

Class 1 DISARM system prevents *Klebsiella pneumoniae* sequencing type 23 from conjugative carbapenemase-encoding plasmids

Canonical hypervirulent sequencing type 23 (ST23) *Klebsiella pneumoniae* causes severe infections, while it generally maintains susceptibility to a broad spectrum of antimicrobials, yet the molecular basis of this phenotypic characteristic remains poorly understood. The purpose of this study was to investigate whether the presence of the DISARM system defends antimicrobial-resistant plasmids invasion in *K. pneumoniae*. Bioinformatic analysis showed that the DISARM system was selectively enriched in ST23 *K. pneumoniae*. PacBio single-molecular real-time sequencing demonstrated that methylase of Class 1 DISARM in ST23 *K. pneumoniae* modified host 5'-GRACRAC-3' motifs which were distributed in all conjugative plasmids in *K. pneumoniae*. The transformation efficiency of plasmid pCOLADuet-MTmotif with the methylated cognate site was reduced 2.9-fold compared with the plasmid pCOLADuet-1 in a ST23 DISARM-positive strain KP2613. The conjugation efficiencies of carbapenemase-encoding plasmids (carrying *bla*_{KPC-2}, *bla*_{NDM-1} and *bla*_{NDM-5}, respectively) between DISARM-positive strains (KP2613 and *Escherichia coli* BL21-DISARM (+)) and DISARM-negative strains (KP2613ΔDISARM and BL21-DISARM (-)) were compared and found that Class 1 DISARM could effectively hinder the antimicrobial resistance plasmids invasion with conjugation efficiency reduced by ranging from 12- to 117-fold in KP2613 and 4.5- to 15.7-fold in BL21. Systematic deletion of individual DISARM genes (*drmA*, *drmB*, *drmC*, *drmD*, and *drmM*) revealed that disruption of any single gene did not fully abolish the DISARM-mediated defense or cause substantial growth defects. Overall, our study demonstrated that Class 1 DISARM provides robust protection against antimicrobial-resistant plasmids, which may be a potential factor underlying the high antimicrobial susceptibility observed in ST23 hypervirulent *K. pneumoniae*.

