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BRIEF REPORT

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

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We report the emergence of colistin resistance in *Klebsiella pneumoniae* carbapenemase (KPC)–producing *Klebsiella pneumoniae* after 8 days of colistin-based therapy, resulting in relapse of bloodstream infection and death. Disruption of the *mgrB* gene by insertion of a mobile genetic element was found to be the mechanism, which was replicated in vitro after exposure to subinhibitory concentrations of colistin and meropenem.

Keywords. carbapenemase; colistin resistance; *Klebsiella pneumoniae*.

Carbapenem-resistant *Klebsiella pneumoniae*, commonly mediated in the United States by production of *Klebsiella pneumoniae* carbapenemase (KPC), is a cause of difficult-to-treat infections associated with high mortality. Among the only currently available agents with reliable activity against KPC-producing *K. pneumoniae* are polymyxin B, colistin, and combinations containing novel inhibitors of KPC that restore the activity of β-lactams. Therefore, reports describing the decreased activity of ceftazidime-avibactam, one of the combinations, and the emergence of resistance to colistin in KPC-producing *K. pneumoniae* are of special concern [1, 2]. 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, bla_{KPC}, bla_{SHV}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{TEM}, $bla_{OXA\text{-}48\text{-}like}$, *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

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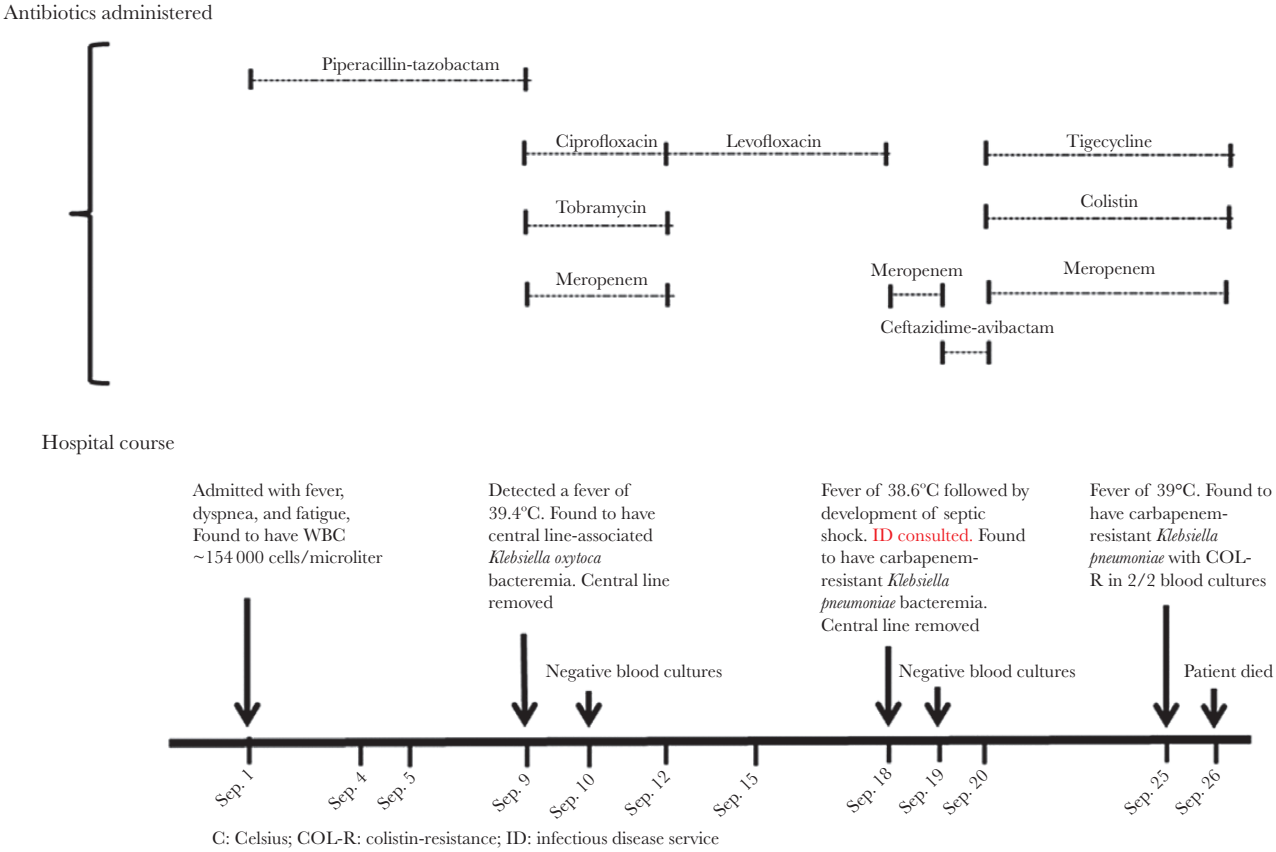

Figure 1. Timeline of clinical events, laboratory findings, and antibiotics administered during the hospital course. Abbreviations: COL-R, colistin resistance; ID, infectious diseases; WBC, white blood cell.

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 *wzi* 29, and shared 98.4% similarity according to rep-PCR. Each harbored bla_{KPC-2}, bla_{SHV-2}, and bla_{TEM-1}. Both isolates possessed FIIK plasmids harboring bla_{KPC-2} on a Tn*4401a* transposon. Neither contained additional carbapenemase genes or *mcr-1/2*. IS*903*, 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 (IS*4*). 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 IS*903*. Similar emergence of colistin resistance was previously reported after 30 days of colistin therapy due to disruptive insertion of IS*4*-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

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 $bla_{\text{KPC-2}}$ 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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