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Fitness and Mechanisms Associated with Host-Dependent Effects of *mcr-10* Plasmids on Colistin Resistance

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Abstract

Background: Plasmid-mediated *mcr* genes have facilitated the global dissemination of colistin resistance. However, the extent to which the bacterial host background influences the resistance phenotype conferred by *mcr-10* plasmids remains poorly understood. This study investigated host-specific effects of *mcr-10* on colistin resistance.

Methods: Three *mcr-10*–carrying plasmids were obtained from clinical isolates of *Enterobacter roggenkampii* (SMC1207-143 and SMC1405-070) and *Enterobacter kobei* (CRE-F75). These plasmids were introduced into colistin-susceptible isolates, *E. roggenkampii* F-1664, *E. kobei* F-913, and *E. coli* DH5α to generate transformants. Antibiotic susceptibility was determined by broth microdilution method. The effects of *mcr-10* plasmid carriage on bacterial fitness were evaluated using growth curve analysis and competition assays. Membrane-associated changes were assessed by zeta potential measurement**.**

Results: Growth curve and competition assays revealed host-dependent differences in bacterial fitness despite the introduction of the same *mcr-10* plasmid. Reduced membrane surface charge was observed *in E. roggenkampii* and *E. kobei* transformants, whereas no significant change was detected in *E. coli* DH5α. This finding suggests host-specific membrane alterations associated with colistin resistance.

Conclusion: These results demonstrate that the phenotypic effects of *mcr-10* plasmids vary depending on the bacterial host background. The findings highlight the importance of host–plasmid interactions in plasmid-mediated colistin resistance.

Methods

Clinical isolates : Eight *mcr-10*-positive clinical isolates, including *E. roggenkampii* (n = 5), *E. kobei* (n = 2), and *E. asburiae* (n = 1), were used in this study. Representative plasmids were selected based on Inc type (IncFIB, IncFII/IncFIB, and IncFII) and introduced into colistin-susceptible recipient strains to generate transformants.

Antibiotic susceptibility testing : Colistin susceptibility was determined by broth microdilution according to CLSI guidelines.

Growth curve: Growth kinetics of recipient strains and their corresponding *mcr-10* plasmid-carrying transformants were monitored by measuring OD_{600} every 2 h for 12 h. Growth curves from biological triplicates were fitted to the Gompertz model, and fitted parameters were compared using the extra sum-of-squares F test.

Competition assay: Competitive fitness was assessed by co-culturing each *mcr-10* plasmid-carrying transformant with DH5α at an equal starting ratio. After 24 h, relative abundance was quantified by CFU counting and used to calculate the competition index.

Zeta potential : Mid-log-phase bacterial cells were harvested by centrifugation, washed twice with Milli-Q water, and resuspended to a standardized cell density before analysis. Zeta potential was measured using a Particle Size & Zeta Potential Analyzer (ELS-Z1000, Otsuka Electronics).

Results

Table 1. Colistin MICs of *mcr-10*–positive clinical isolates

Species	Strain	Plasmid Inc type	COL MIC (mg/L)
E. roggenkampii	SMC1207-143	IncFIB	>64
	SMC1405-070	IncFII/IncFIB	>64
	U1207-164	IncFII/IncFIB	>64
	487	IncFIB	16
	F-1989	IncFIB	>64
E. kobei	CRE-F75	IncFII	>64
	1006	IncFII	>64
E. asburiae	SMC1407-080	IncFII	>64

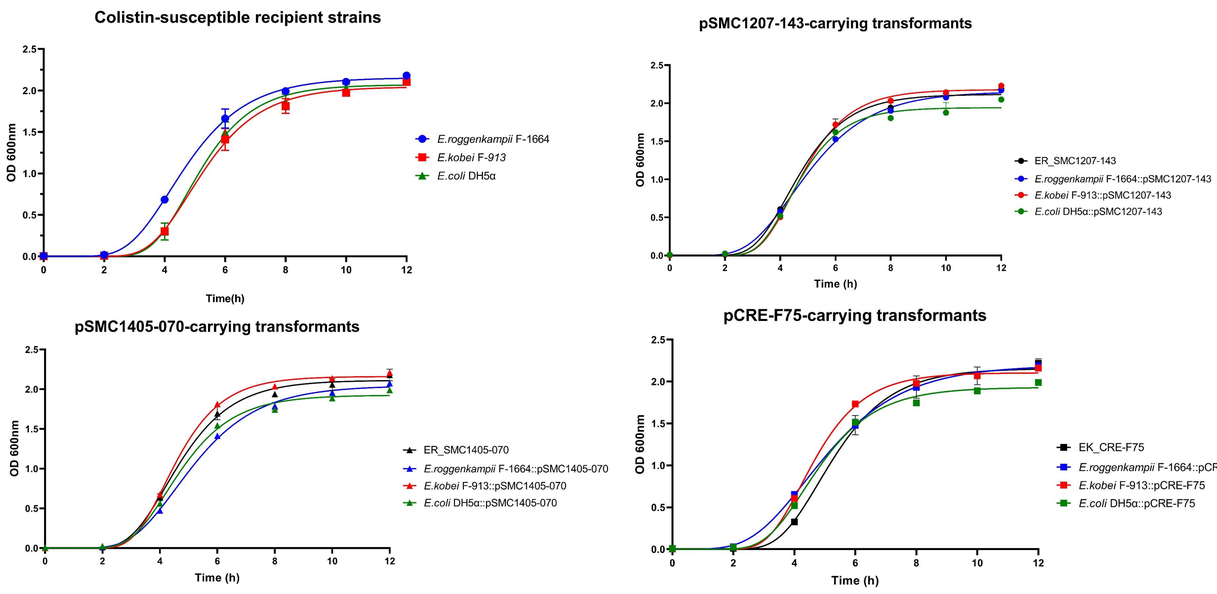
► Most *mcr-10*-positive clinical isolates showed high-level colistin resistance, with MICs of ≥64 mg/L regardless of plasmid Inc type.

