistic pathogen that is often subjected to significant selective pressures from antibiotics in the hospital environment. This may result in chromosomal
mutations and acquisition of resistance genes via horizontal transfer to ensure its survival and spread. *A. baumannii* is intrinsically resistant to several groups of antimicrobials, including glycopeptides, lincosamides, macrolides, and streptogamins. XDR *A. baumannii* may arise through a variety of mechanisms, including efflux pumps, porin expression, antibiotic target mutations, and drug-inactivating enzymes (sections 4.3–4.5.) (Figure 3).

Figure 3. Mechanisms of antibiotic resistance in *A. baumannii*.

### 4.2. Prevalence of and Risk Factors for XDR *A. baumannii* Infection

*A. baumannii* is an important cause of healthcare-associated infections. Determining the prevalence of XDR *A. baumannii* is challenging, in that large multicenter studies investigating antibiotic resistance in *A. baumannii* may not address the XDR phenotype per se. However, one of the hallmarks of XDR *A. baumannii* is carbapenem resistance, as carbapenem resistant isolates are often XDR [119]. According to the National Healthcare Safety Network at the centers for disease control in the US, the overall rate of carbapenem resistant *A. baumannii* was 47% in central line-associated bacteremia and 64% in catheter-associated urinary tract infections [120]. In Europe, a recent report from the European center for disease prevention and control showed that 49% of *A. baumannii* (2861/5853) were carbapenem resistant [121]. There was significant international variability, with countries in southern Europe and the Baltic exhibiting particularly high resistance [121]. The prevalence of carbapenem resistant *A. baumannii* is similarly high in other parts of the world including southern and southeast Asia (40–60%) [122], and Latin America (40–80%) [123]. Risk factors for colonization and/or infection with XDR *A. baumannii* include prior antibiotic use (specifically carbapenem, third generation cephalosporin, or fluoroquinolone use), indwelling central line, mechanical ventilation, tracheostomy, recent surgery, and intensive care unit (ICU) stay [124,125].

### 4.3. Outbreaks of XDR *A. baumannii* Infection

Outbreaks of XDR *A. baumannii* often occur in an ICU setting. The selective pressure of broad-spectrum antibiotics in ICUs and the ease and rapidity of acquiring multiple antibiotic resistance mechanisms are drivers for high antibiotic resistance. The ability of *A. baumannii* to survive on inanimate surfaces for extended periods of time plays a role as well. For example, *A. baumannii* was isolated from hospital bed rails nine days after the infected patient was discharged from the hospital [126]. Multiple ICU outbreaks have been traced back to a contamination source such as respiratory
equipment or the hands of healthcare workers [127]. A. baumannii ICU outbreaks have been reported in cities across Europe, South America, Africa, Asia, the Middle East, and the U.S. [125,128–135]. Additionally, interinstitutional and international spread of such outbreaks have been described in cases of colonized or infected patients who are transferred to another healthcare facility [125,136,137]. Community acquired XDR A. baumannii infections are exceedingly rare [138,139].

4.4. Efflux Pumps and Decreased Outer Membrane Permeability

Increased efflux pump activity is a key mechanism of resistance in A. baumannii. Specifically, overexpression of the adeABC resistance-nodulation-cell division (RND) efflux system enables resistance to several classes of antibiotics, including fluoroquinolones, aminoglycosides, trimethoprim, tetracyclines, and chloramphenicol [140] (Figure 3). When the adeABC efflux pump is over-expressed in A. baumannii harboring carbapenem-hydrolyzing oxacillinases, high level carbapenem resistance is achieved [141]. An additional efflux system-adeIJK may confer resistance to tigecycline. This is particularly the case when adeIJK and adeABC are co-expressed [142]. Efflux pumps encoded by the tet genes are typically associated with mobile genetic elements. TetA confers resistance to tetracycline, while TetB confers resistance to minocycline [143]. The outer membrane protein OmpA is a major A. baumannii porin. Mutations targeting the ompA gene may result in decreased outer membrane permeability, and achieve resistance to chloramphenicol, aztreonam, and nalidixic acid [144] (Figure 3).

4.5. Mutations in Antibiotic Target

Resistance to fluoroquinolones may emerge through mutations in gyrA and parC, subunits of DNA gyrase and DNA topoisomerase, respectively, which decrease the affinity of fluoroquinolones for their targets [145,146] (Figure 3). An international collection of fluoroquinolone-resistant A. baumannii has demonstrated that mutations in genes gyrA and parC are highly prevalent [147]. The role that additional mechanism of resistance, such as overexpression of efflux pumps, plays in the global epidemiology of fluoroquinolone resistance is not known. Mutations in enzymes involved in the biosynthesis of lipopolysaccharide (LPS) leading to the modification or loss of the lipid A portion of LPS may lead to colistin resistance through decreased colistin binding [148,149].

Aminoglycoside-modifying enzymes including acetyltransferases, nucleotidytransferases, and phosphotransferases may be acquired via plasmid exchange, transposons, or class 1 integrons [150,151] (Figure 3). These enzymes may occur in isolation or in combination to enable resistance to aminoglycosides [150]. As described in section 3.5., aminoglycosides bind and inhibit the bacterial 16S rRNA. Modification of the target 16S rRNA is another effective mechanism of resistance (Figure 3). For example, 16S rRNA methyltransferases have been described in A. baumannii and are responsible for high level resistance to all aminoglycosides, including amikacin [152]. ArmA is one such 16S rRNA methyltransferase that is well characterized in A. baumannii and confers resistance via interfering with the binding of aminoglycosides to their site of action [152]. ArmA has been detected worldwide [152–156]. The global epidemiology of the additional 16S methylases (e.g. RmtA, RmtB, RmtC, RmtD, RmtE, RmtF, RmtG, NpmA) is less clear. Of note, isolates harboring 16S rRNA methylases typically also harbor ESBLs or MBLs by nature of their location on transferable plasmids and association of transposable structures [157].

4.6. Antibiotic Degradation

A. baumannii intrinsically possess a chromosomal AmpC cephalosporinase, which hydrolyzes cephalosporins at a low level. Similarly, A. baumannii intrinsically possesses a class D oxacillinase, OXA-51-like enzyme, which hydrolyze penicillins and carbapenems at a low level. Insertion of a strong transcriptional promoter, ISAba1, upstream of the ampC cephalosporinase or OXA-51-like gene results in clinically significant cephalosporin resistance [158,159]. In addition, acquisition of carbapenemases have been well described in A. baumannii. Specifically, class D enzymes such as OXA-23-, OXA-40-, and OXA-58-like enzymes are the most commonly detected carbapenemases in
A. baumannii and may be plasmid or chromosomally encoded [157]. OXA-23-like enzymes are the most widespread carbapenem-hydrolyzing enzymes detected in A. baumannii worldwide and are common drivers of nosocomial outbreaks of carbapenem-resistant A. baumannii [157,160]. These enzymes are typically associated with transposons Tn2006 and Tn2007 [161–163]. Other class D β-lactamases with carbapenemase activity have more regional distributions such as OXA-25, OXA-26, and OXA-40, which are predominantly detected in Europe [164,165] and OXA-72 which is predominantly detected in Asia [166–169]. Less common causes of carbapenem resistance in A. baumannii include the class A and B β-lactamases. Class A KPC-producing A. baumannii are exceedingly rare and have mostly been described in a series of isolates from Puerto Rico [170]. Class B IMP-, VIM-, SIM- (Seoul imipenemase), and NDM-type enzymes have been detected in A. baumannii, though the IMP- and VIM-type enzymes are most commonly isolated and widely distributed [171].

5. Conclusions

Genotyping and whole genome sequencing of highly resistant gram-negative bacteria has revealed many of the diverse ways that these bacteria develop antibiotic resistance. This fundamental knowledge has impacted our understanding of how resistance emerges, how it is transferred to other bacteria and to patients, the development of novel antibiotics, and treatment decisions in select patients. Further integration of bacterial genotyping for resistance mechanisms into the clinical setting will likely impact our therapeutic decisions in meaningful ways.

