27th ECCMID Vienna, Austria 22 – 25 April 2017

The congress of ESCMID

Session: OS055 The complex epidemiology of carbapenemases

Category: 3b. Resistance surveillance & epidemiology: Gram-negatives

23 April 2017, 10:24 - 10:34
OS0296

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

Anna Sheppard*[1], Nicole Stoesser[1], David Eyre[2], Robert Sebra[3], Kasi Vegesana[4], Ian German Mesner[4], John Ainsworth[4], Tim Peto[1], Ann Sarah Walker[5], Derrick Crook[1], Amy Mathers[4]

[1]*University of Oxford; Nuffield Department of Clinical Medicine*

[2]*University of Oxford; Nuffield Department of Medicine*

[3]*Institute for Genomics and Multiscale Biology; Icahn School of Medicine at Mount Sinai*

[4]*University of Virginia*

[5]*Mrc Clinical Trials Unit at Ucl*

Background: *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacterieaceae (KPCE) are a major clinical threat. *bla*$_{KPC}$ is usually contained within the 10kb transposon Tn*4401*, for which several different structural/sequence variants have been described. Plasmid transfer and Tn*4401* transposition are important mediators of *bla*$_{KPC}$ dissemination amongst various host strains/plasmids, with these processes occurring frequently within hospital outbreaks. Variation within Tn*4401* could provide insight into *bla*$_{KPC}$ 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 Tn*4401* was determined using BLASTn comparisons with *de novo* assemblies and mapping of Illumina reads to a Tn*4401*b-1 reference. Tn*4401* flanking sequences were determined by extracting overhanging sequences from the mapped reads. PacBio sequencing was performed for selected isolates (n=4) to confirm *bla*$_{KPC}$ 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 Tn*4401* variants: Tn*4401*b-1 (reference Tn*4401* sequence for this study; n=3 isolates), Tn*4401*b-2 (C8015T relative to Tn*4401*b-1, changing bla_{KPC-2} to bla_{KPC-3}; n=14), Tn*4401*b-8 (T9663C; n=3), Tn*4401*b-9 (C8015T, T9663C; n=1), Tn*4401*b-8_trunc (T9663C, Δ1-1465; n=1), and Tn*4401*b-2_del (C8015T, Δ7068-7153 [novel 86bp deletion upstream of bla_{KPC}]; n=1), with 4/19 (21%) isolates harbouring multiple Tn*4401* variants (Figure 1). There were eight and seven distinct 5bp target site sequences (TSSs) flanking the left and right inverted repeat regions of Tn*4401*, respectively, and 6/19 (32%) isolates had multiple TSSs associated with the same Tn*4401* variant, suggesting multiple Tn*4401* transposition events within KPC-Kp ST196. PacBio sequencing of one KPC-Kp and one KPC-Sm isolate from patient 2 revealed a single bla_{KPC} plasmid in each (pKPC_Kp carrying Tn*4401*b-2 and pKPC_Sm carrying Tn*4401*b-8 respectively). Two PacBio-sequenced KPC-Kp isolates from room C each contained both pKPC_Kp and pKPC_Sm, suggesting bla_{KPC} plasmid transfer from KPC-Sm into an already KPC-producing Kp strain. In one of these, pKPC_Kp harboured Tn*4401*b-1, demonstrating recombination/mutation-mediated Tn*4401* variant switching, and pKPC_Sm contained a deletion truncating Tn*4401*. Additional recombination-mediated Tn*4401* variant/plasmid switching was evidenced by alternate Tn*4401* variant/TSS pairings amongst Illumina-sequenced isolates.

Conclusions: We demonstrate high variation in Tn*4401* within individual host strains/plasmids, mediated by multiple processes including: repeated bla_{KPC} acquisitions by a single strain, recombination between bla_{KPC} plasmids carrying different Tn*4401* variants in the same strain, and deletions involving Tn*4401*. This highlights the dynamic nature of bla_{KPC}/Tn*4401* and has important implications for mobile element-based epidemiological resistance tracking.

