Emergence of Resistance to Colistin During the Treatment of Bloodstream Infection Caused by Klebsiella pneumoniae Carbapenemase–Producing Klebsiella pneumoniae

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Producing Klebsiella pneumoniae Infection Caused by Klebsiella pneumoniae

Emergence of Resistance to Colistin

Although the plasmid-mediated mcr genes are increasingly reported as a cause of colistin resistance in Escherichia coli, the most common mechanism of colistin resistance in K. pneumoniae is insertional inactivation of the mgrB gene [1]. The temporal pace and factors leading to colistin resistance through this mechanism are unknown. We report a case of a 52-year-old man (Figure 1) with neutropenia and chronic myelogenous leukemia who developed a central line–associated bloodstream infection with KPC-producing K. pneumoniae. The organism (isolate 1, Kpn918) had decreased ceftazidime-avibactam susceptibility in the absence of previous treatment with that agent. Despite removal of the line and 8 days of combination therapy with meropenem, tigecycline and colistin (loading dose of 5 mg/kg ideal body weight, followed by 1.75 mg/kg every 12 hours, adjusting for a creatinine clearance <50 mL/min), he relapsed with colistin-resistant KPC–K. pneumoniae bloodstream infection (isolate 2, Kpn926) and died from the infection in the setting of persistent neutropenia.

METHODS

To define the molecular mechanism of treatment-emergent colistin resistance and characterize the genetic background and evolution of colistin resistance, we performed the following microbiological tests. Antibiotic susceptibility testing was performed with disc diffusion assay and broth microdilution. In the case of colistin, broth macrodilution was performed in triplicate [3]. Results were interpreted according to Clinical and Laboratory Standards Institute guidelines, except for tigecycline and colistin minimum inhibitory concentrations (MICs), which were interpreted according to guidelines from the European Committee on Antimicrobial Susceptibility Testing [4, 5]. Multilocus sequence typing (MLST), wzi sequencing, and repetitive sequence-based polymerase chain reaction (rep-PCR) assessed genetic relatedness. Whole-genome sequencing of the isolates Kpn918 and Kpn926 was performed using the MiSeq platform (Illumina Inc., San Diego, CA) and analyzed using ResFinder, PlasmidFinder, and BLAST [6–8]. Additionally, blaKPC, blaSHV, blaNDM, blaVIM, blaoMP, blATM, blOXA-48-Ina, ompK35/36, mcr-1/2, and mgrB genes were queried using established PCR primers, and plasmid replicon typing with PCR was performed [9]. To better understand the evolution of colistin resistance, isolate 1 (Kpn918, colistin-susceptible) was exposed to serial passages with 0.25 µg/mL of colistin and 2 µg/mL of meropenem.

RESULTS

Antibiotic susceptibility testing of both isolates revealed resistance to meropenem (MIC > 8 µg/mL), ceftazidime (MIC > 16 µg/mL; zone of inhibition, 6 mm), aztreonam (MIC > 16 µg/mL), and tigecycline (MIC, 2 µg/mL). Ceftazidime-avibactam displayed decreased susceptibility (MIC, 4 µg/mL; zone of
inhibition, 19 mm). In regards to colistin, isolate 1 demonstrated an MIC of 0.5 µg/mL (susceptible) while isolate 2 had an MIC of 32 µg/mL (resistant). Among antibiotic combinations tested using disc diffusion, only ceftazidime-avibactam combined with aztreonam resulted in an increased zone of inhibition (to 24 mm).

Genetic analysis indicated that Kpn918 and Kpn926 belonged to ST258, contained wzi29, and shared 98.4% similarity according to rep-PCR. Each harbored blaKPC-2, blaSHV-2, and blaTEM-1. Both isolates possessed FIIK plasmids harboring blaKPC-2 on a Tn4401a transposon. Neither contained additional carbapenemase genes or mcr-1/2. IS903, a 1057 base-pair-long sequence was detected within mgrB in isolate 2 (colistin-resistant). Insertional inactivation of mgrB also occurred after 3 serial passages of isolate 1 in 0.25 µg/mL of colistin; however, a different element was found (IS4). In ompK35 and ompK36 from both isolates, we identified mutations that led to a stop codon at amino acid position 50 in ompK35, and insertions encoding glycine and aspartic acid at amino acid positions 134 and 135 in ompK36. Supplementary Figure 1 shows the results of draft whole-genome sequencing.