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References

1. Tumbarello, M.; Viale, P.; Viscoli, C.; Trecarichi, E.M.; Tumietto, F.; Marchese, A.; Spanu, T.; Ambretti, S.; Ginocchio, F.; Cristini, F.; et al. Predictors of mortality in bloodstream infections caused by Klebsiella pneumoniae carbapenemase-producing K. pneumoniae: Importance of combination therapy. Clin. Infect. Dis. 2012, 55, 943–950. doi:10.1093/cid/cis588.
2. Pouch, S.M.; Kubin, C.J.; Satlin, M.J.; Tsapeas, D.S.; Lee, J.R.; Dube, G.; Pereira, M.R. Epidemiology and outcomes of carbapenem-resistant Klebsiella pneumoniae bacteruria in kidney transplant recipients. Transpl. Infect. Dis. 2015, 17, 800–809. doi:10.1111/tid.12450.
3. Gasink, L.B.; Edelstein, P.H.; Lautenbach, E.; Synnestvedt, M.; Fishman, N.O. Risk factors and clinical impact of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. Infect. Control. Hosp. Epidemiol. 2009, 30, 1180–1185, doi:10.1086/648451.
4. Patel, G.; Huprikar, S.; Factor, S.H.; Jenkins, S.G.; Calfee, D.P. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect. Control Hosp. Epidemiol. 2008, 29, 1099–1106, doi:10.1086/592412.
5. Thaden, J.T.; Li, Y.; Ruffin, F.; Maskarinec, S.A.; Hill-Rorie, J.M.; Wanda, L.C.; Reed, S.D.; Fowler, V.G., Jr. Increased Costs Associated with Bloodstream Infections Caused by Multidrug-Resistant Gram-Negative Bacteria Are Due Primarily to Patients with Hospital-Acquired Infections. Antimicrob. Agents Chemother. 2017, 61, e01709–16, doi:10.1128/AAC.01709-16.
6. Evans, H.L.; Lefrak, S.N.; Lyman, J.; Smith, R.L.; Chong, T.W.; McElearney, S.T.; Schulman, A.R.; Hughes, M.G.; Raymond, D.P.; Pruett, T.L.; et al. Cost of Gram-negative resistance. Crit. Care Med. 2007, 35, 89–95, doi:10.1097/01.CCM.0000251496.61520.75.
7. Hu, B.; Ye, H.; Xu, Y.; Ni, Y.; Hu, Y.; Yu, Y.; Huang, Z.; Ma, L. Clinical and economic outcomes associated with community-acquired intra-abdominal infections caused by extended spectrum beta-lactamase (ESBL) producing bacteria in China. Curr. Med. Res. Opin. 2010, 26, 1443–1449, doi:10.1185/03007991003769068.
8. Lautenbach, E.; Patel, J.B.; Bilker, W.B.; Edelstein, P.H.; Fishman, N.O. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae: Risk factors for infection and impact of resistance on outcomes. Clin. Infect. Dis. 2001, 32, 1162–1171, doi:10.1086/319757.
9. Xie, L.; Dou, Y.; Zhou, K.; Chen, Y.; Han, L.; Guo, X.; Sun, J. Coexistence of blaOXA-48 and Truncated blaNDM-1 on Different Plasmids in a Klebsiella pneumoniae Isolate in China. Front. Microbiol 2017, 8, 133, doi:10.3389/fmicb.2017.00133.

10. Kapmaz, M.; Erdem, F.; Abulaila, A.; Yeniaras, E.; Oncul, O.; Aktaş, Z. First detection of NDM-1 with CTX-M-9, TEM, SHV and mTc in Escherichia coli ST471 carrying IncI2, A/C and Y plasmids from clinical isolates in Turkey. J. Glob. Antimicrob. Resist. 2016, 7, 152–153, doi:10.1016/j.jgar.2016.10.001.

11. Marshall, S.; Hujer, A.M.; Rojas, L.J.; Papp-Walace, K.M.; Humphries, R.M.; Spellberg, B.; Hujer, K.M.; Marshall, E.K.; Rudin, S.D.; Perez, F.; et al. Can Ceftazidime-Avibactam and Aztreonam Overcome beta-Lactam Resistance Conferred by Metallo-beta-Lactamases in Enterobacteriaceae? Antimicrob. Agents Chemother. 2017, 61, e02243–16, doi:10.1128/AAC.02243-16.

12. World Health Organization. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics; WHO: Geneva, Switzerland, 2017.

13. Meletis, G.; Chatzidimitriou, D.; Malisiovas, N. Double- and multi-carbapenemase-producers: The excessively armored bacilli of the current decade. Ear J. Clin. Microbiol. Infect. Dis. 2015, 34, 1487–1493, doi:10.1007/s10096-015-2379-9.

14. Albiger, B.; Glaßner, C.; Struelens, M.J.; Grundmann, H.; Monnet, D.L. Carbapenemase-producing Enterobacteriaceae in Europe: Assessment by national experts from 38 countries, May 2015. Euro Surveill. 2015, 20, doi:10.2807/1560-7917.ES.2015.20.45.30062.

15. van Duin, D.; Doi, Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 2017, 8, 460–469, doi:10.1080/21505594.2016.1222343.

16. van der Bij, A.K.; Pitout, J.D. The role of international travel in the worldwide spread of multiresistant Enterobacteriaceae. J. Antimicrob. Chemother. 2012, 67, 2090–2100, doi:10.1093/jac/dks214.

17. Arnold, R.S.; Thom, K.A.; Sharma, S.; Phillips, M.; Kristie Johnson, J.; Morgan, D.J. Emergence of Klebsiella pneumoniae carbapenemase-producing bacteria. South Med. J. 2011, 104, 40–45, doi:10.1097/SMJ.0b013e3181fd7d5a.

18. Chiotos, K.; Tamma, P.D.; Flett, K.B.; Naumann, M.; Karandiar, M.V.; Bilker, W.B.; Zaoutis, T.; Han, J.H. Multicenter Study of the Risk Factors for Colonization or Infection with Carbapenem-Resistant Enterobacteriaceae in Children. Antimicrob. Agents Chemother. 2017, 61, e01440–17, doi:10.1128/AAC.01440-17.

19. Queenan, A.M.; Bush, K. Carbapenemases: The versatile beta-lactamases. Clin. Microbiol. Rev. 2007, 20, 440–458, table of contents, doi:10.1128/CMR.00001-07.

20. Sader, H.S.; Castanheira, M.; Flamm, R.K.; Farrell, D.J.; Jones, R.N. Antimicrobial activity of ceftazidime-avibactam against Gram-negative organisms collected from U.S. medical centers in 2012. Antimicrob. Agents Chemother. 2014, 58, 1684–1692, doi:10.1128/AAC.02429-13.

21. Castanheira, M.; Rhomberg, P.R.; Flamm, R.K.; Jones, R.N. Effect of the beta-Lactamase Inhibitor Vaborbactam Combined with Meropenem against Serine Carbapenemase-Producing Enterobacteriaceae. Antimicrob. Agents Chemother. 2016, 60, 5454–5458, doi:10.1128/AAC.00711-16.

22. Haidar, G.; Clancy, C.J.; Chen, L.; Samanta, P.; Shields, R.K.; Kreiswirth, B.N.; Nguyen, M.H. Identifying Spectra of Activity and Therapeutic Niches for Ceftazidime-Avibactam and Imipenem-Relebactam against Carbapenem-Resistant Enterobacteriaceae. Antimicrob. Agents Chemother. 2017, 61, e00642–17, doi:10.1128/AAC.00642-17.

23. Logan, L.K.; Weinstein, R.A. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. J. Infect. Dis. 2017, 215, S28-S36, doi:10.1093/infdis/jiw282.

24. Naas, T.; Dortet, L.; Iorga, B.I. Structural and Functional Aspects of Class A Carbapenemases. Curr Drug Targets 2016, 17, 1006–1028.

25. Diene, S.M.; Rolain, J.M. Carbapenemase genes and genetic platforms in Gram-negative bacilli: Enterobacteriaceae, Pseudomonas and Acinetobacter species. Clin. Microbiol. Infect. 2014, 20, 831–838, doi:10.1111/1469-0691.12655.

26. Yigit, H.; Queenan, A.M.; Anderson, G.J.; Domenech-Sanchez, A.; Biddle, J.W.; Steward, C.D.; Alberti, S.; Bush, K.; Tenover, F.C. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob. Agents Chemother. 2001, 45, 1151–1161, doi:10.1128/AAC.45.4.1151-1161.2001.

