Rapid reshuffling of Tn4401 transposon variants and plasmids carrying blaKPC in Klebsiella pneumoniae ST196 within a single hospital outbreak

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Background: Klebsiella pneumoniae carbapenemase (KPC)-producing Enterobacteriaceae (KPCE) are a major clinical threat. blaKPC is usually contained within the 10kb transposon Tn4401, for which several different structural/sequence variants have been described. Plasmid transfer and Tn4401 transposition are important mediators of blaKPC dissemination amongst various host strains/plasmids, with these processes occurring frequently within hospital outbreaks. Variation within Tn4401 could provide insight into blaKPC transmission pathways, but relies on the assumption that variants are relatively stable within specific host strains/plasmids.

Material/methods: All ST196 KPC-K. pneumoniae (KPC-Kp) isolates, plus a single KPC-Serratia marcescens (KPC-Sm) isolate from a shared patient, were selected from a larger collection of Illumina-sequenced patient/environmental KPCE from a North American tertiary care hospital (2007-2016). Phylogenies were generated using PhyML. Variation within Tn4401 was determined using BLASTn comparisons with de novo assemblies and mapping of Illumina reads to a Tn4401b-1 reference. Tn4401 flanking sequences were determined by extracting overhanging sequences from the mapped reads. PacBio sequencing was performed for selected isolates (n=4) to confirm blaKPC plasmid structures.
Results: There were 19 KPC-Kp ST196 isolates, from four patients and six environmental locations. Six isolates came from patient 2 over 10 months, and five were sink drain/P-trap isolates from a single room (room C) patient 2 stayed in. There were six Tn4401 variants: Tn4401b-1 (reference Tn4401 sequence for this study; n=3 isolates), Tn4401b-2 (C8015T relative to Tn4401b-1, changing blaKPC-2 to blaKPC-3; n=14), Tn4401b-8 (T9663C; n=3), Tn4401b-9 (C8015T, T9663C; n=1), Tn4401b-8_trunc (T9663C, Δ1-1465; n=1), and Tn4401b-2_del (C8015T, Δ7068-7153 [novel 86bp deletion upstream of blaKPC]; n=1), with 4/19 (21%) isolates harbouring multiple Tn4401 variants (Figure 1). There were eight and seven distinct 5bp target site sequences (TSSs) flanking the left and right inverted repeat regions of Tn4401, respectively, and 6/19 (32%) isolates had multiple TSSs associated with the same Tn4401 variant, suggesting multiple Tn4401 transposition events within KPC-Kp ST196. PacBio sequencing of one KPC-Kp and one KPC-Sm isolate from patient 2 revealed a single blaKPC plasmid in each (pKPC_Kp carrying Tn4401b-2 and pKPC_Sm carrying Tn4401b-8 respectively). Two PacBio-sequenced KPC-Kp isolates from room C each contained both pKPC_Kp and pKPC_Sm, suggesting blaKPC plasmid transfer from KPC-Sm into an already KPC-producing Kp strain. In one of these, pKPC_Kp harboured Tn4401b-1, demonstrating recombination/mutation-mediated Tn4401 variant switching, and pKPC_Sm contained a deletion truncating Tn4401. Additional recombination-mediated Tn4401 variant/plasmid switching was evidenced by alternate Tn4401 variant/TSS pairings amongst Illumina-sequenced isolates.

Conclusions: We demonstrate high variation in Tn4401 within individual host strains/plasmids, mediated by multiple processes including: repeated blaKPC acquisitions by a single strain, recombination between blaKPC plasmids carrying different Tn4401 variants in the same strain, and deletions involving Tn4401. This highlights the dynamic nature of blaKPC/Tn4401 and has important implications for mobile element-based epidemiological resistance tracking.