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Characterization of Variant *mcr-1.11* and Prevalence of *mcr* Genes among *Escherichia coli* and *Klebsiella pneumoniae* Clinical Isolates Collected Worldwide

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Introduction

- The increase in the prevalence of infections caused by gram-negative pathogens that are multidrug resistant has prompted the reconsideration of polymyxins, colistin, and polymyxin B as valid therapeutic options
- Resistance to polymyxins is usually mutation driven and adds a physiologic burden on the cells harboring these mutations
  - In *Klebsiella pneumoniae* and several other species, alterations in the lipid A pathway are largely responsible for polymyxin resistance
- The description of a transferrable polymyxin resistance gene in 2015 caused great concern
  - *Enterobacteriaceae* isolates carrying *mcr* genes encoding phosphoethanolamine-lipid A transferase that codifies resistance to polymyxins have been reported globally among various species
  - Genes *mcr-1* through *mcr-7* and multiple subtypes have been reported to encode proteins that share 30%-70% amino acid identity
- Colistin resistant *Escherichia coli* and *K. pneumoniae* clinical isolates collected worldwide during 2016 as part of the SENTRY Antimicrobial Surveillance Program were screened for the presence of *mcr* and a new *mcr-1* variant was characterized

Materials and Methods

- *E. coli* and *K. pneumoniae* clinical isolates were collected during 2016 from medical centers worldwide according to defined protocols, and 1 isolate per patient was collected
  - Identification was confirmed by matrix assisted laser desorption ionization-time of flight mass spectrometry when necessary
- Isolates were susceptibility tested using reference broth microdilution methods per Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines (M07)
  - Interpretive criteria described by CLSI (M100, 2018) and European Committee on Antimicrobial Susceptibility Testing (EUCAST; 2018) were applied
- Isolates displaying colistin MIC ≥4 µg/mL (resistant per EUCAST criteria) were screened for *mcr-1* and *mcr-2* genes by PCR and sequencing techniques
- The novel variant and *mcr-1* were cloned in pJET1.2 vector (Thermo Fisher Scientific, Waltham, Massachusetts, USA), transformed in an *E. coli* TOP10 host, and susceptibility tested
- The *mcr-1.11* native plasmid and other *mcr-1*-carrying isolates from the same medical center were characterized using next-generation sequencing and analysis (NGS) on MiSeq (Illumina, San Diego, California, USA)
  - Sequences were *de novo* assembled and resistance determinants and plasmid incompatibility group encoding genes were searched using a curated library, applying criteria of >94% sequencing identity and 40% minimum length coverage
  - Plasmid was assembled using a combination of *de novo* and templated assembly
    - Plasmid sequence was analyzed and deposited in GenBank (Accession # KY853650)

Results

- Among 11,493 *E. coli* and *K. pneumoniae* isolates, a total of 199 (1.7%) were resistant to colistin per EUCAST criteria and 12 were positive for *mcr-1*
  - None of the isolates tested were *mcr-2* positive
- Isolates carrying *mcr-1* included 10 *E. coli* (United States [2], Venezuela [3], Peru [3], Colombia [1], Poland [1]) and 2 *K. pneumoniae* (1 each from Spain and Italy; Table 1)
- *mcr-1*-positive isolates were obtained from bloodstream (5), urinary tract (3), and skin and skin structure (3) infections, or patients hospitalized with pneumonia (1)
  - 8/12 isolates displayed colistin MIC values at 4 µg/mL and the remaining isolates had colistin MIC values ≥8 µg/mL
  - Seven isolates were resistant to cephalosporins, but were susceptible to carbapenems
    - 10/12 were resistant to tetracycline and trimethoprim-sulfamethoxazole
    - All isolates were susceptible to tigecycline
- One *E. coli* belonging to ST95 from Peru carried an *mcr-1* gene displaying an insertion encoding for a valine in amino acid position 6
- NGS revealed that this variant, designated *mcr-1.11*, was located on a 63 Kb IncI2 conjugative plasmid (p977565) carrying no other resistance genes (Figure 1)
  - Similar plasmid structures were also observed among 2 other genetically unrelated *E. coli* isolates (ST7954 and ST1485) that carried *mcr-1* from the same hospital
  - The *mcr-1.11*-carrying isolate also harbored *ant(3")-Ia, aph(6)-Ia, aph(6)-Id*, bla_{TEM-1}, *fosA*, *qnrB19*, *sul2*, *tetA,* and *dfrA1*
  - NGS analysis identified 4 other plasmids in this isolate belonging to incompatibility groups ColB512, IncFII, IncFIB, and IncQ1
- The *mcr-1.11*-carrying *E. coli* isolate was susceptible to β-lactams, aminoglycosides, tigecycline, and trimethoprim-sulfamethoxazole, but displayed resistance to tetracycline and quinolones (Table 1)
- The *mcr-1.11* cloned in an *E. coli* TOP10 background exhibited colistin and polymyxin B MIC results (2-4 µg/mL) similar to *mcr-1* (4 µg/mL; Table 2)

