mcr-1 in Multidrug-Resistant *bla*_{KPC-2}-Producing Clinical *Enterobacteriaceae* Isolates in Singapore

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Polymyxin usage is common in Singapore, where carbapenem resistance is prevalent (1). Not surprisingly, polymyxin resistance has been detected in our setting (2). Until recently, polymyxin resistance has been considered rare and largely due to chromosomal mutations. Here, we report the first detection of plasmid-mediated polymyxin resistance in *bla*_{KPC-2}-producing *Enterobacteriaceae* in Singapore.

Polymyxin B resistance, which was interpreted based on the Clinical and Laboratory Standards Institute's non-*Enterobacteriaceae* breakpoints (MICs ≥ 4 $\mu\text{g/ml}$), was detected in 63 of 321 (20%) clinical carbapenem-resistant *Enterobacteriaceae* isolates collected between 2008 and 2015 at our institution (2, 3). Using PCR and sequencing, we detected *mcr-1* in three of the 63 (5%) resistant isolates (4). These isolates were resistant to all tested antibiotics except amikacin, tigecycline, and levofloxacin (EC702 only). Multiple resistance genes, including *bla*_{KPC-2}, were detected in addition to *mcr-1* (Table 1).

Escherichia coli EC249 and EC362 were recovered from two local patients admitted to different wards in a public tertiary care hospital on the same day in 2013, while *Enterobacter aerogenes* EC702 was recovered from a local patient admitted in 2014 (Table 1). All three patients had previous hospital admissions and exposure to broad-spectrum antibiotics, with no recent travel history. None of the patients had prior receipt of polymyxin B. Only *E. coli* EC362 was associated with clinical infection. It is interesting that these patients were colonized/infected with other *Enterobacteriaceae* isolates before or after the isolation of the index *mcr-1*-positive isolates (Table 1). Interestingly, these isolates were all *bla*_{KPC-2} positive. *mcr-1* was absent in all other isolates from these patients, including those which were polymyxin B resistant.

The whole-genome sequences of the three isolates were analyzed (ENA sequence accession no. PRJEB14213). Comparison with complete *mcr-1* plasmid sequences in GenBank indicated that the *mcr-1*-harboring contig from EC249 shared similarity (99.9% identity, 87% query coverage) with plasmid pHNSHP45 (GenBank accession no. KP347127), which was first reported in China. The *mcr-1*-harboring contig from EC702 shared similarity (100% identity, 99% query coverage) with pmcr1-IncX4 (GenBank accession no. KU761327), which was also isolated from a clinical specimen from China (5). There was, however, limited similarity of the *mcr-1*-harboring contig from EC362 with known *mcr-1* plasmid sequences in GenBank. Analysis of the *mcr-1*-neighboring regions revealed that while the *mcr-1*-*pap2* element was present in all three contigs, the IS*ApI1* mobile element was found only in EC362. As suggested in several studies, IS*ApI1* may

not be always associated with *mcr-1* (4, 6). The *nikB* gene was present in EC249. *mcr-1* and *bla*_{KPC-2} were located on distinct unordered contigs for all three isolates. Further plasmid characterization, such as through long-read single-molecule real-time sequencing, is required to determine if *mcr-1* and *bla*_{KPC-2} are located on the same plasmids, in addition to determining the diversity of the *mcr-1*-harboring plasmid backbones of the isolates in this study.

Successful transfer of *mcr-1* was observed in the conjugation assays with *E. coli* J53 Az^r for all three isolates. The transconjugants were positive for *mcr-1* and *bla*_{KPC-2} (confirmed with PCR and sequencing). While the transconjugants had polymyxin B MICs similar to those for the donor isolates, the transconjugants' carbapenem MICs were mostly lower (2 to 8 $\mu\text{g/ml}$).

Comparative genomics suggests that genetic heterogeneity is present in the two *E. coli* strains isolated in the same period. The multilocus sequence type (MLST) profiles of the two *E. coli* isolates were similar—EC249 (ST2006) and EC362 (ST224) are single-locus variants, differing only in *fumC* (*fumC*-371 versus *fumC*-4) (7). However, variant calling by GATK revealed 3,963 single nucleotide polymorphisms (SNPs) between the two isolates, suggesting that they were not identical. The isolates in our study were of different sequence types than those detected previously.

