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Paper Poster Session

Deciphering carbapenem resistance

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

Xavier Basil Britto*¹, Hadi Ameen², Jasmine Coppens¹, Lavanya Vanjari³, Runak Hawrame⁴, Azad O Maged⁵, Youri Glupczynski⁶, Christine Lammens¹, Lakshmi Vemu³, Herman Goossens¹, Surbhi Malhotra-Kumar⁷

¹University of Antwerp, Laboratory of Medical Microbiology, Wilrijk, Belgium

²University of Antwerp, Wilrijk, Belgium

³Nizam's Institute of Medical Sciences, Department of Microbiology, Panjagutta, Hyderabad, Telangana, India

⁴Sulaymanyha Teaching Hospital,, Laboratory of Medical Microbiology, Sulaymanyha, Iraq

⁵Raparin Paediatric Teaching Hospital, Erbil, Iraq

⁶Chu Ucl Namur (Université Catholique de Louvain), Site Godinne, Laboratory of Microbiology, Yvoir, Belgium

⁷University of Antwerp, Laboratory of Medical Microbiology, Wilrijk, Antwerpen, Belgium

Background: 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. 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.

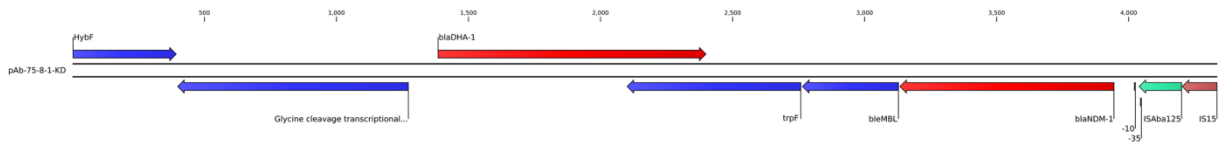
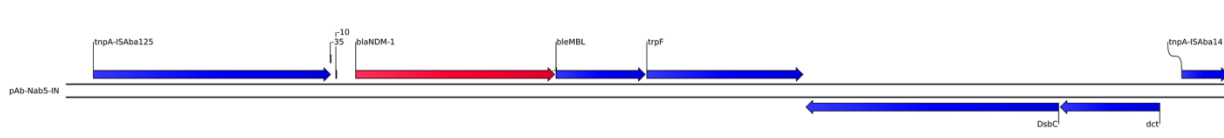
Material/methods: Two strains each of *K. pneumoniae* and *A. baumannii* exhibiting phenotypic carbapenem resistance were isolated from hospitalized patients in Kurdistan (KD), Belgium (BE) and India (IN). The strains (Kpn-169-KD, Ab-Nab5-IN, Ab-75-8-1-KD and Kpn-10197-BE) were screened for carbapenemase 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.

Results: Bla_{NDM-1} was harbored in *K. pneumoniae* on IncF1B (pkpn-10197-BE) and IncFII (pkpn-169-KD) 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 ISAb125 (Figure a and b). However, in contrast to pAb-Nab5-IN, the ISAb125

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. pneumoniae* from Belgium (pkpn-10197-BE) and Iraq (pkpn-169-KD) 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 d). 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*.

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. However 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.

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