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EDITORIAL COMMENTARY

Polymyxin Resistance in *Klebsiella pneumoniae*: Complexity at Every Level

Yohei Doi[1,2,3] and David van Duin[4]

[1]Division of Infectious Diseases and [2]Center for Innovative Antimicrobial Therapy, University of Pittsburgh, School of Medicine, Pennsylvania; [3]Departments of Microbiology and Infectious Diseases, Fujita Health University, Toyoake, Japan; and [4]Division of Infectious Diseases, University of North Carolina, Chapel Hill

(See the Major Article by Macesic et al on pages 2084–91.)

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The emergence and spread of carbapenem-resistant Enterobacteriaceae (CRE) is an important threat to global health, and carbapenem-resistant *Klebsiella pneumoniae* is the most commonly encountered species of CRE. In the United States and many other countries, the CRE epidemic is largely driven by expansion of sequence type (ST) 258 or related clonal lineages of *K. pneumoniae* that produce carbapenemases in the *K. pneumoniae* carbapenemase (KPC) family.

As one of few remaining drug classes with in vitro activity against CRE, polymyxins (polymyxin B and colistin) became key agents in the treatment of carbapenem-resistant *K. pneumoniae* infections. It should be noted that polymyxins were abandoned between the 1960s and 1970s in favor of newer antibiotics [1], which not only had more favorable side-effect profiles but also outperformed polymyxins' limited efficacy [2, 3]. Indeed, the efficacy of colistin-based regimens was dismal in a recent international randomized controlled trial of severe carbapenem-resistant gram-negative infections, with 28-day mortality of colistin monotherapy as high as 35% for CRE [4]. In addition to the less than optimal clinical efficacy, many complex issues surrounding polymyxins remain despite 2 decades of intense research efforts. At the diagnostic level, the only approved susceptibility testing method for colistin is broth microdilution (BMD) since agar diffusion-based methods are unreliable due to the cationic charges of polymyxins [5]. However, few BMD devices are approved in the United States, whereas manual BMD is time consuming and often unfeasible in the clinical laboratory. Furthermore, no clinical breakpoints exist for Enterobacteriaceae including *K. pneumoniae* due to insufficient clinical data to support them. In terms of therapeutic use, dosing of polymyxins, in particular that of colistin, is now becoming standardized based on population pharmacokinetic data [6]. Nonetheless, the now-recommended approach of administering a loading dose followed by higher maintenance doses has not improved patient outcome thus far, yet causes higher nephrotoxicity rates [7]. With all these complexities, it is not surprising that, while limited by small sample sizes or the observational nature of some studies, comparisons between polymyxins and newer anti-CRE antibiotics in the β-lactam and aminoglycoside classes have favored the newer agents [8–12].

Like every other antibiotic, the use of polymyxins—in human infections or as a growth promoter in agriculture—is inevitably followed by bacterial resistance to polymyxins. Polymyxins bind the lipid A component of lipopolysaccharide (LPS), permeabilize the outer membrane, and induce cell death [13]. *K. pneumoniae* can remodel lipid A through the addition of 4-amino-4-deoxy-l-arabinose (Ara4N) by ArnF, a process that reduces the negative charge of the bacterial outer membrane [14]. This physiological process is governed by a complex regulatory network involving 2-component regulatory systems—CrrAB, PmrAB, and PhoPQ—but certain gain-of-function mutations in these systems can result in constitutive modification of lipid A with Ara4N and polymyxin resistance. In addition, PhoPQ is further regulated by negative regulator MgrB in *K. pneumoniae*. Therefore, any loss-of-function genetic changes in MgrB can render the strains resistant to polymyxins.

In this issue of *Clinical Infectious Diseases*, Macesic and colleagues [15] reported the most extensive genome analysis of polymyxin-resistant and -susceptible, carbapenem-resistant *K. pneumoniae* to date using a single-center, longitudinal strain collection at a tertiary medical center in New York City dating back to 2011. The goals of the study were to determine the relative contribution of in-hospital spread of

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Correspondence: Y. Doi, Division of Infectious Diseases, Department of Medicine, University of Pittsburgh School of Medicine, S829 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA 15261 (yod4@pitt.edu).