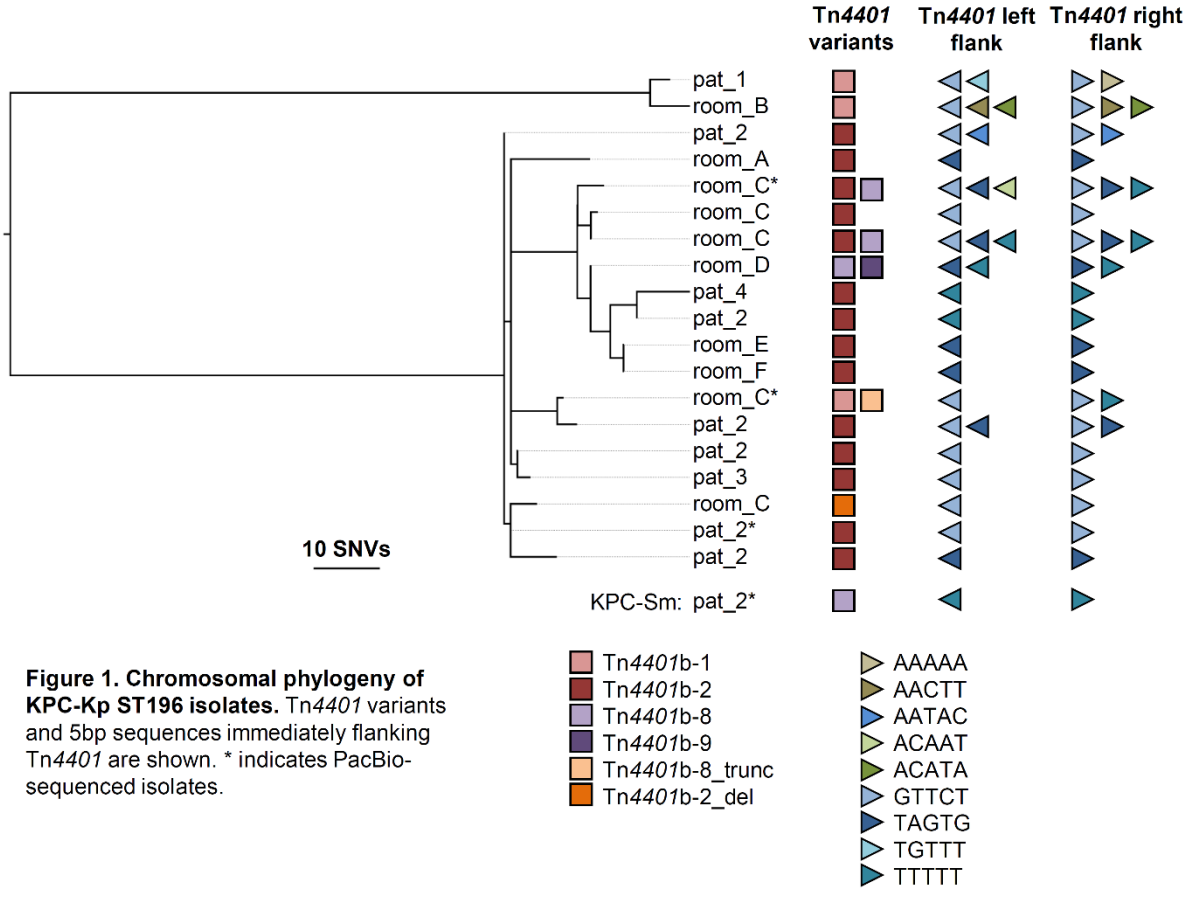

Figure 1. Chromosomal phylogeny of KPC-Kp ST196 isolates. Tn*4401* variants and 5bp sequences immediately flanking Tn*4401* are shown. * indicates PacBio-sequenced isolates.