# Emergence of Colistin-Resistant *Klebsiella* spp., and *Enterobacter* spp. in the Asia-Pacific (APAC) Region: A SENTRY Antimicrobial Surveillance Program Report (2006)

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## Amended Abstract

Background: Colistin has re-established itself as a very important agent for the treatment of multi-drug resistant Gram-negative pathogens worldwide, including the APAC region. We wish to determine whether the reintroduction of this agent into clinical practice has resulted in resistance (R) emergence in our region.

Methods: A total of 2,379 clinically significant Enterobacteriacae (excluding Proteae and *Serratia* spp.) from patients with bacteraemia, pneumonia, complicated skin and skin structure infections (cSSSI), and other infections from 42 medical centres in 10 countries in the APAC region were collected during 2006. All isolates were tested against >25 antimicrobial agents including colistin sulphate and polymyxin B using CLSI methods and broth microdilution panels (TREK Diagnostics). We attempted to validate resistance in strains with colistin BMD MIC > 4 mg/L using an Etest (ET) method similar to that used for staphylococci and vancomycin.

Results: R to colistin was detected in 3 (0.3%) *Klebsiella* spp. and 77 (21%) *Enterobacter* spp. When those R strains were tested by ET, only three strains had an MIC > 4 mg/L. However, 50% of Enterobacters and 25% of Klebsiellas showed significant growth (colonies) inside the ET ellipse, indicating hetero-resistance (h-R). Polymyxin B results were concordant with those of colistin.

Conclusions: In the APAC region R to colistin is common in *Enterobacter* spp., and has also been detected in *Klebsiella* spp. and is frequently manifest as h-R pattern.

## Introduction

The emergence of multidrug-resistant (MDR) Gram-negative pathogens has been increasing worldwide. The recovery of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates susceptible only to polymyxins from critically ill patients has led to the revival of colistin, an antimicrobial forgotten for decades, which appears as the only treatment choice either empirically or as microbiologically documented therapy. Resistance to colistin is currently rare, but is described.[1,2]

We wish to determine whether the reintroduction of this agent into clinical practice has resulted in resistance emergence among Enterobacteriaceae in the Asia-Pacific region (SENTRY Program 2006).

## Methods

### *Isolates*

Enterobacteriaceae (excluding Proteae and *Serratia* spp.) from infected hospitalized patients in 10 countries (42 medical centres) collected during 2006. Isolates came from patients with bacteraemia, pneumonia, complicated skin and skin structure infections, and other infections. All strains were referred to the Women's and Children's Hospital, Adelaide, Australia for testing.

### *Susceptibility testing*

Isolates were tested using custom made dry-form broth microdilution (BMD) panels (TREK Diagnostic Systems) against a wide range of antimicrobials including colistin sulphate (COL) and polymyxin B (POL) according to CLSI standards.[3] Breakpoints for resistance to other antimicrobial agents were those recommended by the CLSI.[4]

Quality control strains utilized included *Escherichia coli* ATCC 25922 and 35218, *P. aeruginosa* ATCC 27853; all MIC results were within CLSI specified ranges.

### *Analysis*

Strains with colistin BMD MIC ≥ 4 mg/L were selected for further analysis using Etest (ET) strips (AB BIODISK, Solna, Sweden). A macro-method (using a 2 McFarland inoculum density) in a manner similar to that used for staphylococci and vancomycin, was used to detect resistant subpopulations.

### *Hetero-resistance*

Isolates with resistant subpopulations inside the Etest ellipse were regarded as heteroresistant.

### References

1. Antoniadou A et al. 2007. Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster. *J Antimicrobiol Chemother*; 59:786-790.
2. Li J, Nation RL, Turnidge JD, et al. (2006). Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infect Dis* 6 (8): 589–601
3. CLSI. 2006. Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria That Grow Aerobically. M7-A7, Wayne, PA.
4. CLSI. 2007. Performance standards for Antimicrobial Susceptibility Testing; 17th Informational Supplement. M100-S17. Wayne, PA.

