basS Mutations linked to Colistin Resistance (ColR) in *Enterobacter Cloacae*

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BACKGROUND

- A treatment option for multi-drug resistant gram negative *Enterobacteriaceae* is the cationic peptide colistin.
- Colistin binds to the lipopolysaccharide (LPS) and phospholipids in the outer cell membrane displacing Ca^{+2} and Mg^{+2} ions from the phosphate groups of membrane lipids leading cell lysis (1).
- Colistin resistance is caused by the addition of amino-4-deoxy-L-arabinose to lipid A
  - Turns the cell membrane charge positive
  - prevents colistin binding(2).
- LPS modifications are controlled by a 2-component regulatory system (Figure 1)
  - Depends on concentration of Mg^{+2}, Al^{+3}, Fe^{+3}, pH, and by the presence of antimicrobial peptides(2).
- One of the most common mutations found in *Klebsiella pneumoniae* has been on the *mgrB* gene.
  - Communicates between *basS*, and *phoQ* (3).
- Resistance usually develops during treatment and is not sustained
  - There may be a fitness cost to it.

Materials and Methods

Isolates: 9 isolates of *E. cloacae* from Colombia showing sustained ColR. We used 3 isolates from different sources as negative controls

Susceptibility testing and Identification: Colistin resistance was verified twice using agar dilution over Cation-Adjusted Muller-Hinton Media. Isolates were verified as *Enterobacter cloacae* by MALDI-TOF MS.

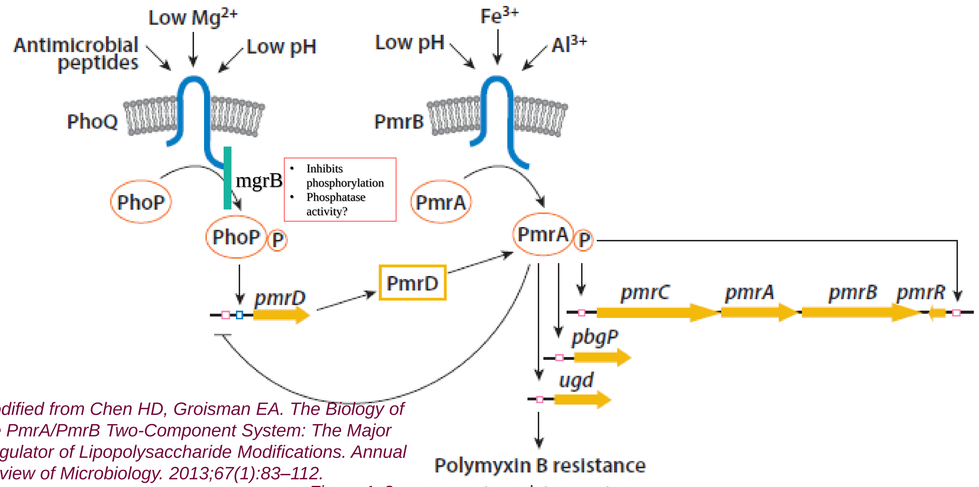
DNA extraction: Extraction was performed using MoBIO UltraClean Microbial DNA isolation kit (Carlsbad, CA, USA) according to manufacturer's instructions.

Bacterial typing: rep-PCR was performed using the Diversilab *Enterobacter kit* (Biomerieux). Products were separated in a Agilent Bionalyzer 2100 using a DNA chip and analyzed using the Diversilab on-line software. *hsp60* sequencing was done on all isolates using primers and conditions published by Rammelkamp et al (4). Sequences were compared between each other and to previously published sequences to define the *hsp60* cluster to which each isolate belonged.

Sequencing of genes related to colistin resistance: we designed primers for sequencing of the following genes: *mgrB*, *basS*, *basR*, *and phoQ*. Standard PCR conditions were used. We used a nested PCR strategy for sequencing of *phoQ*. Dedicated sequencing and amplification primers were used for *basS* and *phoQ*. All sequencing was carried out at a commercial sequencing facility (MCLAB, San Francisco, CA)

Sequence analysis: sequence analysis was done using Lasargene DNASTAR software package. Sequences were assembled with Seqman and aligned with Megalign.

Protein modeling: Computer simulation of the effect of amino-acid substitution was done with on-line software Protein-Predict (5).



Modified from Chen HD, Groisman EA. The Biology of the PmrA/PmrB Two-Component System: The Major Regulator of Lipopolysaccharide Modifications. Annual Review of Microbiology. 2013;67(1):83–112.

Figure 1: 2-component regulatory system.

Results

Susceptibility testing, hsp60 sequencing, and rep-PCR results

Isolate	Colistin AG (µg/mL)	Colistin AG (µg/mL)	hsp60 Cluster
1	0.5	0.25	VIII
8	>128	0.5	IV
9	2	>128	VI
16	2	<0.03	IV
17	>128	>128	XI
19	>128	>128	VI
20	>128	>128	VIII
21	>128	>128	VIII
22	>128	128	VIII
26	>128	>128	VIII
27	>128	>128	VI
28	128	64	IV
48	2	0.125	VIII

Table 1: MIC and *hsp60* cluster of study isolates

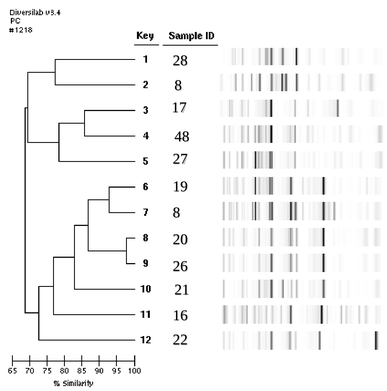


Figure 2: rep-PCR

basS Sequencing

- We identified mutations in the basS gene that resulted in amino-acid changes in 8 positions in the majority of ColR resistant isolates (Figure 3).
  - 5/9 (17,19,20,21 and 26) isolates had the following changes: V93A, TEA170S, T171A, AN232P, PT259A, E270Q, LQ275R, G331A
  - 1 isolate (28) had V93A and PT259A
- No mutations were found in 3 resistant isolates (8, 22, and 27)
- No mutations were found in any of the susceptible isolates
- Resistant isolates were mapped to hsp60 clusters VI, VIII, and XI; and were part of 4 different rep-PCR patterns.

RESULTS

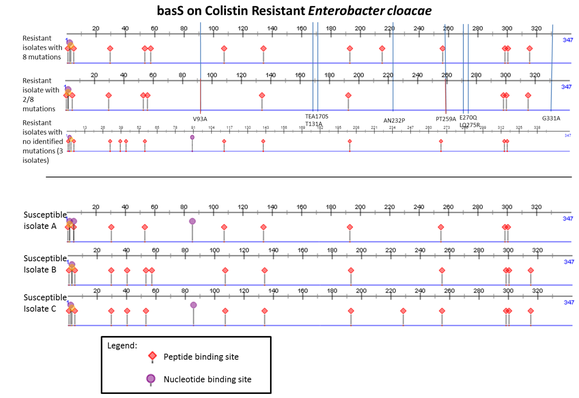


Figure 3: basS Protein representation

mgrB, basR, and phoQ sequencing

- Different mutations in the *mgrB*, *basR*, and *phoQ* genes were found
- None of the mutations produced an amino-acid change

CONCLUSIONS

- An association between ColR in E. cloacae and 8 amino-acid changes on the basS gene was uncovered.
- These single nucleotide changes are independent of the bacterial strain by rep-PCR and nomospecies by hsp60 sequencing and suggest the role of this homologue of pmrB in ColR phenotype in .

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