2 Multidrug-Resistant Gram-Negative Pathogens: The Urgent Need for 'Old' Polymyxins

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Abstract

Antibiotic resistance has presented a major health challenge in the world and many isolates of Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* become resistant to almost all current antibiotics. This chapter provides an overview on the mechanisms of antibiotic resistance in these Gram-negative pathogens and outlines the formidable problem of the genetics of bacterial resistance. Prevalent multidrug-resistance in Gram-negative bacteria underscores the need for optimizing the clinical use of the last-line polymyxins.

Keywords

Antibiotic resistance · Enterobacteriaceae · *Acinetobacter baumannii* · *Pseudomonas aeruginosa* · polymyxin

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Penicillin-resistant bacteria were detected within the first decade of use of this antibiotic. More than 70 years later, antibiotic use for hospitalized patients has switched to agents such as carbapenems, quinolones, aminoglycosides and tigecycline. Unfortunately, the epidemiology of infections has changed so that bacteria resistant to some or all of these antibiotics are now commonplace in many institutions. Typically, units with compromised patients and heavy antibiotic use, such as intensive care units, hematology and transplant wards, and long-term stay units are those in which multidrug-resistant bacteria are most common. Although attention in the past focused on antibiotic-resistant Gram-positive organisms (such as methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus faecium*), the problem bacteria today are the Gram-negative bacteria. The Enterobacteriaceae, *Acinetobacter* spp. and *Pseudomonas aeruginosa* are common causes of healthcare-acquired infections [1] and are frequently resistant to commonly used antibiotics.

The purpose of this chapter is to outline the mechanisms of resistance in these Gram-negative pathogens, as a means of outlining the formidable problem of the genetics of bacterial resistance. It underscores the need for polymyxins, since this class of pathogens is not susceptible to beta-lactams and related resistance mechanisms so frequently seen today.

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2.1 Enterobacteriaceae

Carbapenem antibiotics have typically been regarded as highly stable to beta-lactamases, for example via extended-spectrum beta-lactamases (ESBLs). However, over the last decade, many of the Enterobacteriaceae have become resistant to carbapenems by way of production of carbapenemase enzymes [2].

Early reports of carbapenem-resistant Enterobacteriaceae were as a result of expression of AmpC type beta-lactamases or ESBLs plus loss of outer-membrane proteins in *K. pneumoniae* [3]. However, carbapenem resistance in the Enterobacteriaceae has emerged over the last 15–20 years due to production of beta-lactamases known as carbapenemases [4]. In the United States and some parts of Europe, the most frequently observed type of carbapenemase is the KPC type [5, 6]. KPC-producing strains are typically multidrug-resistant, being resistant to carbapenems, penicillins, cephalosporins, fluoroquinolones and aminoglycosides [6]. Therefore, polymyxins are one of the few options available for use against KPC producers. A single clone of KPC-producing *K. pneumoniae* (known as ST 258 by multilocus sequence typing (MLST)), has been found in the United States, Israel and some parts of Europe (particularly Greece and Italy) [7, 8]. This indicates that a hospital-adapted clone of KPC-producing *K. pneumoniae* was transferred from person to person as a result of breakdown in infection control measures. A new beta-lactamase inhibitor, avibactam, does have activity against the KPC beta-lactamase. However, data are limited as to its clinical effectiveness against KPC producers, and it remains to be seen as to whether it will replace polymyxins as drug of choice for KPC-producing organisms.

Although KPC has been found in China and other parts of Asia, resistance of the Enterobacteriaceae to carbapenems in Asia is more frequently due to production of metallo-beta-lactamases (MBLs) than due to KPC. A variety of MBLs have been detected. Foremost amongst these is the NDM beta-lactamase [9]. Like the KPC-type beta-lactamase, producers of NDM and other MBLs are typically resistant to carbapenems, penicillins, cephalosporins, fluoroquinolones and aminoglycosides. This explains the necessity to use polymyxins in significant infections due to NDM producers.