27. Mirigou, V.; Tzouvelekis, L.S.; Rossiter, S.; Tzelepi, E.; Angulo, F.J.; Whichard, J.M. Imipenem resistance in a Salmonella clinical strain due to plasmid-mediated class A carbapenemase KPC-2. Antimicrob. Agents Chemother. 2003, 47, 1297–1300.
28. Yigit, H.; Queenan, A.M.; Rasheed, J.K.; Biddle, J.W.; Domenech-Sanchez, A.; Alberti, S.; Bush, K.; Tenover, F.C. Carbapenem-resistant strain of Klebsiella oxytoca harboring carbapenem-hydrolyzing beta-lactamase KPC-2. *Antimicrob. Agents Chemother.* 2003, **47**, 3881–3889.

29. Bradford, P.A.; Bratu, S.; Urban, C.; Visalli, M.; Mariano, N.; Landman, D.; Rahal, J.J.; Brooks, S.; Cebular, S.; Quale, J. Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. *Clin. Infect. Dis.* 2004, **39**, 55–60, doi:10.1086/421495.

30. Hossain, A.; Ferraro, M.J.; Pino, R.M.; Dew, R.B.; 3rd; Moland, E.S.; Lockhart, T.J.; Thomson, K.S.; Goering, R.V.; Hanson, N.D. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 in an Enterobacter sp. *Antimicrob. Agents Chemother.* 2004, **48**, 4438–4440, doi:10.1128/AAC.48.11.4438-4440.2004.

31. Bratu, S.; Landman, D.; Alam, M.; Tolentino, E.; Quale, J. Detection of KPC carbapenem-hydrolyzing enzymes in Enterobacter spp. from Brooklyn, New York. *Antimicrob. Agents Chemother.* 2005, **49**, 776–778, doi:10.1128/AAC.49.2.776-778.2005.

32. Centers for Disease Control and Prevention Website: Tracking CRE. Availabe online: https://www.cdc.gov/hai/organisms/cre/TrackingCRE.html (accessed on 22 March 2019).

33. Guh, A.Y.; Bulens, S.N.; Mu, Y.; Jacob, J.T.; Reno, J.; Scott, J.; Wilson, L.E.; Vaeth, E.; Lynfield, R.; Shaw, K.M.; et al. Epidemiology of Carbapenem-Resistant *Enterobacteriaceae* in 7 US Communities, 2012-2013. *JAMA* 2015, **314**, 1479–1487, doi:10.1001/jama.2015.12480.

34. Satlin, M.J.; Chen, L.; Patel, G.; Gomez-Simmonds, A.; Weston, G.; Kim, A.C.; Soo, S.K.; Rosenthal, M.E.; Sperber, S.J.; Jenkins, S.G.; et al. Multicenter Clinical and Molecular Epidemiological Analysis of Bacteremia Due to Carbapenem-Resistant *Enterobacteriaceae* (CRE) in the CRE Epicenter of the United States. *Antimicrob. Agents Chemother.* 2017, **61**, e02349–16 doi:10.1128/AAC.02349-16.

35. Naas, T.; Nordmann, P.; Vedel, G.; Poyart, C. Plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC in a Klebsiella pneumoniae isolate from France. *Antimicrob. Agents Chemother.* 2005, **49**, 4423–4424, doi:10.1128/AAC.49.10.4423-4424.2005.

36. Spyropoulou, A.; Papadimitriou-Olivgeris, M.; Bartzavali, C.; Vamvakopoulou, S.; Marangos, M.; Spiliopoulou, I.; Anastassiou, E.D.; Christofidou, M. A ten-year surveillance study of carbapenemase-producing Klebsiella pneumoniae in a tertiary care Greek university hospital: Predominance of KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. *Antimicrob. Agents Chemother.* 2007, **61**, e02349–16 doi:10.1128/AAC.02349-16.

37. Schwaber, M.J.; Carmeli, Y. An ongoing national intervention to contain the spread of carbapenem-resistant Enterobacteriaceae. *Clin. Infect. Dis.* 2014, **58**, 697–703, doi:10.1093/cid/cit795.

38. Chen, S.; Hu, F.; Xu, X.; Liu, Y.; Wu, W.; Zhu, D.; Wang, H. High prevalence of KPC-2-type carbapenemase coupled with CTX-M-type extended-spectrum beta-lactamases in carbapenem-resistant Klebsiella pneumoniae in a teaching hospital in China. *Antimicrob. Agents Chemother.* 2011, **55**, 2493–2494, doi:10.1128/AAC.00471-11.

39. Baraniak, A.; Izdebski, R.; Fiett, J.; Herda, M.; Derde, L.P.; Bonten, M.J.; Adler, A.; Carmeli, Y.; Goossens, H.; Hryniewicz, W.; et al. KPC-Like Carbapenemase-Producing *Enterobacteriaceae* Colonizing Patients in Europe and Israel. *Antimicrob. Agents Chemother.* 2015, **60**, 1912–1917, doi:10.1128/AAC.02756-15.

40. Kitchel, B.; Rasheed, J.K.; Patel, J.B.; Srinivasan, A.; Navon-Venezia, S.; Carmeli, Y.; Brolund, A.; Giske, C.G. Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: Clonal expansion of multilocus sequence type 258. *Antimicrob. Agents Chemother.* 2009, **53**, 3365–3370, doi:10.1128/AAC.01226-09.

41. Ocampo, A.M.; Chen, L.; Cienfuegos, A.V.; Roncancio, G.; Chavda, K.D.; Kreiswirth, B.N.; Jimenez, J.N. A Two-Year Surveillance in Five Colombian Tertiary Care Hospitals Reveals High Frequency of Non-CG258 Clones of Carbapenem-Resistant Klebsiella pneumoniae with Distinct Clinical Characteristics. *Antimicrob. Agents Chemother.* 2016, **60**, 332–342, doi:10.1128/AAC.01775-15.

42. Deleo, F.R.; Chen, L.; Porcella, S.F.; Martens, C.A.; Kobayashi, S.D.; Porter, A.R.; Chavda, K.D.; Jacobs, M.R.; Mathema, B.; Olsen, R.J.; et al. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 Klebsiella pneumoniae. *Proc. Natl. Acad. Sci. USA* 2014, **111**, 4988–4993, doi:10.1073/pnas.1321364111.
44. Chen, L.; Mathema, B.; Pitout, J.D.; DeLeo, F.R.; Kreiswirth, B.N. Epidemic Klebsiella pneumoniae ST258 is a hybrid strain. *MBio* 2014, 5, e01355–14, doi:10.1128/mBio.01355-14.

45. van Duin, D.; Perez, F.; Rudin, S.D.; Cober, E.; Hanrahan, J.; Ziegler, J.; Webber, R.; Fox, J.; Mason, P.; Richter, S.S.; et al. Surveillance of carbapenem-resistant Klebsiella pneumoniae: Tracking molecular epidemiology and outcomes through a regional network. *Antimicrob. Agents Chemother.* 2014, 58, 4035–4041, doi:10.1128/AAC.02636-14.

46. Kobayashi, S.D.; Porter, A.R.; Freedman, B.; Pandey, R.; Chen, L.; Kreiswirth, B.N.; DeLeo, F.R. Antibody-Mediated Killing of Carbapenem-Resistant ST258 Klebsiella pneumoniae by Human Neutrophils. *MBio* 2018, 9, e00297–18, doi:10.1128/mBio.00297-18.

47. Diago-Navarro, E.; Molty, M.P.; Ruiz-Perez, G.; Yu, W.; Austin, J.; Seco, B.M.S.; Xiao, G.; Chikhalya, A.; Seeberger, P.H.; Fries, B.C. Novel, Broadly Reactive Anticapsular Antibodies against Carbapenem-Resistant Klebsiella pneumoniae Protect from Infection. *MBio* 2018, 9, e00091–18, doi:10.1128/mBio.00091-18.

48. Oteo, J.; Perez-Vazquez, M.; Bautista, V.; Ortega, A.; Zamarron, P.; Saez, D.; Fernandez-Romero, S.; Lara, N.; Ramiro, R.; Arcail, B.; et al. The spread of KPC-producing Enterobacteriaceae in Spain: WGS analysis of the emerging high-risk clones of Klebsiella pneumoniae ST11/KPC-2, ST101/KPC-2 and ST512/KPC-3. *J. Antimicrob. Chemother.* 2016, 71, 3392–3399, doi:10.1093/jac/dkw321.

