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Disruption of *mgrB* and Alterations on *pmrB* Are Most Common Resistance Mechanisms among Colistin-Resistance among *Klebsiella pneumoniae* from a Global Surveillance Program

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Introduction

- *Klebsiella pneumoniae* isolates resistant to most available antimicrobial agents have led to an increased use of the polymyxin class (colistin [polymyxin E] and polymyxin B) in serious infections caused by these organisms
- Resistance to polymyxins in *K. pneumoniae* is usually caused by chromosomal mutations leading to modifications in the lipopolysaccharide (LPS) that becomes positively charged and interacts poorly with polymyxins
  - Mutations in the 2 component systems PmrA-PmrB and PhoP-PhoQ as well as inactivation of MgrB decrease the affinity of the LPS for polymyxins
    - PmrA-PmrB and PhoQ-PhoP regulate the *pmrHFIJKLM* operon, which controls modification of the outer membrane
    - MgrB is a small transmembrane protein that exerts negative feedback on the *pmrHFIJKLM* operon by interaction with the sensor kinase PhoQ at the periplasmic level
  - Additionally, the presence of *mcr*, a plasmid-mediated gene that increases positive charges in the LPS, has been reported in *K. pneumoniae*
- We evaluated colistin resistance mechanisms using whole genome sequence (WGS) analysis for 144 *K. pneumoniae* isolates collected worldwide during 2016 as part of the SENTRY Program

Materials and Methods

- A total of 4,049 *K. pneumoniae* clinical isolates collected during 2016 from 159 hospitals located in North America (n=2,199), Europe (n=1,204), Asia-Pacific (n=297), and Latin America (n=349) were included in the study
- Isolates were limited to 1 per patient episode and were collected from bloodstream infections (n=1,089), intra-abdominal infections (n=184), pneumonia in hospitalized patients (n=1,140), skin and skin structure infections (n=490), urinary tract infections (n=1,024), and other sources (n=122)
- Species identification was confirmed, when necessary, by matrix-assisted laser desorption ionization-time of flight mass spectrometry
- Isolates were tested for susceptibility against colistin and comparator agents using the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; M07, 2018)
  - Quality control (QC) was performed according to CLSI guidelines, and all QC MIC results were within acceptable ranges, as published in CLSI documents (M100, 2018)
  - Categorical interpretations for all comparator agents were those found in CLSI criteria in M100 (2018), EUCAST breakpoint tables (version 7.0, January 2018), and/or the US FDA website
- A total of 144 *K. pneumoniae* isolates displaying colistin MIC values from 0.5 to >8 mg/L (Figure 1) were submitted to WGS on a MiSeq (Illumina, San Diego, California, USA) instrument targeting a 30X coverage
  - Sequences were *de novo* assembled and searched for the presence of acquired carbapenemases using a curated library and applying criteria of >94% sequencing identity and 40% minimum length coverage
  - Isolates were screened for mutations in *pmrA*, *pmrB*, *phoQ*, *phoP*, *mgrB*, *ccrA*, *ccrB* and the presence of *mcr* variants

Results

- Among 328 *K. pneumoniae* isolates with colistin MIC values ranging from 0.5 (100 isolates) to >8 (84) mg/L, 144 isolates collected in 54 hospitals were analyzed for the presence of *mcr* and intrinsic colistin resistance mechanisms (Table 1)
- Only 1 isolate from Italy and displaying a colistin MIC at >8 mg/L carried *mcr-1*
  - No other colistin resistance mechanisms were detected in this isolate that also carried $bla_{CTX\text{-}M\text{-}15}$
  - Other *mcr* variants were not observed
- Disruption of *mgrB* was detected in 56 isolates all displaying colistin MIC values at 4 mg/L or greater (Figure 2)
  - *mgrB* was disrupted by insertion sequences (IS; 40 isolates), nonsense mutations (13), or frameshifts (3 isolates; all starting at position 25)
  - The most common ISs disrupting *mgrB* were IS*903*, IS*1294*, IS*1R*, and IS*Kpn26*
- Substitutions on MgrB previously associated with colistin resistance were detected in 10 isolates and included W20R/S, C28R/S/Y, G37S and D31N
- A total of 87 isolates had *pmrB* mutations encoding R256G substitution, including 46 *mgrB*-disrupted isolates (Figure 2)
  - PmrB R256G substitution with other MgrB, PmrB, or PhoQ alterations occurred among 8, 1, and 1 isolates, respectively, and these isolates had colistin MIC values ≥4 mg/L
  - 26 isolates carried the PmrB R256G alteration alone, and the colistin MIC results of these isolates ranged from 0.5 to >8 mg/L
- One isolate displaying a colistin MIC value >8 carried amino acid substitutions in PhoP (V3F) and PhoQ (L96R) previously associated with colistin resistance
- 25/41 isolates values at 0.5 and 1 µg/mL had no resistance mechanisms and the remaining isolates carried alterations MgrB (D31N; 1 isolates), PhoP (S86L, 1 isolate) and 14 isolates harboring PmrB R256G alterations alone (Figure 2)
- Colistin resistance mechanisms were not identified in 15 isolates with colistin MIC values of 4 to >8 mg/L (Table 2)
  - 11 of these isolates had alterations on PmrA in the receiver domain that is potentially involved in colistin resistance
  - Further studies are being performed to identify potential colistin resistance mechanism in these isolates