Table 1.Colistin MIC Distribution using broth microdilution

| Organism | Total | Colistin MIC (mg/L) <=0.5 | 1 | 2 | 4 | >4 | % ≥ 4 mg/L |
|---|---|---|---|---|---|---|---|
| *Klebsiella* species | 905 | 859 | 40 | 3 | | 3 | 0.3 |
| *Enterobacter* species | 361 | 269 | 10 | 3 | 2 | 77 | 21.9 |
| *Escherichia coli* | 918 | 910 | 8 | | | | 0.0 |
| *Citrobacter* species | 80 | 75 | 3 | 1 | | 1 | 1.3 |
| *Pantoea* species | 5 | 6 | | | | | 0.0 |
| *Salmonella* species | 108 | 90 | 6 | | 10 | 2 | 11.1 |
| *Kluyvera* species | 2 | 2 | | | | | 0.0 |

Table 2. Colistin Etest MIC distributions on selected strains

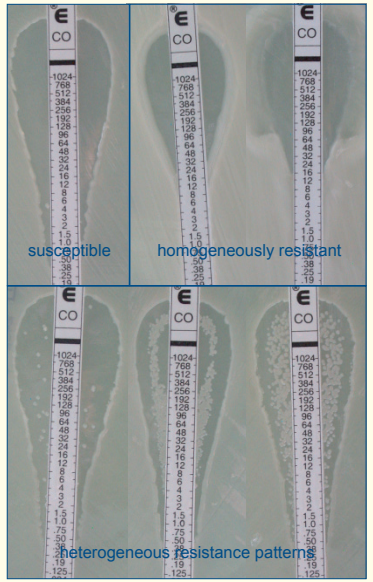
| Organism | BMD MIC (mg/L) | No. | Colistin MIC (mg/L) 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | %h-R[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Klebsiella pneumoniae* | ≤0.5 | 5 | 1 | 1 | 1 | 2 | | | | | | |
| hetero-resistant | | | | | | | | | | | | 0.0 |
| | ≥4 | 3 | | | | | | | 1 | 1 | 1 | |
| hetero-resistant | | | | | | | | | | | | 0.0 |
| *Enterobacter* spp. | ≤0.5 | 50 | 4 | 44 | 2 | | | | | | | |
| hetero-resistant | | | | | | | | | | | | 0.0 |
| | ≥4 | 69 | 2 | 50 | 16 | 1 | | | | | | |
| hetero-resistant | | | 1 | 23 | 8 | 1 | | | | | | 47.8 |

[a] % h-R = % of isolates with > 1 colony inside the ellipse

## Results

- A total of 2,379 Enterobacteriaceae (excluding Proteae and *Serratia* spp.) were collected from the APAC region during 2006.
- Colistin resistance (MIC ≥ 4 mg/L) was common among *Enterobacter* spp. (21.9%) (Table 1). Resistance was seen in all countries except Singapore; ranging from 13.8% (India) to 50% (Philippines).
- A bi-modal colistin MIC distribution was observed among *Salmonella* spp. Overall, 11% of all *Salmonella* had colistin MIC ≥ 4 mg/L. The strains with elevated MICs were from five separate countries.
- Three *K. pneumoniae* isolates had colistin MICs of 16, 32 and 64 mg/L. These strains were from Thailand, India and Korea, respectively.
- Colistin Etests demonstrating resistant subpopulations are shown in Figure 1.
- Resistant subpopulations were observed for approximately half of all *Enterobacter* spp. tested that had colistin BMD MIC ≥ 4 mg/L (Table 2). Interestingly, these subpopulations were seen at all Etest MIC levels.
- A selection of 50 *Enterobacter cloacae* isolates with BMD MIC ≤ 0.5 mg/L did not express resistant subpopulations using the macro Etest method.
- No correlation between colistin resistance and multi-drug resistance was observed.
- Polymyxin B results were concordant with those of colistin.

Figure 1.
Patterns of colistin susceptibility observed using the macro Etest method




## Conclusions

- In the Asia-Pacific region resistance to colistin is common in *Enterobacter* spp.
- Resistance is frequently manifested as hetero-resistance.
- Colistin resistance has also been detected in *Klebsiella* spp. in three countries.
- The apparent discrepancy between broth microdilution and Etest MICs requires further investigation.