Table 2. Colistin MICs of transformants carrying *mcr-10*–positive plasmids

strain	Plasmid	Plasmid Inc type	Col MIC (mg/L)
E. roggenkampii F-1664	WT	-	0.5
	pSMC1207-143	IncFIB	64
	pSMC1405-070	IncFII/IncFIB	>64
	pCRE-F75	IncFII	>64
E.kobei F-913	WT	-	0.5
	pSMC1207-143	IncFIB	>64
	pSMC1405-070	IncFII/IncFIB	64
	pCRE-F75	IncFII	64
E.coli DH5α	WT	-	0.25
	pSMC1207-143	IncFIB	4
	pSMC1405-070	IncFII/IncFIB	4
	pCRE-F75	IncFII	4

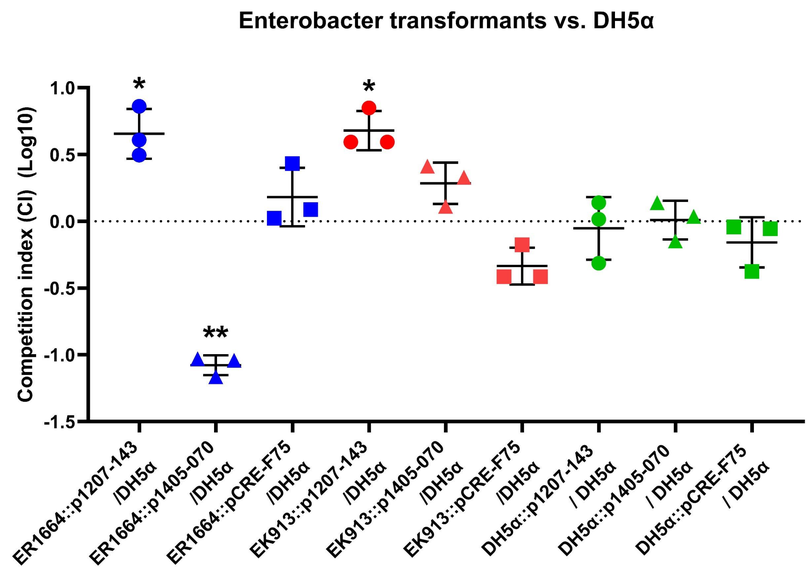
► *mcr-10* plasmid-mediated colistin resistance varied by host background, with higher MICs observed in *Enterobacter* transformants.

Figure 1. Growth kinetics of recipient strains and *mcr-10* plasmid-carrying transformants.



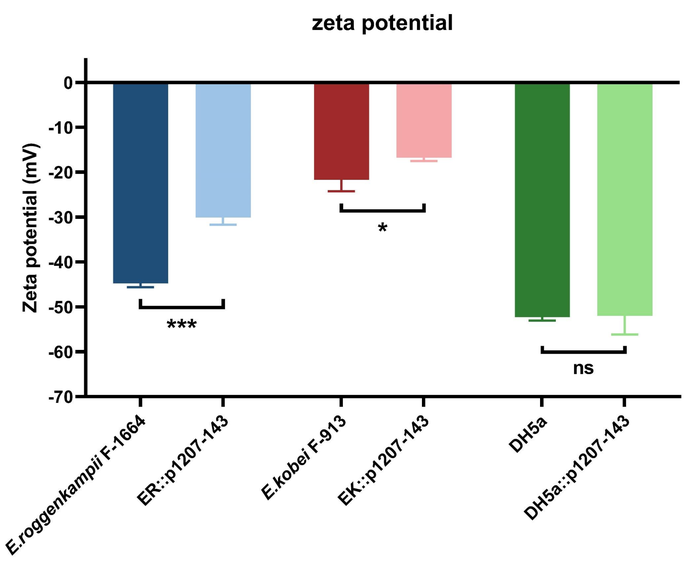
► Growth kinetics varied depending on both host background and plasmid type, indicating host-dependent fitness effects of *mcr-10*-positive plasmids.

Figure 2. Competition index of *mcr-10* plasmid-carrying *Enterobacter* transformants relative to DH5α.



► *Enterobacter* transformants showed plasmid-and host-dependent competitive fitness against DH5α, with pSMC1207-143 generally conferring a fitness advantage.

Figure 3. Zeta potential of recipient strains and pSMC1207-143-carrying transformants.



► pSMC1207-143 altered membrane surface charge in *Enterobacter* hosts, but not in *E. coli* DH5α.

Conclusion

Clinical isolates harboring *mcr-10* exhibited elevated colistin MICs across diverse plasmid backgrounds. Transfer of the same *mcr-10* plasmids resulted in host-dependent differences in colistin MICs. Growth and competition assays showed that plasmid-associated fitness effects varied by host–plasmid combination. pSMC1207-143 altered membrane surface charge in *Enterobacter* hosts, but not in *E. coli* DH5α. These findings suggest that host background plays a key role in shaping *mcr-10*-mediated colistin resistance and its associated fitness effects.