Nonclonal Emergence of Colistin-Resistant *Klebsiella pneumoniae* Isolates from Blood Samples in South Korea

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In vitro activities of colistin and other drugs were tested against 221 *Klebsiella pneumoniae* isolates that were collected between 2006 and 2007 in nine tertiary care South Korean hospitals from patients with bacteremia. The clonality of colistin-resistant *K. pneumoniae* (CRKP) isolates was assessed by multilocus sequence typing (MLST). We found that 15 isolates (6.8%) were resistant to colistin. MLST showed that CRKP isolates were nonclonal, with colistin resistance in *K. pneumoniae* occurring independently and not by clonal spreading.

The lack of effective antimicrobial agents effective against infections by Gram-negative pathogens has led to the revival of polymyxins, such as colistin and polymyxin B, which had been abandoned because of their nephrotoxicity and neurotoxicity (12). Although low colistin resistance rates have been reported in many parts of the world (8), some reports have indicated high polymyxin resistance rates for *K. pneumoniae* in New York (United States) and in Greece has been reported (1, 3). Additionally, it was recently reported that resistance to polymyxin B developed during treatment for *K. pneumoniae* infection (11).

As a part of a nationwide multicenter surveillance study, a total of 221 *K. pneumoniae* isolates were obtained from blood samples in nine South Korean university hospitals: Samsung Medical Center, Kangbuk Samsung Hospital, Konkuk University Hospital, Kyungpook National University Hospital, Chonnam National University Hospital, Chungnam National University Hospital, Chungbuk National University Hospital, Gyeongsang National University Hospital, and Jeju National University Hospital. Only the first isolate obtained from each patient was included in the study.

Antimicrobial susceptibility testing was performed by a broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (5). Colistin sulfate and nine other antimicrobial agents were tested. The susceptibility interpretive criteria used were those established in CLSI standard M100-S18 (5). Regarding colistin, interpretive criteria were defined based on the British Society for Antimicrobial Chemotherapy (BSAC) breakpoint criteria for *Enterobacteriaceae* (resistant, >4 mg/liter), *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were used as control strains. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial agents, excluding colistin. Extended-spectrum β-lactamase (ESBL) activity was detected by the double-disc synergy test, as recommended by the CLSI (5). To detect metallo-β-lactamase (MBL) activity, the EDTA-imipenem disc synergy test was performed for all colistin-resistant isolates (4).

To investigate the clonality of colistin-resistant *K. pneumoniae* isolates, multilocus sequence typing (MLST) was performed for colistin-resistant *K. pneumoniae* (CRKP) isolates, as described by Diancourt et al. (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) (6). Fisher’s exact *t* test was used to determine significant differences in resistance by using SPSS for Windows (version 11.5 software package; SPSS Inc., Chicago, IL).

As a whole, the 221 *K. pneumoniae* isolates from blood samples showed 10% to 20% resistance rates for most antimicrobial agents except imipenem and colistin (Table 1). Only one isolate showed imipenem resistance (MIC, 16 mg/liter). Fifteen *K. pneumoniae* isolates (6.8%) showed colistin resistance. For most antimicrobial agents, excluding ciprofloxacin and trimethoprim-sulfamethoxazole, colistin susceptible *K. pneumoniae* (CSKP) isolates showed antimicrobial resistance rates that were higher than those of CRKP isolates, although this difference was not statistically significant (Table 1). In addition, while only two MDR isolates were identified among the 15 CRKP isolates (13.3%), 44 CSKP isolates were MDR (21.4%). There was also one CRKP isolate that showed ESBL activity and was resistant to all antimicrobials but imipenem. No isolates showed MBL activity.

MLST analysis revealed that 15 CRKP isolates showed 14 different sequence types (STs) (ST11, ST27, ST37, ST218, ST354, ST356, ST358, ST359, ST363, ST364, ST365, ST366, ST367, and ST369). Only two isolates, which were from the same hospital (Samsung Medical Center, Seoul, South Korea), showed the same ST, ST359. However, these two ST359 isolates showed different antimicrobial resistance profiles: one showed resistance to ciprofloxacin and trimethoprim-sulfam-
methoxazole, while the other was susceptible to both of these drugs. Generally, the colistin resistance rate for \textit{K. pneumoniae} has been reported to be low. However, 10% to 25% of MDR \textit{K. pneumoniae} isolates in New York City showed resistance to polymyxins (2, 3). In addition, an outbreak of multilocal CRKP isolates in a Greek intensive care unit was reported (1). Furthermore, a study found that CRKP isolates in Singapore were not uncommon (6%) (17). In this study, the colistin resistance among \textit{K. pneumoniae} isolates from blood samples was 6.8%, according to the BSAC breakpoint criteria. By applying the MIC breakpoint of the Société Française de Microbiologie (resistance, >2 mg/liter), 24 isolates (10.9%) would be classified as resistant to colistin (7). This finding may indicate that colistin resistance is as common in \textit{K. pneumoniae} in South Korea as in \textit{Acinetobacter baumannii} (10). It should be noted that CRKP isolates were identified in only four of the nine participating hospitals. In particular, two hospitals showed the most frequent emergence of CRKP isolates. Four out of 9 \textit{K. pneumoniae} isolates from blood samples taken in the Chungnam National University Hospital were CRKP, and 3 out of 11 isolates from the Konkuk University Hospital were colistin resistant. Further studies on the relationships between emergence of colistin resistance and colistin use should be performed.

Decreased antimicrobial resistance in colistin-resistant isolates has also been shown for \textit{A. baumannii} (13, 14). Li and colleagues have speculated that changes in the outer membrane coupling with colistin resistance may allow susceptibility to other antimicrobials (13). However, one isolate was resistant to all tested antimicrobial agents but one, imipenem. This isolate showed ESBL activity. Our finding indicates the possibility of colistin resistance in MDR \textit{K. pneumoniae} isolates, which would be of great concern in clinical settings.

Based on MLST analysis, we found that the emergence of CRKP isolates appeared to be independent. The emergence of multilocal CRKP isolates was also reported in a Greek study (1), and independent emergence of colistin resistance was also shown for \textit{Acinetobacter} sp. isolates (15). Such nonclonal occurrence of colistin resistance in \textit{Acinetobacter} sp. as well as \textit{K. pneumoniae} may indicate that colistin resistance is mainly due to selective pressure by increasing and inadequate use of colistin (1). In fact, cases indicating that colistin resistance is provoked by exposure of both \textit{K. pneumoniae} and \textit{Acinetobacter} spp. to colistin have been reported (9, 11). Thus, appropriate dosage regimens for colistin should be determined in order to prevent colistin resistance. In addition, combination therapy, such as colistin combined with rifampin, would be important not only to effectively treat infections by Gram-negative bacteria but also to prevent colistin resistance in these pathogens (11, 16).

Our study showed the emergence of colistin resistance in invasive \textit{K. pneumoniae} isolates in South Korea, most likely by an independent acquisition mechanism.

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### REFERENCES


