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

Meiping Ye*, Baixing Ding, Hongliang Qian, Minggui Wang

1Fudan University, Huashan Hospital; Institute of Antibiotics
2Institute of Antibiotics; Huashan Hospital, Fudan University
3Shanghai Jiaotong University
4Institute of Antibiotics; Huashan Hospital, Fudan University

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.