49. Naas, T.; Vandel, L.; Songakoff, W.; Livermore, D.M.; Nordmann, P. Cloning and sequence analysis of the gene for a carbapenem-hydrolyzing class A beta-lactamase, Sme-I, from Serratia marcescens S6. *Antimicrob. Agents Chemother.* 1994, 38, 1262–1270.

50. Walther-Rasmussen, J.; Hoiby, N. Class A carbapenemases. *J. Antimicrob. Chemother.* 2007, 60, 470–482, doi:10.1093/jac/dkm226.

51. Rasmussen, B.A.; Bush, K.; Keeney, D.; Yang, Y.; Hare, R.; O’Gara, C.; Medeiros, A.A. Characterization of IMI-1 beta-lactamase, a class A carbapenem-hydrolyzing enzyme from Enterobacter cloacae. *Antimicrob. Agents Chemother.* 1996, 40, 2080–2086.

52. Nordmann, P.; Poirel, L. Emerging carbapenemases in Gram-negative aerobes. *Clin. Microbiol. Infect.* 2002, 8, 321–331.

53. Meini, M.R.; Llarrull, L.I.; Vila, A.J. Overcoming differences: The catalytic mechanism of metallo-beta-lactamases. *FEBS Lett.* 2015, 589, 3419–3432, doi:10.1016/j.febslet.2015.08.015.

54. Walsh, T.R.; Toleman, M.A.; Poirel, L.; Nordmann, P. Metallo-beta-lactamases: The quiet before the storm? *Clin. Microbiol. Rev.* 2005, 18, 306–325, doi:10.1128/CMR.18.2.306-325.2005.

55. Kohira, N.; West, J.; Ito, A.; Ito-Horiyama, T.; Nakamura, R.; Sato, T.; Rittenhouse, S.; Tsuji, M.; Yamano, Y. In Vitro Antimicrobial Activity of a Siderophore Cephalosporin, S-649266, against Enterobacteriaceae Clinical Isolates, Including Carbapenem-Resistant Strains. *Antimicrob. Agents Chemother.* 2016, 60, 729–734, doi:10.1128/AAC.01695-15.

56. Wang, X.; Zhang, F.; Zhao, C.; Wang, Z.; Nichols, W.W.; Testa, R.; Li, H.; Chen, H.; He, W.; Wang, Q.; et al. In vitro activities of ceftazidime-avibactam and aztreonam-avibactam against 372 Gram-negative bacilli collected in 2011 and 2012 from 11 teaching hospitals in China. *Antimicrob. Agents Chemother.* 2014, 58, 1774–1778, doi:10.1128/AAC.02123-13.

57. Kumarasamy, K.K.; Toleman, M.A.; Walsh, T.R.; Bagaria, J.; Butt, F.; Balakrishnan, R.; Chaudhary, U.; Doumith, M.; Giske, C.G.; Irfan, S.; et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect. Dis.* 2010, 10, 597–602, doi:10.1016/S1473-3099(10)70143-2.

58. Yong, D.; Toleman, M.A.; Giske, C.G.; Cho, H.S.; Sundman, K.; Lee, K.; Walsh, T.R. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob. Agents Chemother.* 2009, 53, 5046–5054, doi:10.1128/AAC.00774-09.

59. Ranjan, A.; Shaik, S.; Mondal, A.; Nandanwar, N.; Hussain, A.; Semmler, T.; Kumar, N.; Tiwari, S.K.; Jadhav, S.; Wieler, L.H.; et al. Molecular Epidemiology and Genome Dynamics of New Delhi Metallo-beta-Lactamase-Producing Extraintestinal Pathogenic Escherichia coli Strains from India. *Antimicrob. Agents Chemother.* 2016, 60, 6795–6805, doi:10.1128/AAC.01345-16.

60. Rahman, M.; Shukla, S.K.; Prasad, K.N.; Ovejero, C.M.; Pati, B.K.; Tripathi, A.; Singh, A.; Srivastava, A.K.; Gonzalez-Zorn, B. Prevalence and molecular characterisation of New Delhi metallo-beta-lactamases NDM-1, NDM-5, NDM-6 and NDM-7 in multidrug-resistant Enterobacteriaceae from India. *Int. J. Antimicrob. Agents* 2014, 44, 30–37, doi:10.1016/j.ijantimicag.2014.03.003.
Antimicrob. Agents Chemother. 2014, 58, 5589–5593, doi:10.1128/AAC.02425-14.

Deshpande, L.M.; Rhomberg, P.R.; Sader, H.S.; Jones, R.N. Emergence of serine carbapenemases (KPC and VIM-2) in Enterobacteriaceae and Acinetobacter baumannii isolates from Pakistan. Antimicrob. Agents Chemother. 2014, 58, 5589–5593, doi:10.1128/AAC.02425-14.

Walsh, T.R.; Weeks, J.; Livermore, D.M.; Toleman, M.A. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study. Lancet Infect. Dis. 2011, 11, 355–362, doi:10.1016/s1473-3099(11)70059-7.

Lauretti, L.; Riccio, M.L.; Mazzariol, A.; Cornaglia, G.; Amicosante, G.; Fontana, R.; Rossolini, G.M. Cloning and characterization of blaVIM, a new integron-borne metallo-beta-lactamase gene from a Pseudomonas aeruginosa clinical isolate. Antimicrob. Agents Chemother. 1999, 43, 1584–1590.

Matsumura, Y.; Peirano, G.; Devinney, R.; Bradford, P.A.; Motyl, M.R.; Adams, M.D.; Chen, L.; Kreiswirth, B.; Pitout, J.D.D. Genomic epidemiology of global VIM-producing Enterobacteriaceae. J. Antimicrob. Chemother. 2017, 72, 2249–2258, doi:10.1093/jac/dkx148.

Poirel, L.; Potron, A.; Nordmann, P. OXA-48-like carbapenemases: The phantom menace. J. Antimicrob. Chemother. 2012, 67, 1597–1606, doi:10.1093/jac/dks121.

Poirel, L.; Heritier, C.; Tolun, V.; Nordmann, P. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob. Agents Chemother. 2004, 48, 15–22.

Cizmeci, Z.; Aktas, E.; Otu, B.; Acikgoz, O.; Ordecki, S. Molecular characterization of carbapenem-resistant Enterobacteriaceae yields increasing rates of NDM-1 carbapenemases and colistin resistance in an OXA-48-endemic area. J. Chemother. 2017, 29, 344–350, doi:10.1080/1120009X.2017.1323149.

Baran, I.; Aksu, N. Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Ann. Clin. Microbiol. Antimicrob. 2016, 15, 20, doi:10.1186/s12941-016-0136-2.

Li, X.Z.; Plesiut, P.; Nikaido, H. The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. Clin. Microbiol. Rev. 2015, 28, 337–418, doi:10.1128/CMR.00117-14.

Goessens, W.H.; van der Bij, A.K.; van Boxtel, R.; Pitout, J.D.; van Ulsen, P.; Melles, D.C.; Tommassen, J. Antibiotic trapping by plasmid-encoded CMY-2 beta-lactamase combined with reduced outer membrane permeability as a mechanism of carbapenem resistance in Escherichia coli. Antimicrob. Agents Chemother. 2013, 57, 3941–3949, doi:10.1128/AAC.02459-12.

Martinez-Martinez, L.; Pascual, A.; Hernandez-Alles, S.; Alvarez-Diaz, D.; Suarez, A.I.; Tran, J.; Benedi, V.J.; Jacoby, G.A. Roles of beta-lactamases and porins in activities of carbapenems and cephalosporins against Klebsiella pneumoniae. Antimicrob. Agents Chemother. 1999, 43, 1669–1673.

Livermore, D.M. Bacterial resistance to carbapenems. Adv. Exp. Med. Biol. 1995, 390, 25–47.

Cornaglia, G.; Russell, K.; Satta, G.; Fontana, R. Relative importances of outer membrane permeability and group 1 beta-lactamase as determinants of meropenem and imipenem activities against Enterobacter cloacae. Antimicrob. Agents Chemother. 1999, 35, 350–355.