Figure 1 Distribution of all *K. pneumoniae* isolates tested in 2016 and isolates screened

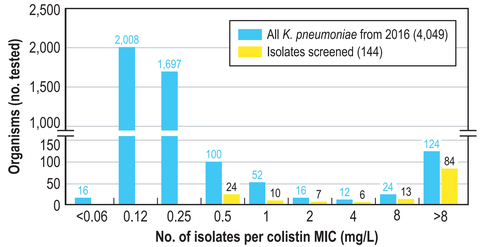
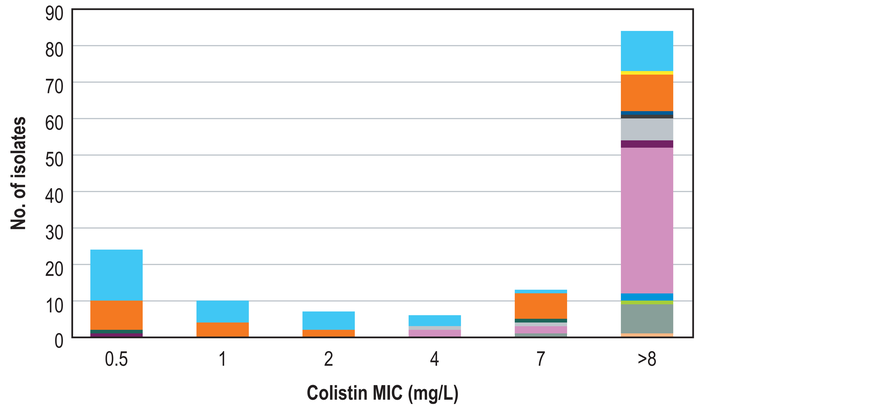


Figure 2 Distribution of colistin resistance mechanisms by colistin MIC value



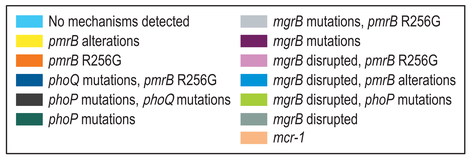


Table 1 Alterations associated with colistin resistance in isolates not harboring *mrgB* disruption alone or PmrB R256G alterations alone

		Amino acid alteration			
Colistin resistance mechanism profile	**Colistin MIC results (mg/L)**	**MgrB**	**PhoP**	**PhoQ**	**PmrB**
mgrB disrupted, *phoP* mutations	>8		V3F		
mgrB disrupted, *pmrB* alterations	>8				R256G, S208R
mgrB disrupted, *pmrB* alterations	>8				T157P, V287 to A289 deletion, R256G
mgrB mutations	>8	C28R			
mgrB mutations	0.5	D31N			
mgrB mutations	>8	C28R			
mgrB mutations, *pmrB* R256G	4	W20S			R256G
mgrB mutations, *pmrB* R256G	>8	C28Y			R256G
mgrB mutations, *pmrB* R256G	>8	C28R			R256G
mgrB mutations, *pmrB* R256G	>8	G37S			R256G
mgrB mutations, *pmrB* R256G	>8	W20S			R256G
mgrB mutations, *pmrB* R256G	>8	C28R			R256G
mgrB mutations, *pmrB* R256G	8	C28S			R256G
mgrB mutations, *pmrB* R256G	>8	W20R			R256G
phoP mutations	8		E195D,S86L		
phoP mutations	0.5		E195D,S86L		
phoP mutations, *phoQ* mutations	>8		V3F,E195D	L96R	
phoQ mutations, *pmrB* R256G	>8			L348Q	R256G
pmrB alterations	>8				T157P, R256G

Table 2 Amino acid substitutions observed in colistin-resistant isolates applying the EUCAST breakpoints without previously described colistin resistance mechanisms

	Amino acid alteration						
Colistin MIC results (mg/L)	**CrrA**	**CrrB**	**MgrB**	**PhoP**	**PhoQ**	**PmrA***	**PmrB**
>8	I219V	L295F,Q287K		E195D		L38R	A246T
>8				E195D	G385S		A246T
8				E195D		A41T	L213M
>8				E195D		A41T	L213M
>8				E195D		A41T	L213M
4				E195D		A41T	L213M
4				E195D		A41T	L213M
>8				E195D		A41T	M285R,L213M
>8				E195D			T112P
>8				Q33K,E195D		E57G	
>8			V1 deletion	E195D		E57G	
>8				E195D		E57G	
>8				E195D		T189P,E57G	
4				E195D	K46N		
>8				E195D,M126I			

* All PmrA alterations are located in the receiver domain

Conclusions

- Disruption of *mgrB* was the most common colistin resistance mechanism in isolates with colistin-resistant MIC values (EUCAST breakpoint; MIC, >2 mg/L)
  - A few did not carry known colistin resistance mechanisms
- PmrB R256G, whether alone or in combination with other mechanisms, was the most common alteration previously associated with colistin resistance; many isolates displaying MIC results as low as 0.5 mg/L also had this alteration
  - This finding could suggest that this alteration alone might not be responsible for elevated colistin MIC results
- Resistance mechanisms that have been associated with elevated colistin MIC values could not be identified in 15 isolates
- Development of colistin resistance adds to the continued challenges for treating multidrug-resistant isolates and it is important to understand these mechanisms and their epidemiology

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