The NDM-type β-lactamase was first isolated in 2009 from a Swedish patient returning from India [9]. The patient was infected with NDM producing *K. pneumoniae,* resistant to multiple antibiotics including all carbapenems. The bla_{NDM} gene has now spread to all inhabited continents and is carried by multiple Gram-negative species [10]. NDM producing organisms have been strongly linked with the Indian subcontinent (India, Pakistan, Bangladesh and Nepal) [11]. China is also known to be a reservoir country for NDM producers, although surveillance data is not yet complete – at this stage it does not appear that NDM producers are as widely prevalent in China as in the Indian subcontinent. The Balkan states (for example, Serbia, Montenegro, and Bosnia-Herzegovina) may also be considered as a reservoir area for bla_{NDM} acquisition since a number of cases have been reported with no travel history to Asia [10]. In general, travel appears to be the major means by which NDM producing bacteria have spread throughout the world. Europe provided the first case in 2009 in Sweden and shortly after, many other countries began reporting travel related NDM acquisition from the Indian Subcontinent or the Balkan states. Unlike the case with KPC-producing *K. pneumoniae*, various *K. pneumoniae* sequence types (STs) have been reported to harbor bla_{NDM} [10].

A variety of other MBLs have been found to lead to carbapenem resistance in the Enterobacteriaceae. These include the VIM-type (with worldwide distribution, but particularly noteworthy in Greece), the IMP-type (with IMP-4 particularly prominent in Australia) and the SPM-type (almost exclusively found in Brazil).

Standard susceptibility testing may sometimes categorize KPC- or MBL-producing Enterobacteriaceae as susceptible to carbapenems. This issue is particularly pertinent to another group of carbapenemases found in the Enterobacteriaceae – OXA-48, and related enzymes [12]. The OXA-48 like carbapenemases

are widespread in North Africa, the Middle East and India [12, 13]. There has now been significant spread to Europe [12].

The genes encoding the carbapenemases frequently reside on mobile genetic elements (such as plasmids), which are capable of transferring resistance genes from one bacterial cell to another [10]. Other resistance genes which can be co-harbored on the same genetic elements as carbapenemases include ESBLs, AmpC, quinolone resistance mechanisms, aminoglycoside modifying enzymes and 16S ribosomal RNA methylases. Chromosomally encoded mechanisms may also occur in strains with mobile genetic elements. For example, quinolone resistance in Enterobacteriaceae is usually due to chromosomally encoded alterations in target enzymes (DNA gyrase and/or topoisomerase IV) or to impaired access to the target enzymes, occurring either because of changes in porin expression or because of efflux mechanisms.

The end-result of the proliferation of this multitude of resistance mechanisms is truly multidrug-resistant Enterobacteriaceae. It is not surprising, therefore, that in settings where carbapenem-resistant Enterobacteriaceae are highly prevalent, polymyxin use becomes a necessity.

2.2 *Acinetobacter* spp.

A. baumannii and newly described species, *A. pittii* and *A. nosocomialis*, also possess a wide array of antimicrobial resistance mechanisms. Intrinsically, *Acinetobacter* species are resistant to first and second generation cephalosporins, aztreonam and ertapenem, due to a combination of poor permeability to these antibiotics and intrinsic beta-lactamase production. Antibiotics with activity against wild-type strains of *Acinetobacter* include sulbactam, meropenem, ciprofloxacin, aminoglycosides, tetracyclines, tigecycline and trimethoprim/sulfamethoxazole [14]. Acquired resistance mechanisms frequently originate from *Pseudomonas* spp., *E. coli* and other Gram-negative species, and may be localized to large resistance islands [14].

Carbapenem resistance in *Acinetobacter* spp. is a common indication for polymyxin use. As is the case with the Enterobacteriaceae, carbapenem resistance is typically mediated by production of carbapenemases.

The OXA-type beta-lactamases (especially OXA-23) are the most common mechanisms of carbapenem resistance in *Acinetobacter*. Although the KPC type carbapenemases are widely found in the Enterobacteriaceae, they are rarely found in *Acinetobacter*. However, the OXA-type carbapenemases, especially OXA-23, predominate. OXA-23 was first isolated in *Acinetobacter* spp. in the United Kingdom in 1985 [14, 15]. This carbapenemase is typically found in an internationally prevalent clone termed international clone (IC) 2. OXA-27 and OXA-49 are closely related enzymes that make up the $bla_{\text{OXA-23}}$ gene cluster in *A. baumannii* [14]. Other OXA-type genes may have carbapenemase activity including $bla_{\text{OXA-24}}$- (OXA-24, -25, -26, -40) and the $bla_{\text{OXA-58}}$-like [14] carbapenemase genes. Additionally, a chromosomally encoded gene, $bla_{\text{OXA-51}}$, is intrinsic to *A. baumannii* – its contribution to carbapenem resistance is dependent on the presence of an insertion sequence, IS*Aba1* [16]. In the absence of this insertion sequence, $bla_{\text{OXA-51}}$ does not lead to carbapenem resistance [14].