Shields, R.K.; Nguyen, M.H.; Potoski, B.A.; Press, E.G.; Chen, L.; Kreiswirth, B.N.; Clarke, L.G.; Eschenauer, G.A.; Clancy, C.J. Doripenem MICs and ompK36 porin genotypes of sequence type 258, KPC-producing Klebsiella pneumoniae may predict responses to carbapenem-colistin combination therapy among patients with bacteremia. Antimicrob. Agents Chemother. 2015, 59, 1797–1801, doi:10.1128/AAC.03894-14.

Clancy, C.J.; Chen, L.; Hong, J.H.; Cheng, S.; Hao, B.; Shields, R.K.; Farrell, A.N.; Doi, Y.; Zhao, Y.; Perlin, D.S.; et al. Mutations of the ompK36 porin gene and promoter impact responses of sequence type 258, KPC-2-producing Klebsiella pneumoniae strains to doripenem and doripenem-colistin. Antimicrob. Agents Chemother. 2013, 57, 5258–5265, doi:10.1128/AAC.01069-13.

Clancy, C.J.; Hao, B.; Shields, R.K.; Chen, L.; Perlin, D.S.; Kreiswirth, B.N.; Nguyen, M.H. Doripenem, gentamicin, and colistin, alone and in combinations, against gentamicin-susceptible, KPC-producing Klebsiella pneumoniae strains with various ompK36 genotypes. Antimicrob. Agents Chemother. 2014, 58, 3521–3525, doi:10.1128/AAC.01949-13.

Deshpande, L.M.; Romberg, P.R.; Sader, H.S.; Jones, R.N. Emergence of serum carbapenemases (KPC and SME) among clinical strains of Enterobacteriaceae isolated in the United States Medical Centers: Report from the MYSTIC Program (1999–2005). Diagn. Microbiol. Infect. Dis. 2006, 56, 367–372, doi:10.1016/j.diagmicrobio.2006.07.004.
79. Evans, M.E.; Feola, D.J.; Rapp, R.P. Polymyxin B sulfate and colistin: Old antibiotics for emerging multiresistant gram-negative bacteria. *Ann. Pharmacother.* 1999, 33, 960–967, doi:10.1345/aph.18426.

80. Poirel, L.; Jayol, A.; Nordmann, P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. *Clin. Microbiol. Rev.* 2017, 30, 557–596, doi:10.1128/CMR.00064-16.

81. Sun, J.; Zhang, H.; Liu, Y.H.; Feng, Y. Towards Understanding MCR-like Colistin Resistance. *Trends Microbiol.* 2018, 26, 794–808, doi:10.1016/j.tim.2018.02.006.

82. Rossolini, G.M.; Mantengoli, E. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. *Clin. Microbiol. Infect.* 2005, 11 (Suppl. 4), 17–32, doi:10.1111/j.1469-0691.2005.01161.x.

83. Falagas, M.E.; Rafaillidis, P.I.; Matthaiou, D.K.; Vritzili, S.; Nikita, D.; Michalopoulos, A. Pandrug-resistant * Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections: Characteristics and outcome in a series of 28 patients. *Int. J. Antimicrob. Agents* 2008, 32, 450–454, doi:10.1016/j.ijantimicag.2008.05.016.

84. Palavutitotai, N.; Jitmuang, A.; Tongsai, S.; Kiratisin, P.; Angkasekwinai, N. Epidemiology and risk factors of extensively drug-resistant *Pseudomonas aeruginosa* infections. *PLoS ONE* 2018, 13, e0193431, doi:10.1371/journal.pone.0193431.

85. Zavascki, A.P.; Carvalhaes, C.G.; Picao, R.C.; Gales, A.C. Multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Resistance mechanisms and implications for therapy. *Expert Rev. Anti Infect. Ther.* 2010, 8, 71–93, doi:10.1586/eri.09.108.

86. Cabot, G.; Ocampo-Sosa, A.A.; Dominguez, M.A.; Gago, J.F.; Juan, C.; Tubau, F.; Rodriguez, C.; Moya, B.; Pena, C.; Martinez-Martinez, L.; et al. Genetic markers of widespread extensively drug-resistant *Pseudomonas aeruginosa* high-risk clones. *Antimicrob. Agents Chemother.* 2012, 56, 6349–6357, doi:10.1128/AAC.01388-12.

87. Samonis, G.; Vardakas, K.Z.; Kofteridis, D.P.; Dimopoulou, D.; Andrianaki, A.M.; Chatzinikolaou, I.; Katsanevaki, E.; Maraki, S.; Falagas, M.E. Characteristics, risk factors and outcomes of adult cancer patients with extensively drug-resistant *Pseudomonas aeruginosa* infections. *Infection* 2014, 42, 721–728, doi:10.1007/s15010-014-0635-z.

88. Saderi, H.; Owlia, P. Detection of Multidrug Resistant (MDR) and Extremely Drug Resistant (XDR) *P. aeruginosa* Isolated from Patients in Tehran, Iran. *Iran J. Pathol.* 2015, 10, 265–271.

89. Safaei, H.G.; Moghim, S.; Isfahani, B.N.; Fazeli, H.; Poursina, F.; Yadegari, S.; Nasirmoghadas, P.; Hosseininassab Nodoushan, S.A. Distribution of the Strains of Multidrug-resistant, Extensively Drug-resistant, and Pandrug-resistant *Pseudomonas aeruginosa* Isolates from Burn Patients. *Adv. Biomed. Res.* 2017, 6, 74, doi:10.4103/abr.abr_239_16.

90. Bodro, M.; Sabe, N.; Tubau, F.; Llado, L.; Baliellas, C.; Gonzalez-Costello, J.; Cruzado, J.M.; Carratala, J. Extensively drug-resistant *Pseudomonas aeruginosa* bacteremia in solid organ transplant recipients. *Transplantation* 2015, 99, 616–622, doi:10.1097/TP.0000000000000366.

91. Park, Y.S.; Lee, H.; Chin, B.S.; Han, S.H.; Hong, S.G.; Hong, S.K.; Kim, H.Y.; Uh, Y.; Shin, H.B.; Choo, E.J.; et al. Acquisition of extensive drug-resistant *Pseudomonas aeruginosa* among hospitalized patients: Risk factors and resistance mechanisms to carbapenems. *J. Hosp. Infect.* 2011, 79, 54–58, doi:10.1016/j.jhin.2011.05.014.

92. Ziha-Zarifi, I.; Llanes, C.; Kohler, T.; Pechere, J.C.; Plesiat, P. In vivo emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA-MexB-OprM. *Antimicrob. Agents Chemother.* 1999, 43, 287–291.

93. Livermore, D.M. Of *Pseudomonas*, porins, pumps and carbapenems. *J. Antimicrob. Chemother.* 2001, 47, 247–250.

94. Fruci, M.; Poole, K. Aminoglycoside-inducible expression of the mexAB-oprM multidrug efflux operon in *Pseudomonas aeruginosa*: Involvement of the envelope stress-responsive AmgRS two-component system. *PLoS ONE* 2018, 13, e0205036, doi:10.1371/journal.pone.0205036.

95. Jo, J.T.; Brinkman, F.S.; Hancock, R.E. Aminoglycoside efflux in *Pseudomonas aeruginosa*: Involvement of novel outer membrane proteins. *Antimicrob. Agents Chemother.* 2003, 47, 1101–1111.

96. Masuda, N.; Sakagawa, E.; Ohya, S. Outer membrane proteins responsible for multiple drug resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 1995, 39, 645–649, doi:10.1128/aac.39.3.645.

97. Masuda, N.; Ohya, S. Cross-resistance to meropenem, cephems, and quinolones in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 1992, 36, 1847–1851, doi:10.1128/aac.36.9.1847.
98. Akasaka, T.; Tanaka, M.; Yamaguchi, A.; Sato, K. Type II topoisomerase mutations in fluoroquinolone-resistant clinical strains of Pseudomonas aeruginosa isolated in 1998 and 1999: Role of target enzyme in mechanism of fluoroquinolone resistance. Antimicrob. Agents Chemother. 2001, 45, 2263–2268, doi:10.1128/AAC.45.8.2263-2268.2001.

99. Morais Cabral, J.H.; Jackson, A.P.; Smith, C.V.; Shikotra, N.; Maxwell, A.; Liddington, R.C. Crystal structure of the breakage-reunion domain of DNA gyrase. Nature 1997, 388, 903–906, doi:10.1038/42294.

