The Biological Costs and Plasmid Sequence of Two *Klebsiella pneumoniae* bla_{KPC-2}- and bla_{VIM-1}-Carrying Plasmids in Different *Escherichia coli* Clinical Isolates

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/ INTRODUCTION

Resistance to carbapenems has increased globally in the past decades, representing a major concern to human health (1). In Enterobacteriaceae, the recurring association between carbapenemase genes and mobile genetic elements (e.g. plasmids) facilitates the efficient inter- and intra-species dissemination of these resistance determinants (2). However, the carriage of plasmids impose a fitness cost to the bacterial cell, which is a key factor that determines if a newly horizontally acquired plasmid will be stably maintained in a bacterial population over time (3).

/ OBJECTIVES

To determine the sequence and investigate the biological cost of two carbapenemase-encoding plasmids containing bla_{KPC-2} (pG12-KPC-2) and bla_{VIM-1} (pG06-VIM-1) isolated from *Klebsiella pneumoniae* when newly acquired by uropathogenic *Escherichia coli* clinical isolates of different genetic backgrounds.

/ MATERIALS AND METHODS

Two *K. pneumoniae* plasmids encoding bla_{KPC-2} and bla_{VIM-1} were transferred by electroporation (4) into *E. coli* clinical strains of different phylogroups and MLST sequence types. Transformants were characterized phenotypically and genotypically. Fitness costs of the plasmids in *E. coli* were estimated in head-to-head competition experiments (5). Plasmid sequences were determined using NGS technology. Assembly, gap closure, annotation and comparative analyses were performed.

/ REFERENCES

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/ RESULTS

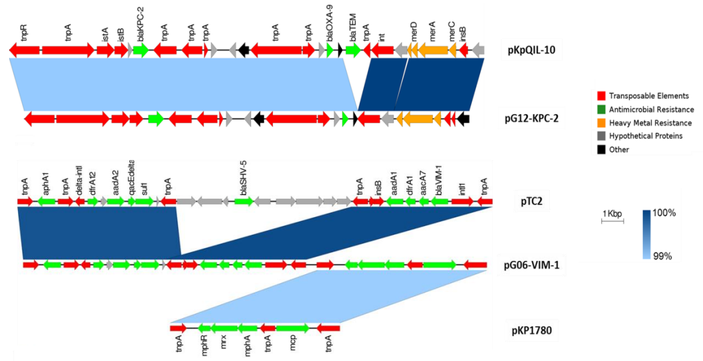
// Strains and susceptibility profiles

pG12-KPC-2 was successfully transformed into two different genetic backgrounds. pG06-VIM-1 was transformed into three different genetic backgrounds of which one background (ST69) was the same as for one bla_{KPC-2} transformant.

					Antimicrobial susceptibility profiles (MIC, mg/L)											
Isolate	**Species**	**ST**	**Phylogroup**	**Comment**	**Antibiotic**											
					AMP	**AMX**	**TZP**	**CAZ**	**ATM**	**MEM**	**IPM**	**ETP**	**GEN**	**TOB**	**AMK**	**CIP**
K47-25	*K. pneumoniae*	ST258	-	Host strain of pG12-KPC-2	≥256	128	≥256	≥256	≥256	8	4	16	2	32	64	≥32
AO-15200	*K. pneumoniae*	ST147	-	Host strain of pG06-VIM-1	≥256	≥256	≥256	≥256	256	32	32	8	2	16	16	≥32
K56-43	*E. coli*	ST537	B2	Recipient strain for transformation	2	2	1	0.25	0.064	0.016	0.25	0.004	0.5	1	4	0.008
K56-65	*E. coli*	ST10	A	Recipient strain for transformation	2	2	1	0.125	0.032	0.016	0.25	0.008	0.25	0.5	2	0.016
K56-68	*E. coli*	ST95	B2	Recipient strain for transformation	4	4	2	0.5	0.125	0.032	0.25	0.008	0.5	1	2	0.032
K56-75	*E. coli*	ST69	D	Recipient strain for transformation	4	4	2	0.25	0.064	0.032	0.25	0.008	0.5	1	2	0.016
G1-13	*E. coli* K56-65	ST10	A	pG12-KPC-2 transformant	≥256	32	32	2	8	0.25	0.5	0.5	0.25	0.5	1	0.016
G1-14	*E. coli* K56-75	ST69	D	pG12-KPC-2 transformant	≥256	32	128	4	16	0.5	0.5	0.5	0.5	1	2	0.016
G1-15	*E. coli* K56-43	ST537	B2	pG06-VIM-1 transformant	≥256	16	16	≥256	0.064	0.25	0.5	0.25	1	4	8	0.008
G1-16	*E. coli* K56-68	ST95	B2	pG06-VIM-1 transformant	≥256	32	256	≥256	0.125	0.5	1	0.25	1	8	8	0.016
G1-17	*E. coli* K56-75	ST69	D	pG06-VIM-1 transformant	≥256	32	128	128	0.064	0.25	1	0.25	1	8	8	0.016

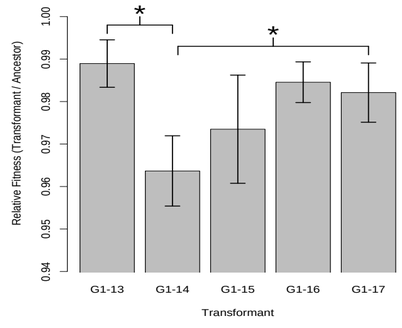
// Plasmid analysis

- pG12-KPC-2 was a multireplicon plasmid (IncFI and IncFII) with a backbone similarly organized to pKpQIL-10 (6). The resistance region included bla_{KPC-2} as part of Tn*4401a*.
- pG06-VIM-1 belonged to the IncR group. The scaffold showed high similarity scores with pKP1780 while the bla_{VIM-1} region was part of a mosaic structure highly similar to that of plasmid pTC2 from *Providencia stuartii* (7).



// Cost of plasmid carriage

- Both plasmids were stably maintained in *E. coli* and resulted in low to moderate reductions in host fitness (1.1 to 3.6%).
- For pG12-KPC-2, a difference in fitness cost was observed between two different genetic backgrounds whereas pG06-VIM-1 displayed no fitness difference between three different genetic backgrounds.
- A difference was observed between pG12-KPC-2 and pG06-VIM-1 in the same genetic background.



/ CONCLUSION

In a non-selective environment, plasmid carriage caused low to moderate fitness reduction in different plasmid-host combinations. This cost was dependent on the genetic background of the host and/or the specific plasmid. Both bla_{KPC-2-} and bla_{VIM-1-}encoding plasmids combine a diverse host range, maintenance capacities, plasticity of the MDR region and a wide variety of resistance genes, properties that may contribute to the acquisition and the spread of resistance determinants.