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Overcoming β -lactam resistance in Gram-negative pathogens

“...antibacterial drug discovery and development must continue to identify novel therapeutic approaches, as well as novel compounds, in our efforts to try to manage these highly resistant Gram-negative pathogens.”

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Resistance has been driving the development of new antibiotics ever since penicillin-resistant staphylococci began to be reported in the mid-1940s. Although early antibiotics were targeted primarily against the Gram-positive cocci, their use soon selected for β -lactam-resistant Gram-negative bacteria. Due to the threats posed by a growing diversity of β -lactam-hydrolyzing β -lactamases in the enteric bacteria, antibiotic development programs placed greater emphasis on the identification of naturally occurring non- β -lactam antibiotics such as aminoglycosides and tetracyclines, followed by optimization of (fluoro)quinolone molecules as a means of circumventing β -lactam resistance in Gram-negative bacteria [1]. In spite of these efforts, more potent β -lactams continued to replenish the antibiotic armamentarium, serving to drive selection of broader spectrum β -lactamases which appeared on mobile genetic elements in most Gram-negative bacteria. Unfortunately, many of these newer enzymes appeared on transposons or plasmids that carried additional resistance determinants, thereby resulting in multidrug-resistant Gram-negative pathogens that could be selected by one of a number of antibiotics [2].

Within the decade spanning the beginning of the 21st century, susceptibility to some of the most reliable antibacterial agents eroded significantly. In surveillance studies reported in 2001, low resistance rates (2.4–5.8%) were reported for levofloxacin and gentamicin in large collections of sequentially collected clinical isolates of *Escherichia coli* and *Klebsi-*

ella pneumoniae from the USA. Resistance to expanded-spectrum cephalosporins, piperacillin-tazobactam and imipenem in these isolates was also low, with no carbapenem resistance reported, and resistance to the cephalosporins peaking at 6.8% in *K. pneumoniae* [3,4]. However, this situation changed dramatically in a short period of time. Isolates collected from US pneumonia patients in 2012 demonstrated elevated resistance rates for all the drugs tested; in *K. pneumoniae* isolates levofloxacin and gentamicin resistance was 21–24%, and for the β -lactams, resistance increased to 31% for ceftazidime and 8.9% for meropenem, while in *E. coli* levofloxacin resistance was 34% [5]. In a set of Polish hospitals, carbapenem resistance in the Enterobacteriaceae isolates rose to 17% by 2013 [6]; resistance to the expanded-spectrum cephalosporins was reported to be 47% among Enterobacteriaceae isolates from Shanghai nursing home residents [7]. This increase in β -lactam resistance is due largely to the exponential increase in β -lactamases that collectively are capable of inactivating all β -lactams [1].

Most disconcerting among the β -lactamases are the carbapenem-hydrolyzing enzymes, the carbapenemases that emerged on plasmids in the early 1990s and that have now proliferated over the globe. These enzymes hold the ability to hydrolyze virtually every β -lactam-containing molecule [2]. They belong to two different molecular classes depending on their hydrolysis mechanisms: those that utilize serine at

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their active site and those that contain at least one zinc ion to facilitate hydrolysis. The latter, named metallo- β -lactamases, or MBLs, include the NDM-1 MBL that recently emerged from India [8] and that has rapidly spread worldwide in multidrug-resistant Gram-negative pathogens [9]. Almost without exception the carbapenemases exist in concert with at least one other β -lactamase, and usually with additional resistance determinants [2]. As a result, carbapenem-resistant Enterobacteriaceae are being reported as 91% resistant to levofloxacin, 63% resistant to amikacin and, most unsettling, 26% resistant to colistin [6].

Drugs of last resort in the last decade for the treatment of multidrug-resistant Gram-negative bacteria became colistin and tigecycline, with 99–100% susceptibility recorded for enteric bacteria in the 2012 study of pneumonia isolates; 1.5% colistin resistance was recorded for *Pseudomonas aeruginosa* and up to 8% resistance in *Acinetobacter* isolates [5]. Resistance to both these agents, however, continues to increase, with multiple resistance mechanisms described for both agents [10]. Perhaps the most disturbing development within the past year has been the identification of colistin-resistant isolates carrying the plasmid-encoded *mcr-1* gene that encodes a phosphoethanolamine transferase, the first plasmid-borne resistance determinant for polymyxins [11]. The gene, first described in food animals from China, and now reported from multiple animal and human sources throughout the world, is frequently identified in isolates that are β -lactam resistant, with co-transfer of both *mcr-1* and β -lactamase genes. Although co-resistance has been reported to have been acquired during therapy with cephalosporins [12], the origin of the *mcr-1* gene likely has arisen through environmental transfer. The question then arises as to what is available to treat infections caused by these highly resistant bacteria.

“Novel β -lactams in development include the monobactam BAL30072 and the cephalosporin S-649266, both of which are siderophore-containing β -lactam molecules.”