100. Kato, J.; Suzuki, H.; Ikeda, H. Purification and characterization of DNA topoisomerase IV in Escherichia coli. J. Biol. Chem. 1992, 267, 25676–25684.

101. Yokoyama, K.; Doi, Y.; Yamano, K.; Kurokawa, H.; Shibata, N.; Shibayama, K.; Yagi, T.; Kato, H.; Arakawa, Y. Acquisition of 16S rRNA methylase gene in Pseudomonas aeruginosa. Lancet 2003, 362, 1888–1893, doi:10.1016/s0140-6736(03)14959-8.

102. Doi, Y.; de Oliveira Garcia, D.; Adams, J.; Paterson, D.L. Coproduction of novel 16S rRNA methylase RmtD and metallo-beta-lactamase SPM-1 in a panresistant Pseudomonas aeruginosa isolate from Brazil. Antimicrob. Agents Chemother. 2007, 51, 852–856, doi:10.1128/AAC.01345-06.

103. Gurung, M.; Moon, D.C.; Tamang, M.D.; Kim, J.; Lee, Y.C.; Seol, S.Y.; Cho, D.T.; Lee, J.C. Emergence of 16S rRNA methylase gene armA and co-carriage of bla(IMP-1) in Pseudomonas aeruginosa isolates from South Korea. Diagn. Microbiol. Infect. Dis. 2010, 68, 468–470, doi:10.1016/j.diagmicrobio.2010.07.021.

104. Olaitan, A.O.; Morand, S.; Rolain, J.M. Mechanisms of polymyxin resistance: Acquired and intrinsic resistance in bacteria. Front. Microbiol. 2014, 5, 643, doi:10.3389/fmicb.2014.00643.

105. Bush, K. Alarming beta-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr. Opin. Microbiol. 2010, 13, 558–564, doi:10.1016/j.mib.2010.09.006.

106. Poole, K. Aminoglycoside resistance in Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 2005, 49, 479–487, doi:10.1128/AAC.49.2.479-487.2005.

107. Aggen, J.B.; Armstrong, E.S.; Goldblum, A.A.; Dozzo, P.; Linsell, M.S.; Gliedt, M.J.; Hildebrandt, D.J.; Feeney, L.A.; Kubo, A.; Matias, R.D.; et al. Synthesis and spectrum of the neoglycoside ACHN-490. Antimicrob. Agents Chemother. 2010, 54, 4636–4642, doi:10.1128/AAC.00572-10.

108. Mulet, X.; Cabot, G.; Ocampo-Sosa, A.A.; Dominguez, M.A.; Zamorano, L.; Juan, C.; Tubau, F.; Rodriguez, C.; Moya, B.; Pena, C.; et al. Biological markers of Pseudomonas aeruginosa epidemic high-risk clones. Antimicrob. Agents Chemother. 2013, 57, 5527–5535, doi:10.1128/AAC.01481-13.

109. Pena, C.; Cabot, G.; Gomez-Zorrilla, S.; Zamorano, L.; Ocampo-Sosa, A.; Murillas, J.; Almirante, B.; Pomar, V.; Aguilar, M.; Granados, A.; et al. Influence of virulence genotype and resistance profile in the mortality of Pseudomonas aeruginosa bloodstream infections. Clin. Infect. Dis. 2015, 60, 539–548, doi:10.1093/cid/ciu866.

110. Oliver, A.; Mulet, X.; Lopez-Causape, C.; Juan, C. The increasing threat of Pseudomonas aeruginosa high-risk clones. Drug Resist. Updat. 2015, 21-22, 41-59, doi:10.1016/j.drup.2015.08.002.

111. Kos, V.N.; Deraspe, M.; McLaughlin, R.E.; Whiteaker, J.D.; Roy, P.H.; Alm, R.A.; Corbeil, J.; Gardner, H. The resistome of Pseudomonas aeruginosa in relationship to phenotypic susceptibility. Antimicrob. Agents Chemother. 2015, 59, 427–436, doi:10.1128/AAC.00934-14.

112. Weldhagen, G.F.; Poirel, L.; Nordmann, P. Ambler class A extended-spectrum beta-lactamases in Pseudomonas aeruginosa: Novel developments and clinical impact. Antimicrob. Agents Chemother. 2003, 47, 2385–2392.

113. Nordmann, P.; Ronco, E.; Naas, T.; Duport, C.; Michel-Briand, Y.; Labia, R. Characterization of a novel extended-spectrum beta-lactamase from Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 1993, 37, 962–969.

114. Vanegas, J.M.; Cienfuegos, A.V.; Ocampo, A.M.; Lopez, L.; del Corral, H.; Roncancio, G.; Sierra, P.; Echeverri-Toro, L.; OSPina, S.; Maldonado, N.; et al. Similar frequencies of Pseudomonas aeruginosa isolates producing KPC and VIM carbapenemases in diverse genetic clones at tertiary-care hospitals in Medellin, Colombia. J. Clin. Microbiol. 2014, 52, 3978–3986, doi:10.1128/JCM.01879-14.

115. Correa, A.; Del Campo, R.; Perenguez, M.; Blanco, V.M.; Rodriguez-Banos, M.; Perez, F.; Maya, J.J.; Rojas, L.; Canton, R.; Arias, C.A.; et al. Dissemination of high-risk clones of extensively drug-resistant Pseudomonas aeruginosa in Colombia. Antimicrob. Agents Chemother. 2015, 59, 2421–2425, doi:10.1128/AAC.03926-14.

116. Guzvinec, M.; Izdebski, R.; Butic, I.; Jelic, M.; Abram, M.; Koscak, I.; Baraniak, A.; Hryniewicz, W.; Gniadkowski, M.; Tambic Andrasevic, A. Sequence types 235, 111, and 132 predominate among multidrug-
resistant *Pseudomonas aeruginosa* clinical isolates in Croatia. *Antimicrob. Agents Chemother.* 2014, 58, 6277–6283, doi:10.1128/AAC.03116-14.

117. Garcia-Castillo, M.; Del Campo, R.; Morosini, M.I.; Riera, E.; Cabot, G.; Willems, R.; van Mansfeld, R.; Oliver, A.; Canton, R. Wide dispersion of ST175 clone despite high genetic diversity of carbapenem-nonsusceptible *Pseudomonas aeruginosa* clinical strains in 16 Spanish hospitals. *J. Clin. Microbiol.* 2011, 49, 2905–2910, doi:10.1128/JCM.00753-11.

118. Viedma, E.; Juan, C.; Villa, J.; Barrado, L.; Orellana, M.A.; Sanz, F.; Otero, J.R.; Oliver, A.; Chaves, F. VIM-2-producing multidrug-resistant *Pseudomonas aeruginosa* ST175 clone, Spain. *Emerg. Infect. Dis.* 2012, 18, 1235–1241, doi:10.3201/eid1808.111234.

119. Wong, D.; Nielsen, T.B.; Bonomo, R.A.; Pantapalangkoor, P.; Luna, B.; Spellberg, B. Clinical and Pathophysiological Overview of *Acinetobacter* Infections: A Century of Challenges. *Clin. Microbiol. Rev.* 2017, 30, 409–447, doi:10.1128/CMR.00058-16.

120. Weiner, L.M.; Webb, A.K.; Limbago, B.; Dudeck, M.A.; Patel, J.; Kallen, A.J.; Edwards, J.R.; Sievert, D.M. Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011-2014. *Infect. Control Hosp. Epidemiol.* 2016, 37, 1288–1301, doi:10.1017/ice.2016.174.

121. European Centre for Disease Prevention and Control. Surveillance of Antimicrobial Resistance in Europe 2017. European Center for Disease Prevention, Sona, Sweden. Available online: https://ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2017. (accessed on 1 January 2019).

122. Hsu, L.Y.;Apisarnthanarak, A.; Khan, E.; Suwantarat, N.; Ghafur, A.; Tambyah, P.A. Carbapenem-Resistant *Acinetobacter baumannii* and *Enterobacteriaceae* in South and Southeast Asia. *Clin. Microbiol. Rev.* 2017, 30, 1–22, doi:10.1128/CMR.00042-16.

