Genetic context of *bla*$_{NDM-1}$ isolated from *Acinetobacter baumannii* and *Klebsiella pneumoniae* from India, Belgium and Iraq

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Introduction

Widespread dissemination of carbapenem resistance due to rapidly spreading resistance determinants such as *bla*$_{NDM}$ (New Delhi metallo-β-lactamase) is now a global crisis. *bla*$_{NDM}$ is harbored on various multi-drug resistant plasmids that transfer easily within and between Gram-negative bacterial species[1]. Here, we investigated the genetic context of the *bla*$_{NDM-1}$ gene present in *A. baumannii* and *K. pneumoniae* isolated from hospitalized patients in Kurdistan (Iraq), India and Belgium.

Methods

Two strains each of *K. pneumoniae* and *A. baumannii* exhibiting phenotypic carbapenem resistance were isolated from hospitalized patients in Kurdistan (KD), Belgium (BE) and India (IN). The strains (Kpn-169-KD, Ab-Nab5-IN, Ab-75-8-1-KD and Kpn-10197-BE) were screened for carbapenemase genes by PCR and sequencing. Whole genome sequencing (WGS) of strains was performed via 2×150b paired end sequencing (Nextera XT sample) preparation kit and Miseq, Illumina. The sequences of strains were independently assembled using SPAdes v3.6.1 (denovo assembly). *De novo* contigs were screened for plasmid origin by using Plasmid Finder (https://cge.cbs.dtu.dk//services/PlasmidFinder/). The identified plasmid specific contigs were used as reference template for reference mapping using CLC Genomics Workbench v7.5.1 (CLCbio, Denmark). Mapped reads were extracted and *de novo* assembly was performed, then contigs were annotated using online server BASys to understand the genetic context. The IS elements were identified using https://www-is.biotoul.fr/. Prophages were identified using http://phast.wishartlab.com/

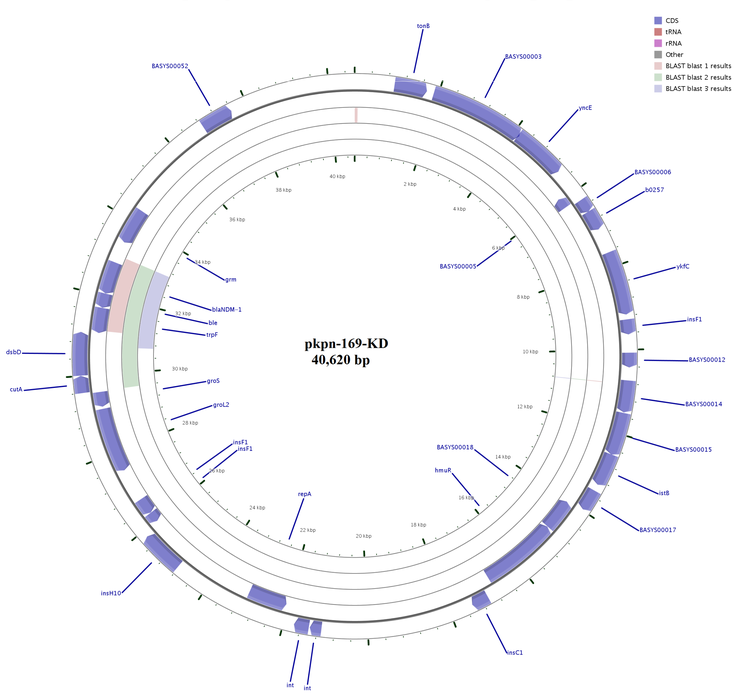

Figure 1. Comparative analysis of NDM strains: Outer ring with annotation shows plasmid pkpn-169-KD that was used as reference. BLAST comparison were done for NDM carrying genetic element (transposon) strains as follows blast1 (pAb-75-8-1-KD), blast2 (pAb-Nab5-IN) and blast3 (pkpn-10197-BE) shown as subsequent inner rings and similar regions were marked in different colours as mentioned in the legend. Image was generated using http://stothard.afns.ualberta.ca/cgview_server/

