The mgrB gene as a key target for acquired resistance to colistin in Klebsiella pneumoniae

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INTRODUCTION

- Colistin is increasingly used in endemic areas for treating infections caused by carbapenemase-producing Klebsiella pneumoniae and reports of colistin resistance isolates in that species are on the rising trend (1).
- Alterations in the PhoPQ two-component regulatory system may be associated with polymyxin resistance in K. pneumoniae (2).
- mgrB is a small transmembrane protein of 47 amino acids produced upon activation of the PhoPQ signalling system, and acting as a negative regulator on this system (3).
- A recent study showed that insertional inactivation of the mgrB gene was involved in the acquired resistance to colistin in K. pneumoniae (4).

Matterials and Methods

- Bacterial isolates: a collection of 47 colistin-resistant K. pneumoniae isolates recovered from different countries, with a focus on the mgrB gene.
- MICs of colistin were determined by Etest.
- Clonal relationship was determined using Multilocus Sequence Typing and Diversilab analysis.
- mgrB gene was analyzed by PCR and Sequencing.
- Complementation assays were performed by cloning a wild-type mgrB gene into a cloning vector, and then electroporating this recombinant plasmid into the colistin-resistant clinical isolates.

PURPOSE

We aimed to investigate the mechanisms responsible for colistin resistance in a series of K. pneumoniae isolates recovered from different countries, with a focus on the mgrB gene.

RESULTS

1. Truncation of the mgrB gene by diverse insertion sequences in seven isolates.

(A) T1a (wild-type)

(B) T1b, 1118, 2009, C9, C10

(C) T1b, 1118, 2009, C9, C10

(D) C21

(E) C22

- Three different insertion sequences
- Two different locations

2. Diversilab and MLST analysis identified four clonally-related isolates with the same IS5-like insertion sequence.

ST-512, France
ST-258, France
ST-258, Colombia
ST-258, Turkey

3. Modification of the mgrB expression through the insertion of an IS10R insertion sequence upstream of the mgrB gene in two clonally-related isolates.

- The arrow indicates the target site for insertion of IS10R

4. Truncation of the mgrB gene by a premature codon stop in three unclonally-related isolates.

- The three isolates were identified as T1a, C11, and Sa.

5. Complementation with a wild-type MgrB protein generated a complete reversion to colistin susceptibility.

- The table shows the MICs of colistin (µg/ml) before and after supplementation with pTOPO-mgrB.

CONCLUSIONS

This study identified mutations inside the coding sequence but also the promoter region of the mgrB gene, both leading to colistin resistance among K. pneumoniae isolates. The MgrB protein therefore appears to be a key target for acquired resistance to colistin in K. pneumoniae.

REFERENCES