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polymyxin-resistant strains and de novo emergence of polymyxin resistance in the maintenance of endemicity, and to provide a full description of genetic changes that occur in canonical polymyxin resistance genes. To accomplish these goals, the authors sequenced and analyzed the genomes of 164 polymyxin-resistant *K. pneumoniae* isolates identified from 88 patients. These were patients who were quite ill and who had been in the hospital for an average of 21 days prior to identification of the first polymyxin-resistant isolate, and 69% of them were infected, as opposed to colonized by polymyxin-resistant *K. pneumoniae*. Sixty percent of the patients had prior polymyxin exposure, and 60% were treated with polymyxin-containing regimens despite known polymyxin resistance, reflecting the sheer lack of treatment options especially in the earlier years of the study when the new generation β-lactam/β-lactamase inhibitor combinations (BLBLIs) were not yet available. While limited by the small sample size, mortality rates were only slightly higher in patients with infection versus those with colonization from polymyxin-resistant *K. pneumoniae*. This suggests that polymyxin resistance may be primarily a marker for advanced illness in the patient, rather than a direct cause of poor outcomes.

In the genome analysis, the bla_{KPC} gene was detected in 97%, and the vast majority of the isolates belonged to ST258, ST17, ST307, or ST392, which is overall reflective of the known epidemiology of carbapenem-resistant *K. pneumoniae* in the United States. Eighty-three variants in the canonical polymyxin resistance genes (*crrAB*, *mgrB*, *pmrAB*, *phoPQ*), 67 of which were newly identified in this study, were found exclusively among polymyxin-resistant isolates, suggesting their possible role in resistance. Many of them appeared in combinations, and 36% of patients with serial isolates available had more than 1 genetic combination, suggesting that the emergence of polymyxin resistance is a dynamic and heterogenous process even within a single host. In phylogenetic analysis, there was no evidence of a generalized outbreak of polymyxin-resistant isolates, and 10 of 14 phylogenetically defined clusters containing more than 1 polymyxin-resistant isolates had isolates with different genetic combinations of canonical polymyxin resistance genes. Only 6 clusters contained isolates with identical genetic combinations, representing 16 patients and 24 isolates. These findings overall suggested that, in nonoutbreak settings, polymyxin resistance emerges independently for the most part, likely in response to selective pressure from polymyxin B or colistin, which the majority of the patients had received, rather than through clonal transmission of polymyxin-resistant isolates, which has been reported in outbreaks involving polymyxin and carbapenem-resistant *K. pneumoniae* [16].

The authors are commended for taking on the extensive genomic analyses of polymyxin-resistant *K. pneumoniae*, which fully supports the findings of previous smaller studies implicating de novo genetic changes as the main driver of polymyxin resistance in *K. pneumoniae*, as has also been found with *Acinetobacter baumannii*, another carbapenem-resistant pathogen for which polymyxins are used for therapy [17]. In addition, the study identified a large number of previously unknown genetic changes that may be associated with polymyxin resistance, which opens up venues for further research at the molecular levels.

What are the implications of these findings, at a time when newer BLBLIs such as ceftazidime-avibactam are increasingly recognized as preferred treatment options? First, despite accumulating clinical data demonstrating the superiority of ceftazidime-avibactam–based therapy over polymyxin-based therapy in the outcome of patients infected with carbapenem-resistant *K. pneumoniae*, polymyxins still continue to be widely used to combat carbapenem-resistant *K. pneumoniae* infections today due to factors including access, costs, and provider awareness among others. Ceftazidime-avibactam is also prone to resistance, with rates exceeding 10% among treatment-experienced patients [18]. Indeed, 4 of 11 patients treated with this agent in this study developed ceftazidime-avibactam–resistant *K. pneumoniae* isolates. Ceftazidime-avibactam is also not active against strains producing metallo-β-lactamases, such as NDM-1, and there are some concerning signs that increasing use of ceftazidime-avibactam may be selecting for metallo-β-lactamase–producing organisms in regions where those producing KPC previously prevailed [19]. Second, the results of this study clearly indicate antimicrobial stewardship as the priority in controlling and curbing the emergence and spread of polymyxin resistance over infection-prevention efforts to mitigate clonal spread, even though both interventions are clearly important and go hand in hand. Third, given that one-third of the patients had isolates with different genetic changes in the canonical resistance genes, some of which dictated susceptibility to polymyxins, repeating polymyxin susceptibility testing would be beneficial when patients are receiving a polymyxin and continue to have positive cultures. Finally, the extensive diversity of genetic changes associated with polymyxin resistance makes it difficult, if not impossible, to develop a genetic testing method to predict polymyxin resistance. Alternative approaches to rapid diagnosis may include colorimetric phenotypic assays [20] and detection of the lipid A signature that results from these genetic changes, Ara4N modification in the case of *K. pneumoniae*, through mass spectrometry [21].

The highly heterogenous genetic changes leading to polymyxin resistance in *K. pneumoniae* reported here are stark reminders of the complexity of polymyxins on multiple fronts—mechanisms of action and resistance, diagnostics, pharmacokinetics, and clinical efficacy—all of which will require continued and collaborative investigation before clinicians can utilize them with full confidence.

Notes

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