Table 1 Characteristics of *mcr-1*-producing isolates

				MIC (µg/mL):											
Organism	Country	MLST	Infection type	COL	CAZ	CTX	CPM	P-T	IMI	CIP	GEN	TOB	TET	TIG	T-S
Escherichia coli	Colombia	ST131	BSI	8 (R)	0.12 (S)	≤0.06 (S)	≤0.12 (S)	2 (S)	0.25 (S)	≤0.03 (S)	1 (S)	1 (S)	2 (S)	0.12 (S)	>4 (R)
	Peru[a]	ST95	BSI	4 (R)	0.12 (S)	≤0.06 (S)	≤0.12 (S)	1 (S)	≤0.12 (S)	>4 (R)	1 (S)	1 (S)	>16 (R)	0.12 (S)	1 (S)
	Peru	ST7954	SSSI	4 (R)	0.12 (S)	≤0.06 (S)	≤0.12 (S)	2 (S)	≤0.12 (S)	>4 (R)	>8 (R)	4 (S)	>16 (R)	0.25 (S)	>4 (R)
	Peru	ST1485	SSSI	4 (R)	>8 (R)	>8 (R)	>16 (R)	2 (S)	≤0.12 (S)	1 (S)	1 (S)	1 (S)	>16 (R)	0.12 (S)	>4 (R)
	Poland	ST410	UTI	4 (R)	8 (R)	>8 (R)	8 (R)	1 (S)	≤0.12 (S)	>4 (R)	1 (S)	0.5 (S)	>16 (R)	0.5 (S)	>4 (R)
	USA	ST58	BSI	4 (R)	0.25 (S)	>8 (R)	2 (S)	2 (S)	≤0.12 (S)	0.06 (S)	1 (S)	1 (S)	>16 (R)	0.25 (S)	>4 (R)
	USA	ST1148	UTI	4 (R)	>8 (R)	>8 (R)	2 (S)	2 (S)	≤0.12 (S)	>4 (R)	0.25 (S)	0.5 (S)	>16 (R)	0.12 (S)	>4 (R)
	Venezuela	ND	BSI	4 (R)	2 (S)	>8 (R)	4 (R)	1 (S)	≤0.12 (S)	>4 (R)	0.5 (S)	1 (S)	>16 (R)	0.25 (S)	>4 (R)
	Venezuela	ND	SSSI	4 (R)	1 (S)	>8 (R)	2 (S)	1 (S)	≤0.12 (S)	>4 (R)	0.25 (S)	0.5 (S)	>16 (R)	0.12 (S)	>4 (R)
	Venezuela	ND	PIHP	8 (R)	0.12 (S)	≤0.06 (S)	≤0.12 (S)	2 (S)	≤0.12 (S)	4 (R)	0.5 (S)	0.5 (S)	>16 (R)	0.25 (S)	>4 (R)
Klebsiella pneumoniae	Italy	ST219	UTI	>8 (R)	>8 (R)	>8 (R)	>16 (R)	4 (S)	0.25 (S)	1 (S)	0.5 (S)	1 (S)	>16 (R)	0.5 (S)	>4 (R)
	Spain	ND	BSI	>8 (R)	0.12 (S)	≤0.06 (S)	≤0.12 (S)	2 (S)	≤0.12 (S)	≤0.03 (S)	0.25 (S)	0.25 (S)	4 (S)	0.5 (S)	≤0.5 (S)

BSI, bloodstream infection; SSSI, skin and skin structure infection; UTI, urinary tract infection; PIHP, pneumonia in hospitalized patient; COL, colistin; CAZ, ceftazidime; CTX, ceftriaxone; CPM, cefepime; P-T, piperacillin-tazobactam; IMI, imipenem; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; TET, tetracycline; TIG, tigecycline; T-S, trimethoprim-sulfamethoxazole; ND, not determined; (R), resistant and (S), susceptible per CLSI/EUCAST criteria
[a] *E. coli* harboring *mcr-1.11*.

Table 2 Susceptibilities of transformants carrying *mcr-1* and *mcr-1.11* expressed in the same background

	MIC (µg/mL)	
Isolate	Colistin	Polymyxin B
E. coli TOP10 (pJET1.2-*mcr-1.11*)	4	2
E. coli TOP10 (pJET1.2-*mcr-1*)	4	4
E. coli TOP10 recipient strain	0.12	≤0.5

Conclusions

- Isolates carrying *mcr-1* were identified in only 0.1% of the isolates tested, mostly in *E. coli*
- The new variant, *mcr-1.11*, encoded similar activity against colistin when compared to *mcr-1*
  - The *mcr-1* variant likely emerged via spontaneous mutation within an endemic plasmid structure
- Although the prevalence of *mcr*-carrying isolates is low, the transferability of this colistin-resistance gene is worrisome, and this study emphasizes the diversity and widespread nature of this resistance determinant

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References

Castanheira M, Griffin MA, Deshpande LM, et al. (2016). Detection of *mcr-1* among *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates collected worldwide as part of the SENTRY Antimicrobial Surveillance Program during 2014–2015. *Antimicro Agents Chemother* 60: 5623-5624.

Clinical and Laboratory Standards Institute (2018). *M100Ed28E. Performance standards for antimicrobial susceptibility testing: 28th informational supplement.* Wayne, PA: CLSI.

Clinical and Laboratory Standards Institute (2018). *M07Ed11E. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—eleventh edition.* Wayne, PA: CLSI.

EUCAST (2018). Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, January 2018. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf. Accessed January 2018.

Wang R, van Dorp L, Shaw LP, et al. (2018). The global distribution and spread of the mobilized colistin resistance gene *mcr-1. Nat Commun* 9: 1179.

Figure 1 Schematic representation of IncI2 plasmids carrying *mcr-1*-like genes detected in *E. coli* isolates resistant to colistin isolated from a Peruvian medical center