In conclusion, we report the first cases of *mcr-1* in clinical isolates in Singapore. While polymyxin resistance in local carbapenem-resistant *Enterobacteriaceae* isolates is high, it appears that plasmid-mediated resistance is rare and sporadic. Cocarriage of highly transmissible resistance determinants such as *mcr-1* and various carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, and *bla*_{OXA-48}) in human infections is increasingly being reported globally in regions such as Europe, the Americas, China, and South Africa (5, 8–14). Close attention is warranted for these multidrug-resistant infections, for which there are few therapeutic options remaining.

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TABLE 1 Characteristics of patients with *mcr-1*-positive isolates^c

Characteristic	Patient no. (isolate)		
	1 (EC249)	2 (EC362)	3 (EC702)
Comorbidities	End-stage renal failure, hypertension, hyperlipidemia, ischemic heart disease, gout	Diabetes, hypertension, congestive heart failure, gastroesophageal reflux disease	Hypertension, chronic renal failure, atrial fibrillation, ischemic heart disease, Parkinson's disease
Reason for admission	Fall	Infected sacral sore	Recurrent urosepsis
Culture period	August 2013	August 2013	April 2014
Organism with <i>mcr-1</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. aerogenes</i>
Culture site	Surveillance rectal swab	Intraoperative sacral sore tissue	Urine
Concomitant organism(s)	Pb-sensitive <i>Klebsiella pneumoniae</i> (blood)	Pb-resistant <i>E. coli</i> / <i>Enterococcus faecium</i> (sacral sore), Pb-sensitive <i>Klebsiella pneumoniae</i> (urine), Pb-sensitive <i>Klebsiella pneumoniae</i> (same intraoperative culture as <i>mcr-1</i> isolate)	Pan-sensitive <i>Proteus</i> spp. (urine), <i>Klebsiella pneumoniae</i> (stool)
Outcome	Discharged against medical advice before completion of treatment for pneumonia with <i>Klebsiella pneumoniae</i> bacteremia	Died due to uncontrolled bleeding 2 days after the operation	Discharged after 2 weeks of antibiotic treatment; <i>mcr-1 E. aerogenes</i> was isolated shortly before discharge and was not treated
MLST	ST2006	ST224	No scheme available
Other resistance genes detected	<i>aadA1</i> , <i>aadA2</i> , <i>bla</i> _{TEM-1A} , <i>bla</i> _{KPC-2} , <i>mph</i> (A), <i>cmlA1</i> , <i>sul3</i> , <i>dfrA12</i>	<i>aadA17</i> , <i>aph</i> (3')-Ic, <i>bla</i> _{TEM-1A} , <i>bla</i> _{KPC-2} , <i>qnrS1</i> , <i>lnu</i> (F), <i>mph</i> (A), <i>floR</i> , <i>sul3</i> , <i>tet</i> (A), <i>dfrA12</i> , <i>dfrA14</i>	<i>strA</i> , <i>strB</i> , <i>aac</i> (6')Ib-cr, <i>aac</i> (3)-IIa, <i>bla</i> _{TEM-1B} , <i>bla</i> _{CTXM-15} , <i>bla</i> _{OXA-1} , <i>bla</i> _{KPC-2} , <i>qnrB66</i> , <i>mph</i> (A), <i>catB3</i> , <i>sul2</i> , <i>tet</i> (A), <i>dfrA14</i>
Plasmid scaffold types	ColpVC, IncFII, IncI2, IncN	IncFII, IncXI	IncFII, IncX4
MIC (μg/ml) of drug ^a			
Cefepime	≥256	≥256	≥256
Piperacillin-tazobactam	≥128	≥128	≥128
Aztreonam	≥64	≥64	≥64
Ertapenem	≥32	≥32	16
Imipenem	≥32	8	16
Meropenem	≥32	16	≥32
Doripenem	8	8	8
Levofloxacin	≥32	≥32	1
Amikacin	2	2	8
Trimethoprim-sulfamethoxazole	≥4/76	≥4/76	≥4/76
Rifampin ^b	≥64	32	≥64
Tigecycline	≤0.25	0.50	0.5
Fosfomycin ^b	12	≥2,048	8
Pb	4	4	8
Colistin	4	4	4

^a Determined using CLSI broth dilution method, unless otherwise stated.^b Determined using Etest.^c Abbreviations: Pb, polymyxin B; MLST, multilocus sequence type.

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We declare no competing interests.

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