123. Rodriguez, C.H.; Nastro, M.; Famiglietti, A. Carbapenemases in *Acinetobacter baumannii*. Review of their dissemination in Latin America. *Rev. Argent. Microbiol.* 2018, 50, 327–333, doi:10.1016/j.ram.2017.10.006.

124. Mahgoub, S.; Ahmed, J.; Glatt, A.E. Underlying characteristics of patients harboring highly resistant *Acinetobacter baumannii*. *Am. J. Infect. Control* 2002, 30, 386–390.

125. Manikal, V.M.; Landman, D.; Saurina, G.; Oydna, E.; Lal, H.; Quale, J. Endemic carbapenem-resistant *Acinetobacter* species in Brooklyn, New York: Citywide prevalence, interinstitutional spread, and relation to antibiotic usage. *Clin. Infect. Dis.* 2000, 31, 101–106, doi:10.1086/313902.

126. Catalano, M.; Quelle, L.S.; Jeric, P.E.; Di Martin, A.; Maimone, S.M. Survival of *Acinetobacter baumannii* on bed rails during an outbreak and during sporadic cases. *J. Hosp. Infect.* 1999, 42, 27–35, doi:10.1053/jhin.1998.0535.

127. Villegas, M.V.; Hartstein, A.I. *Acinetobacter* outbreaks, 1977–2000. *Infect. Control Hosp. Epidemiol.* 2003, 24, 284–295, doi:10.1086/502205.

128. Bianco, A.; Quirino, A.; Giordano, M.; Marano, V.; Rizzo, C.; Liberto, M.C.; Foca, A.; Pavia, M. Control of carbapenem-resistant *Acinetobacter baumannii* outbreak in an intensive care unit of a teaching hospital in Southern Italy. *BMC Infect. Dis.* 2016, 16, 747, doi:10.1186/s12879-016-2036-7.

129. Ansaldi, F.; Canepa, P.; Bassetti, M.; Zancolli, M.; Molinari, M.P.; Talamini, A.; Ginocchio, F.; Durando, P.; Mussap, M.; Oreno, G.; et al. Sequential outbreaks of multidrug-resistant *Acinetobacter baumannii* in intensive care units of a tertiary referral hospital in Italy: Combined molecular approach for epidemiological investigation. *J. Hosp. Infect.* 2011, 79, 134–140, doi:10.1016/j.jhin.2011.05.027.

130. Tanguy, M.; Kouatchet, A.; Tanguy, B.; Pichard, E.; Fanello, S.; Joly-Guillou, M.L. Management of an *Acinetobacter baumannii* outbreak in an intensive care unit. *Med. Mal. Infect.* 2017, 47, 409–414, doi:10.1016/j.medmal.2017.06.003.

131. LoDel, K.; Rice, T.W.; Munoz-Price, L.S.; Quinn, J.P. Multicity outbreak of carbapenem-resistant *Acinetobacter baumannii* isolates producing the carbapenemase OXA-40. *Antimicrob. Agents Chemother.* 2006, 50, 2941–2945, doi:10.1128/AAC.01116-06.

132. Naas, T.; Bogaerts, P.; Bauraing, C.; Dehghelde, Y.; Glupczynski, Y.; Nordmann, P. Emergence of PER and VEB extended-spectrum beta-lactamases in *Acinetobacter baumannii* in Belgium. *J. Antimicrob. Chemother.* 2006, 58, 178–182, doi:10.1093/jac/dkl178.

133. Coelho, J.M.; Turton, J.F.; Kaufmann, M.E.; Glover, J.; Woodford, N.; Warner, M.; Palepou, M.F.; Pike, R.; Pitt, T.L.; Patel, B.C.; et al. Occurrence of carbapenem-resistant *Acinetobacter baumannii* clones at multiple hospitals in London and Southeast England. *J. Clin. Microbiol.* 2006, 44, 3623–3627, doi:10.1128/JCM.00699-06.
134. Chen, Y.; Ai, L.; Guo, P.; Huang, H.; Wu, Z.; Liang, X.; Liao, K. Molecular characterization of multidrug resistant strains of Acinetobacter baumannii isolated from pediatric intensive care unit in a Chinese tertiary hospital. BMC Infect. Dis. 2018, 18, 614, doi:10.1186/s12879-018-3511-0.

135. Saharman, Y.R.; Karuniawati, A.; Sedono, R.; Aditianingsih, D.; Sudarmono, P.; Goessens, W.H.F.; Claassen, C.H.W.; Verbrugh, H.A.; Severin, J.A. Endemic carbapenem-nonsusceptible Acinetobacter baumannii-calcoaceticus complex in intensive care units of the national referral hospital in Jakarta, Indonesia. Antimicrob. Resist. Infect. Control 2018, 7, 5, doi:10.1186/s13756-017-0296-7.

136. Landelle, C.; Legrand, P.; Lesprit, P.; Cizeau, F.; Ducellier, D.; Gout, C.; Brehaut, P.; Soing-Altrach, S.; Girou, E.; Brun-Buisson, C. Protracted outbreak of multidrug-resistant Acinetobacter baumannii after intercontinental transfer of colonized patients. Infect. Control Hosp. Epidemiol. 2013, 34, 119–124, doi:10.1086/669093.

137. Wybo, I.; Blommaert, L.; De Beer, T.; Soetens, O.; De Regt, J.; Lacor, P.; Pierard, D.; Lauwers, S. Outbreak of multidrug-resistant Acinetobacter baumannii in a Belgian university hospital after transfer of patients from Greece. J. Hosp. Infect. 2007, 67, 374–380, doi:10.1016/j.jhin.2007.09.012.

138. Chen, M.Z.; Hsueh, P.R.; Lee, L.N.; Yu, C.J.; Yang, P.C.; Luh, K.T. Severe community-acquired pneumonia due to Acinetobacter baumannii. Chest 2001, 120, 1072–1077.

139. Leung, W.S.; Chu, C.M.; Tsang, K.Y.; Lo, F.H.; Lo, K.F.; Ho, P.L. Fulminant community-acquired Acinetobacter baumannii pneumonia as a distinct clinical syndrome. Chest 2006, 129, 102–109, doi:10.1378/chest.129.1.102.

140. Magnat, S.; Courvalin, P.; Lambert, T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in Acinetobacter baumannii strain BM4454. Antimicrob. Agents Chemother. 2001, 45, 3375–3380, doi:10.1128/AAC.45.12.3375-3380.2001.

141. Lee, Y.; Yum, J.H.; Kim, C.K.; Yong, D.; Jeon, E.H.; Jeong, S.H.; Ahn, J.Y.; Lee, K. Role of OXA-23 and AdeABC efflux pump for acquiring carbapenem resistance in an Acinetobacter baumannii strain carrying the blaOXA-66 gene. Antimicrob. Agents Chemother. 1998, 42, 43–48.

142. Damier-Piolle, L.; Magnat, S.; Bremont, S.; Lambert, T.; Courvalin, P. AdeIJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in Acinetobacter baumannii. Antimicrob. Agents Chemother. 2008, 52, 557–562, doi:10.1128/AAC.00732-07.

143. Huys, G.; Chockaert, M.; Vaneechoutte, M.; Woodford, N.; Nemec, A.; Dijkshoorn, L.; Swings, J. Distribution of tetracycline resistance genes in genotypically related and unrelated multiresistant Acinetobacter baumannii strains from different European hospitals. Res Microbiol. 2005, 156, 348–355, doi:10.1016/j.resmic.2004.10.008.

144. Smani, Y.; Fabrega, A.; Roca, I.; Sanchez-Encinales, V.; Vila, J.; Pachon, J. Role of OmpA in the multidrug resistance phenotype of Acinetobacter baumannii. Antimicrob. Agents Chemother. 2014, 58, 1806–1808, doi:10.1128/AAC.02101-13.

145. Vila, J.; Ruiz, J.; Goni, P.; Jimenez de Anta, T. Quinolone-resistance mutations in the topoisomerase IV parC gene of Acinetobacter baumannii. J. Antimicrob. Chemother. 1997, 39, 757–762.

146. Vila, J.; Ruiz, J.; Goni, P.; Marcos, A.; Jimenez de Anta, T. Mutation in the gyrA gene of quinolone-resistant clinical isolates of Acinetobacter baumannii. Antimicrob. Agents Chemother. 1995, 39, 1201–1203.