Results

Bla$_{NDM-1}$ was harbored in *K. pneumoniae* on IncF1B (pkpn-10197-BE) and IncFII (pkpn-169-KD) (Figure 1) plasmids while the same for *A. baumannii* could not be defined indicating that the gene might be chromosomally integrated. *Bla*$_{NDM-1}$ harboring elements isolated from the *A.baumannii* strains from Iraq (pAb-75-8-1-KD) and India (pAb-Nab5-IN) were highly similar and in both strains, the gene was harbored along with ISAba125 (Figure 2a and 2b). However, in contrast to pAb-Nab5-IN, the ISAba125 in pAb-75-8-1-KD was truncated by IS15 and also co- harbored other resistance genes such as the cephalosporinase, *bla*$_{DHA-1}$ that showed a 73 amino-acid long C-terminal extension. The *bla*$_{NDM-1}$ plasmids in *K. pneumonaie* from Belgium (pkpn-10197-BE) and Iraq (pkpn-169-KD) (Figure 1) also differed in the genes that were co-carried. Of note, the pkpn-169-KD harbored an intact shiga-toxin (Stx2)-encoding bacteriophage, the *rmtC* 16S rRNA methyltransferase as well as the *senB* gene and the *cjr* operon (Figure 2d). The shiga-toxin genes are a major virulence attribute of enterohemorrhagic (EHEC) *Escherichia coli*, while the *senB*-encoded enterotoxin and the *cjr* operon have been shown to be crucial during early pathogenesis and invasion of the urinary tract by uropathogenic (UPEC) *E. coli*.

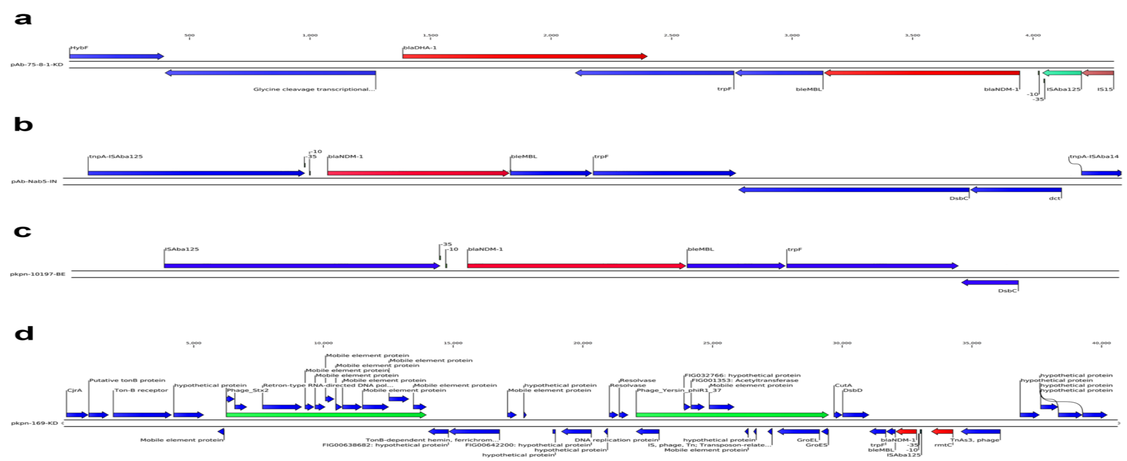

Figure 2: Genetic organization *bla*$_{NDM-1}$ gene in different strains.

Conclusions

We demonstrate here remarkable differences in the genes co-harbored along with *bla*$_{NDM-1}$ in *A. baumannii* and *K. pneumoniae* isolated from different geographic locations. Presence of major virulence factors of EHEC and UPEC *E.coli* on the *bla*$_{NDM-1}$ plasmid isolated from *K. pneumoniae* in Iraq might imply an enhanced virulence capacity of these multi-drug resistant strains and is of special concern.

References

1.Mathers AJ, et al. The Role of Epidemic Resistance Plasmids and International High-Risk Clones in the Spread of Multidrug-Resistant Enterobacteriaceae. *Clinical Microbiology Reviews* 2015; **28**: 565-91.

Funding

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