Historically, one approach to the treatment of β -lactam-resistant infections was to introduce β -lactamase inhibitors in combination with broad-spectrum penicillins. Since 2014, two new β -lactamase inhibitor combinations have been approved for clinical use in the USA with indications that include the treatment of infections caused by Gram-negative bacteria [13]. The introduction of ceftolozane-tazobactam has provided a potent combination for treatment of infections caused by *P. aeruginosa* [13] with less than 6% resistance reported in recent USA and European pseudomonal isolates [5]. Susceptibility to the

combination in enteric bacteria, however, is lower due to the lability of ceftolozane to hydrolysis by extended-spectrum β -lactamases (ESBLs) in *K. pneumoniae* [5]. Although ceftolozane resistance due to mutations in the chromosomal AmpC β -lactamase from *P. aeruginosa* has recently been described [14], this resistance is less common. The ceftazidime-avibactam cephalosporin- β -lactamase inhibitor combination was approved in 2015 after only Phase II data had been compiled. This combination includes avibactam, a novel diazabicyclo[3.2.1]octanone (DBO) non- β -lactam β -lactamase inhibitor that has been used as a model platform by a number of medicinal chemistry groups in attempts to improve upon the cephalosporin-avibactam inhibitor combination [13]. Avibactam, and its most notable followers relebactam and OP0595, represent major improvements over earlier β -lactamase inhibitors that were only effective against narrow spectrum β -lactamases. The DBOs include those same enzymes in their inhibitory spectrum, but have additional potent inhibitory activity against the serine carbapenemases and the AmpC cephalosporinases [13]. They are being developed with cephalosporins, monobactams and carbapenems in order to obtain the broadest spectrum of activity to cover resistant Gram-negative pathogens. The advantage of the avibactam-monobactam combination is the observed stability of aztreonam to hydrolysis by MBLs, providing a β -lactam-related option to counteracting resistance in MBL-producing Gram-negative bacteria [13]. In addition to the DBOs in development, vaborbactam represents a novel approach to β -lactamase inhibition by using a unique cyclic boronic acid template to inhibit serine β -lactamases, especially targeting serine carbapenemases [13]. For the carbapenem-inhibitor combinations such as the relebactam-imipenem and vaborbactam-meropenem combinations, development times may be extended, as enrollment in clinical trials to demonstrate efficacy against resistant pathogens is often slow [15], especially when the absolute number of carbapenem-resistant pathogens may be quite low at many recruitment sites.

Novel β -lactams in development include the monobactam BAL30072 and the cephalosporin S-649266, both of which are siderophore-containing β -lactam molecules [13]. Historically, β -lactams decorated with various siderophores have not been successful in advancing into therapeutic clinical trials. The antibiotic literature over the past 30 years has multiple examples of penicillins, cephalosporins, carbapenems and monobactams designed with siderophore structures to allow utilization of iron transport systems as a means of facilitating entry into Gram-negative bacteria [16]. Many of these molecules failed to advance due to the rapid selection of resistance in various genes involved with iron transport. However, both BAL30072 and

S-649266 have demonstrated reduced selection frequencies compared with earlier studies [13]. In addition, S-649266 has demonstrated stability to a variety of β -lactamases, with an expanded spectrum of activity against carbapenem-resistant Enterobacteriaceae including a number of strains producing MBLs [17]. It will be interesting to follow the development of this novel cephalosporin through therapeutic clinical trials to determine whether its attractive *in vitro* profile can be maintained during the course of human infections.

Other promising agents active against multidrug-resistant Gram-negative bacteria include the novel aminoglycoside plazomicin, and the tetracyclines eravacycline and omadacycline. Plazomicin and eravacycline have demonstrated potentially useful antibacterial activity against carbapenem-producing Enterobacteriaceae and *Acinetobacter* spp. [18,19]. They represent the possibility of using drugs outside the β -lactam area, a requirement for patients who may not be able to tolerate β -lactam therapy. However, resistance issues have been identified for each drug, with eravacycline sharing common resistance mechanisms with tigecycline, and plazomicin being vulnerable to resistance caused by rRNA methyltransferases that are often transferred on the same plasmid(s) as the NDM-1 (MBL)-encoding gene [20]. Because of the past success of the polymyxins, other antimicrobial peptides or peptide mimetics could be of interest. Although many of these molecules have encountered issues in their development as a therapeutic agent, brilacidin with its broad-spectrum antibacterial activity is perhaps one of the most promising of the current investigational agents in this class [21].

As noted above, a number of antibacterial agents with activity against many β -lactam-resistant Gram-negative pathogens have recently been approved or are in late-stage development. This is good. However, the downside is that these molecules are all closely related to drug classes that have been used extensively in the past. This is not necessarily bad. Although there have been many suggestions that ‘newer is better’, the older drugs have

served the infectious disease community well, and are still somewhat effective. Susceptibility rates for amikacin and carbapenems in large populations of European Enterobacteriaceae are still >95% [6]. However, it is the 5% resistant population that demands attention. Resistance is being transferred not only within the nosocomial setting, or from long-term care facilities, but also from person to person through international travel that allows dissemination of the worst of the resistance determinants. Resistance genes in food animals in the Asia-Pacific region may be transferred in meat that is sold in North America. Resistance is global and will not diminish unless major containment efforts are made on a global scale. For the future, new drugs will continue to be needed. Combination therapy will need to be considered, not just for β -lactams but across drug classes. Treatment of infections caused by non-fermentative bacteria in particular may routinely need to be treated with drug combinations, perhaps an aminoglycoside plus a β -lactam, in order to counteract colistin-resistant organisms [19]. Finally, antibacterial drug discovery and development must continue to identify novel therapeutic approaches, as well as novel compounds, in our efforts to try to manage these highly resistant Gram-negative pathogens.

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