147. Seward, R.J.; Towner, K.J. Molecular epidemiology of quinolone resistance in Acinetobacter spp. Clin. Microbiol. Infect. 1998, 4, 248–254, doi:10.1111/j.1469-0691.1998.tb00552.x.

148. Adams, M.D.; Nickel, G.C.; Bajaksouzian, S.; Lavender, H.; Murthy, A.R.; Jacobs, M.R.; Bonomo, R.A. Resistance to colistin in Acinetobacter baumannii associated with mutations in the PmrAB two-component system. Antimicrob. Agents Chemother. 2009, 53, 3628–3634, doi:10.1128/AAC.00284-09.

149. Moffatt, J.H.; Harper, M.; Harrison, P.; Hale, J.D.; Vinogradov, E.; Seemann, T.; Henry, R.; Crane, B.; St Michael, F.; Cox, A.D.; et al. Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production. Antimicrob. Agents Chemother. 2010, 54, 4971–4977, doi:10.1128/AAC.00834-10.

150. Nemec, A.; Dolzani, L.; Brisse, S.; van den Broek, P.; Dijkshoorn, L. Diversity of aminoglycoside-resistance genes and their association with class 1 integrons among strains of pan-European Acinetobacter baumannii clones. J. Med. Microbiol. 2004, 53, 1233–1240, doi:10.1099/jmm.0.45716-0.

151. Seward, R.J.; Lambert, T.; Towner, K.J. Molecular epidemiology of aminoglycoside resistance in Acinetobacter spp. J. Med. Microbiol. 1998, 47, 455–462, doi:10.1099/00222615-47-5-455.
152. Doi, Y.; Adams, J.M.; Yamane, K.; Paterson, D.L. Identification of 16S rRNA methylase-producing *Acinetobacter baumannii* clinical strains in North America. *Antimicrob. Agents Chemother.* 2007, 51, 4209–4210, doi:10.1128/AAC.00560-07.

153. Zhou, H.; Du, X.X.; Yang, Q.; Zhou, J.Y.; Yu, Y.S.; Li, L.J. Study on carbapenemase and 16S rRNA methylase of imipenem-resistant *Acinetobacter baumannii*. *Zhonghua Liu Xing Bing Xue Za Zhi* 2009, 30, 269–272.

154. Brigante, G.; Migliavacca, R.; Bramati, S.; Motta, E.; Nucleo, E.; Manenti, M.; Migliorino, G.; Pagani, L.; Luzzaro, F.; Vigano, F.E. Emergence and spread of a multidrug-resistant *Acinetobacter baumannii* clone producing both the carbapenemase OXA-23 and the 16S rRNA methylase ArmA. *J. Med. Microbiol.* 2012, 61, 653–661, doi:10.1099/jmm.0.040980-0.

155. Aghazadeh, M.; Rezaee, M.A.; Naheai, M.R.; Mahdian, R.; Pajand, O.; Saffari, F.; Hassan, M.; Hojabri, Z. Dissemination of aminoglycoside-modifying enzymes and 16S rRNA methylases among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates. *Microb. Drug Resist.* 2013, 19, 282–288, doi:10.1089/mdr.2012.0223.

156. Bakour, S.; Touati, A.; Bachiri, T.; Sahli, F.; Tiouit, D.; Naim, M.; Azouaou, M.; Rolain, J.M. First report of 16S rRNA methylase ArmA-producing *Acinetobacter baumannii* and rapid spread of metallo-beta-lactamase NDM-1 in Algerian hospitals. *J. Infect. Chemother.* 2014, 20, 696–701, doi:10.1016/j.jiac.2014.07.010.

157. Poirel, L.; Nordmann, P. Carbapenem resistance in *Acinetobacter baumannii*: Mechanisms and epidemiology. *Clin. Microbiol. Infect.* 2006, 12, 826–836, doi:10.1111/j.1469-0691.2006.01456.x.

158. Corvec, S.; Caroff, N.; Espaze, E.; Giraudieu, C.; Drugeon, H.; Reynaud, A. AmpC cephalosporinase hyperproduction in *Acinetobacter baumannii* clinical strains. *J. Antimicrob. Chemother.* 2003, 52, 629–635, doi:10.1093/jjac/dkg407.

159. Turton, J.F.; Woodford, N.; Glover, J.; Yarde, S.; Kaufmann, M.E.; Pitt, T.L. Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. *J. Clin. Microbiol.* 2006, 44, 2974–2976, doi:10.1128/JCM.01021-06.

160. Olaitan, A.O.; Berrazeg, M.; Fagade, O.E.; Adelowo, O.O.; Alli, J.A.; Rolain, J.M. Emergence of multidrug-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase, Nigeria. *Int. J. Infect. Dis.* 2013, 17, e469–e470, doi:10.1016/j.ijid.2012.12.008.

161. Poirel, L.; Naas, T.; Nordmann, P. Diversity, epidemiology, and genetics of class D beta-lactamases. *Antimicrob. Agents Chemother.* 2010, 54, 24–38, doi:10.1128/AAC.01512-08.

162. Corvec, S.; Poirel, L.; Naas, T.; Drugeon, H.; Nordmann, P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 2007, 51, 1530–1533, doi:10.1128/AAC.01132-06.

163. Merino, M.; Poza, M.; Roca, I.; Barba, M.J.; Sousa, M.D.; Vila, J.; Bou, G. Nosocomial outbreak of a multiresistant *Acinetobacter baumannii* expressing OXA-23 carbapenemase in Spain. *Microb. Drug Resist.* 2014, 20, 259–263, doi:10.1089/mdr.2013.0127.

164. Afzal-Shah, M.; Woodford, N.; Livermore, D.M. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 2001, 45, 583–588, doi:10.1128/AAC.45.2.583-588.2001.

165. Heritier, C.; Poirel, L.; Aubert, D.; Nordmann, P. Genetic and Functional Analysis of the Chromosome-Encoded Carbapenem-Hydrolyzing Oxacillinase OXA-40 of *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 2003, 47, 268–273, doi:10.1128/ajas.47.1.268-273.2003.

166. Tada, T.; Miyoshi-Akiyama, T.; Shimada, K.; Shimojima, M.; Kirikae, T. Dissemination of 16S rRNA methylase ArmA-producing *Acinetobacter baumannii* and emergence of OXA-72 carbapenemase coproducers in Japan. *Antimicrob. Agents Chemother.* 2014, 58, 2916–2920, doi:10.1128/AAC.01212-13.

167. de Sa Cavalcanti, F.L.; Almeida, A.C.; Vilela, M.A.; de Morais Junior, M.A.; de Morais, M.M.; Leal-Balbino, T.C. Emergence of extensively drug-resistant OXA-72-producing *Acinetobacter baumannii* in Recife, Brazil: Risk of clonal dissemination? *Diagn. Microbiol. Infect. Dis.* 2013, 77, 250–251, doi:10.1016/j.diagmicrobio.2013.07.022.

168. Povilonis, J.; Seputiene, V.; Krasauskas, R.; Juskaite, R.; Miskinyte, M.; Suziedelis, K.; Suziedeliene, E. Spread of carbapenem-resistant *Acinetobacter baumannii* carrying a plasmid with two genes encoding OXA-72 carbapenemase in Lithuanian hospitals. *J. Antimicrob. Chemother.* 2013, 68, 1000–1006, doi:10.1093/jac/dks499.

169. Goic-Barisic, I.; Towner, K.J.; Kovacic, A.; Sisko-Kraljevic, K.; Tonkic, M.; Novak, A.; Punda-Polic, V. Outbreak in Croatia caused by a new carbapenem-resistant clone of *Acinetobacter baumannii* producing OXA-72 carbapenemase. *J. Hosp. Infect.* 2011, 77, 368–369, doi:10.1016/j.jhin.2010.12.003.
170. Robledo, I.E.; Aquino, E.E.; Sante, M.I.; Santana, J.L.; Otero, D.M.; Leon, C.F.; Vazquez, G.J. Detection of KPC in Acinetobacter spp. in Puerto Rico. *Antimicrob. Agents Chemother.* **2010**, *54*, 1354–1357, doi:10.1128/AAC.00899-09.

171. Potron, A.; Poirel, L.; Nordmann, P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology. *Int. J. Antimicrob. Agents* **2015**, *45*, 568–585, doi:10.1016/j.ijantimicag.2015.03.001.

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