**DISCUSSION**

This report describes the occurrence of treatment-emergent colistin-resistant KPC–K. pneumoniae after 8 days of colistin-based combination therapy and highlights important observations. First, colistin resistance emerged due to disruption of mgrB by IS903. Similar emergence of colistin resistance was previously reported after 30 days of colistin therapy due to disruptive insertion of IS4-like insertion sequence into mgrB [1]. Insertion sequences are self-transmissible elements that can integrate into and excise from the chromosomes. The strain of K. pneumoniae infecting this patient belongs to ST258, the most common strain of KPC-producing K. pneumoniae in the United States and other countries, and insertional events may represent the genetic basis of its success [10, 11]. We hypothesize that this strain is prone to random transposition of insertion sequences, explaining the variability in insertion sequences found in isolate 2 in vitro during exposure to subinhibitory concentrations of colistin. The cause of transposition of insertion sequences is not known, although exposure to subinhibitory concentrations of antibiotics may be associated with such events, as reproduced in vitro in the original isolate. Antibiotic-induced bacterial stress responses can lead to insertional mutations that contribute to methicillin resistance in Staphylococcus aureus and to deletions of regulatory genes in E. coli resistant to fluoroquinolones [12, 13]. Second, we found that the porin genes ompK35 and ompK36 in both

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**Table 1.** antibiotics administered during the hospital course. **Abbreviations:** COL-R, colistin resistance; ID, infectious diseases; WBC, white blood cell.

| Antibiotics administered | Hospital course |
|--------------------------|-----------------|
| Admitted with fever, dyspnea, and fatigue, Found to have WBC ~154,000 cells/microliter | Sept 1 |
| Detected a fever of 39.4°C. Found to have central line-associated *Klebsiella oxytoca* bacteremia. Central line removed | Sept 4 |
| Fever of 38.6°C followed by development of septic shock. ID consulted. Found to have carbapenem-resistant *Klebsiella pneumoniae* bacteremia. Central line removed | Sept 5 |
| Negative blood cultures | Sept 9 |
| Negative blood cultures | Sept 10 |
| Negative blood cultures | Sept 12 |
| Negative blood cultures | Sept 13 |
| Patient died | Sept 19 |
| Fever of 39°C. Found to have carbapenem-resistant *Klebsiella pneumoniae* with COL-R in 2/2 blood cultures | Sept 25 |

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**Figure 1.** Timeline of clinical events, laboratory findings, and antibiotics administered during the hospital course. **Abbreviations:** COL-R, colistin resistance; ID, infectious diseases; WBC, white blood cell.
isolates contained mutations previously linked to reduced susceptibility to ceftazidime-avibactam and carbapenems [2]. Of note, our patient had not been treated with ceftazidime-avibactam; therefore, reinfection with a new strain or horizontal gene transfer from a strain previously exposed to ceftazidime-avibactam cannot be dismissed. Approximately 5 cases of carbapenem-resistant K. pneumoniae occur at our hospital monthly. Although analyses of genetic relatedness suggest that both isolates from this case are clonally related, genome sequencing of KPC-producing K. pneumoniae from our hospital reveal that highly similar subpopulations can coexist over time, including isolates containing blaKPC-2 in IncFIIK plasmids, as in this patient [14]. In conclusion, clinicians should strongly suspect emergence of resistance to colistin during treatment with colistin-based combination therapies in patients with persistent or relapsing KPC–K. pneumoniae bloodstream infection. These observations highlight the dynamic nature of resistance to colistin, often a “last-line agent” in critically ill patients.

Supplementary Data
Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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