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In-vivo development of tigecycline resistance in Klebsiella pneumoniae due to deletion of ribosomal binding site of ramR

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Background: Tigecycline resistance among *Klebsiella pneumoniae* isolates is emerging, but knowledge on the mechanisms underlying *in vivo* development of tigecycline resistance is limited. Here, we report a new mechanism of tigecycline resistance in *K. pneumoniae* evolved during tigecycline therapy.

Material/methods: *K. pneumoniae* isolates were consecutively collected before and throughout of 48 days of tigecycline treatment course from urine samples of a patient with scrotal abscess and urinary tract infection. Minimum inhibitory concentration of tigecycline was determined by broth microdilution. RT-qPCR was used to measure the transcriptional levels of *ramA*, *acrB*, *ramR* and *kpgB*. Immunoblotting was performed to determine RamR protein level in *K. pneumoniae*. The *xylE* reporter system was used to determine the effect of mutations on gene translation.

Results: Two tigecycline-resistant *K. pneumoniae* strains (KP-3R and KP-4R, MIC=8 μg/ml) were isolated after 41 and 47 days of tigecycline therapy. These isolates had the same sequence type (ST11) and PFGE patterns with the tigecycline-susceptible strains (KP-1S and KP-2S, MIC=2 μg/ml) that were isolated initially from the patient. Compared to KP-1S and KP-2S, KP-3R and KP-4R exhibited higher expression level of the efflux pump gene *acrB*. Sequence comparative analysis of its repressor gene *ramR* revealed that KP-3R and KP-4R harbored a 12-bp deletion upstream of *ramR*,

including the loss of the ribosomal binding site (RBS) TGAGG. Quantitative real-time PCR and immunoblotting analyses showed that KP-3R and KP-4R had normal level of *ramR* mRNA, but had defect in RamR protein production. Further *xylE* reporter gene assay further supported that the 12-bp deletion upstream of *ramR* abolished *ramR* translation. Complementing KP-3R and KP-4R with a functional *ramR* gene suppressed the *acrAB* efflux pump, and subsequently increased tigecycline susceptibility.

Conclusions: This is the first report identifies deletion of RBS of *ramR* as the mechanism contributing to rapid emergence of tigecycline resistance in *K. pneumoniae* during tigecycline therapy.