MBLs have also been well described in *Acinetobacter* spp., although they are not as frequent a cause of resistance as OXA-23 [14]. However, the carbapenem hydrolyzing activity of the MBLs is typically much more potent than that of the OXA-type carbapenemases [14]. The NDM type MBLs are particularly noteworthy since they may have originated within the genus *Acinetobacter* [10]. As noted previously, the NDM enzymes are prominent in the Indian subcontinent but have now spread widely. IMP, VIM and SIM MBLs have also been found in *Acinetobacter* spp. [17]. Additionally, *Acinetobacter* isolates may co-produce both MBL and OXA type carbapenemases [14]. Most commonly, MBLs produced by *Acinetobacter* are encoded within integrons, especially class 1 integrons. These genetic structures typically encode a wide variety of resistance genes, and

contribute to the multidrug resistance typical of *Acinetobacter* [14].

Carbapenemase-producing *Acinetobacter* isolates are usually resistant to all beta-lactam antibiotics. Aminoglycoside resistance is also common, via the production of aminoglycoside modifying enzymes or 16S rRNA methylases. Ciprofloxacin resistance is typically mediated by changes in the chromosomally encoded quinolone resistance determining regions (QRDRs). Upregulated efflux pumps may also contribute to aminoglycoside, quinolone, tigecycline and beta-lactam resistance. Finally, the *sul* gene may lead to sulfamethoxazole resistance. The end-result of this multiplicity of resistance genes may be *Acinetobacter* strains resistant to all antibiotics. Hence, polymyxins play a major role in the armamentarium against the carbapenem-resistant *Acinetobacter* isolates commonly observed in clinical practice. Unfortunately, an *A. baumannii* strain has now been described which is resistant to polymyxins and all commercially available antibiotics [18].

2.3 *Pseudomonas aeruginosa*

P. aeruginosa is an organism with enhanced virulence characteristics and is the sixth most commonly isolated organism responsible for hospital-acquired infections [1]. Unlike the case with Enterobacteriaceae or *Acinetobacter* spp., the most common mechanism of carbapenem resistance in *P. aeruginosa* appears to be mutational loss of the OprD porin [19]. The primary function of OprD is importation of arginine, but it is also the major entry point for carbapenems [19]. Mutations in *oprD* can result in loss of porin function. Thus, uptake of carbapenems by *P. aeruginosa* is substantially reduced and typically confers resistance to this antibiotic class. Efflux mechanisms such as MexAB-OprM, MexXY-OprM, and the regulator MexZ may play a contributory role in carbapenem resistance, as may carbapenemase production. MBLs (such as VIM or NDM) and KPC-type beta-lactamases may be produced by *P. aeruginosa*, while OXA-type beta-lactamases are rarely seen [10, 19].

Quinolone resistance in *P. aeruginosa* is typically found to be due to mutations in the chromosomally encoded QRDRs [19]. First-step mutations occurring in the *gyrA* QRDR appear to be the most significant in causing quinolone resistance, while subsequent mutations are believed to further decrease susceptibility levels. Aminoglycoside resistance in *P. aeruginosa* is associated with efflux by the MexXY-OprM transporter, aminoglycoside modifying enzymes or 16S rRNA methyltransferases. Typically, mechanisms of quinolone and aminoglycoside resistance occur in isolates with OprD loss and/or production of carbapenemases leading to multidrug or extensively drug resistant (XDR) strains. Given that tigecycline is ineffective against *P. aeruginosa* by way of intrinsic efflux mechanisms, the polymyxins are one of the very few treatment options available for multidrug resistant strains.

2.4 Conclusions

A wide variety of issues have contributed to the problem of multidrug resistance in Gram-negative bacilli. Pharmaceutical company disinvestment in antibiotic discovery has led to fewer new options for clinical use. At the same time, proliferation of genetic elements encoding resistance has continued unabated. Exacerbators of this problem have included heavy agricultural use of antibiotics, over the counter availability of antibiotics and environmental contamination by antibiotics themselves as well as antibiotic resistant organisms in water, food and hospital environments. In particular, the recent discovery of plasmid-mediated polymyxin resistance via multiple *mcr* genes indicate that polymyxin resistance exists in food animals and patients [20–22]. The polymyxins are not perfect treatment options by any means. However, they have become the only option for many patients in this current era of resistance.

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