Non-clonal emergence of colistin-resistant *Klebsiella pneumoniae* isolates from blood in Korea

Short title: Colistin-resistant *K. pneumoniae* isolates in Korea

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Abstract

In vitro activities of colistin and other drugs were tested against 221 *Klebsiella pneumoniae* isolates that were collected between 2006 and 2007 in 9 tertiary-care Korean hospitals from patients with bacteremia. The clonality of colistin-resistant *K. pneumoniae* (CRKP) isolates was assessed by MLST. We found that 15 isolates (6.8%) were resistant to colistin. MLST showed that CRKP isolates were non-clonal, 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 a low colistin resistance rate has been reported in many parts of the world (8), some indicated high polymyxin resistance rates in *Acinetobacter* spp. (10). Low polymyxin resistance was also reported for *Klebsiella pneumoniae* (8), but emergence of polymyxin-resistant *K. pneumoniae* isolates has been reported in New York of the United States and in Greece (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 in nine university Korean 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 another nine antimicrobial agents were tested. Susceptibility interpretive criteria used were those established in CLSI standard M100-S17 (5). Regarding colistin, interpretive criteria were defined based on the British Society for Antimicrobial Chemotherapy (BSAC) breakpoint criteria for Enterobacteriaceae (resistant, >4 mg/L). *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and
Staphylococcus aureus ATCC29213 were used as control strains. MDR was defined as resistant to three or more antimicrobial agents except 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, EDTA-imipenem disc synergy test was performed for all colistin-resistant isolates (4).

To investigate the clonality of colistin-resistant K. pneumoniae isolates, MLST was performed for colistin-resistant K. pneumoniae (CRKP) isolates, as described in 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 using SPSS for Windows (version 11.5 software package; SPSS Inc., Chicago, IL, USA).

As a whole, 221 K. pneumoniae isolates from blood 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/L). Fifteen K. pneumoniae isolates (6.8%) showed colistin resistance. For most antimicrobial agents except ciprofloxacin and trimethoprim-sulfamethoxazole, colistin-susceptible K. pneumoniae (CSKP) isolates showed higher antimicrobial resistance rates than 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) (Supplement). Only two isolates, which were from the same hospital (Samsung
Medical Center, Seoul), showed the same ST, ST359. However, these two ST359 isolates showed different antimicrobial resistance profiles; one showed resistance to ciprofloxacin and trimethoprim-sulfamethoxazole while the other was susceptible to both of these drugs.

Generally, the colistin resistance rate in *K. pneumoniae* has been reported to be low. However, 10% to 25% of MDR *K. pneumoniae* isolates in New York City showed resistance to polymyxins (2, 3). In addition, an outbreak of multiclonal CRKP isolates was reported in a Greek intensive care unit (1). Furthermore, in Singapore, CRKP isolates were not uncommon (6%) (17). In this study, colistin resistance in *K. pneumoniae* isolates from blood was 6.8%, according to the BSAC breakpoint criteria. Applying the MIC breakpoint of the Société Française de Microbiologie (resistance, >2 mg/L), 24 isolates (10.9%) would be classified as resistant to colistin (7). This finding may indicate that colistin resistance is as common in *K. pneumoniae* in Korea, as in *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 nine *K. pneumoniae* isolates from blood taken in the Chungnam National University Hospital were CRKP, and three 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 in *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 *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. Emergence of multiclonal CRKP isolates was also reported in a Greek study (1) and independent emergence of colistin resistance was also shown in *Acinetobacter* spp. isolates (15). Such non-clonal occurrence of colistin resistance in *Acinetobacter* spp. as well as *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 to colistin have been reported both in *K. pneumoniae* and *Acinetobacter* spp. (9, 11). Thus, appropriate dosage regimens for colistin should be determined 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 as well (11, 16).

Our study showed the emergence of colistin resistance in invasive *K. pneumoniae* isolates in Korea, most likely by an independent acquisition mechanism.

**Acknowledgments**

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References


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Table 1. In vitro antimicrobial resistances of 221 *K. pneumoniae* isolates from blood taken in 9 Korean hospitals

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Total (n=221)</th>
<th>CSKP a (n=206)</th>
<th>CRKP b (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance (%)</td>
<td>MIC₅₀ (mg/L)</td>
<td>MIC₉₀ (mg/L)</td>
<td>Resistance (%)</td>
</tr>
<tr>
<td>Colistin</td>
<td>6.8</td>
<td>1</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>91.4</td>
<td>64</td>
<td>&gt;64</td>
<td>92.2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>16.3</td>
<td>0.5</td>
<td>&gt;64</td>
<td>17.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>17.7</td>
<td>≤0.06</td>
<td>64</td>
<td>17.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>17.7</td>
<td>0.25</td>
<td>&gt;64</td>
<td>18.5</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>14.5</td>
<td>≤0.12</td>
<td>128</td>
<td>15.1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>16.7</td>
<td>≤0.06</td>
<td>&gt;64</td>
<td>17.5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5</td>
<td>0.25</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>19.5</td>
<td>0.25/0.75</td>
<td>&gt;32/608</td>
<td>18.9</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>19.5</td>
<td>4/4</td>
<td>&gt;256/4</td>
<td>16.0</td>
</tr>
</tbody>
</table>

a CSKP, colistin-susceptible *K. pneumoniae*
b CRKP, colistin-resistant *K. pneumoniae*