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Hi-C metagenomic study of antimicrobial resistance genes spread in critically ill patients

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Motivation and Aim: Antimicrobial resistance (AMR) is one of the main reasons for the increased mortality of patients in medical institutions. Human microbiome may play a key role in the accumulation and propagation of antibiotic resistance [1]. Therefore, studying mechanisms of AMR genes transmission within bacterial communities will open up new opportunities for improving methods of controlling and limiting their spread in clinical settings. Hi-C metagenomics appears promising in this context, combining information about the genetic and taxonomic composition of a microbiome with the spatial arrangement of genomic fragments [2].

Methods and Algorithms: A total of 12 Hi-C metagenomes of stool samples from patients treated in the ICU were analyzed. In addition, the complete genomes of microorganisms inhabiting sputum were isolated and sequenced (WGS) from 5 of these patients. Metagenome-assembled genomes (MAGs) were reconstructed from the metagenomes. Using information about the strength of Hi-C contacts, we established connections between plasmids and their bacterial hosts. We also conducted a comparative analysis of complete genomes and plasmids found in the lungs and intestines to investigate the evidence of their possible transmission. AMR genes bound to these contigs were also studied.

Results: Combining information on plasmid-to-MAG linkage in gut metagenome for all patients, we built an interaction network of the bacterial genera through plasmids. Final graph included 28 genera from 7 different phyla, showing signs of intra-phylum clusterization. *Firmicutes* and *Proteobacteria* had the highest rate of intra-phylum plasmid transfer, most prevalent genera being opportunistic *Klebsiella*, *Enterococcus*, *Clostridia* and *Streptococcus*, as well as commensal *Limosilactobacillus* and *Lactobacillus*. Also, our data suggest that a significant proportion of plasmids could

potentially cross phylum boundaries. The *Proteobacteria-to-Firmicutes* appeared to be most common among these interactions.

Across the analyzed plasmids, we found a total of 54 unique antibiotic resistance genes belonging to 34 AMR gene families and 21 drug classes. Sixteen genera from 4 phylums carried AMR genes, among them, *Proteobacteria* (composed of *Klebsiella* and *Escherichia*) carried their greatest diversity (37 out of 54). Small fraction of genes were also distributed among the genera related to different phyla.

About 60 of the plasmids were detected in gut metagenome and lung isolates sequences of the same subject, which may indicate their translocation or co-occupation of various niches of the human organism. Interestingly, just one of those events was accompanied by co-colonization with the same bacterial strain of two organs. For a number of other cases, we did not observe species or even genus overlap between the bacterial compositions of two patient's samples. In addition, genes with resistance to beta-lactams, quinolones and sulfonamides antibiotics were associated with intestine-lung intersecting plasmids. Intriguingly, we found same plasmids carrying OXA-1 and CTX-M-15 genes associated with both genomes of *Klebsiella pneumoniae* detected in feces and sputum alongside with *Proteus mirabilis* from lung sample, presenting to us a possible picture of translocation pathways of discussed resistance genes.

Conclusion: Hi-C metagenomics is proving to be a perspective tool in studying the mechanism of plasmid transfer between bacteria. Application of this technology to clinical data will deepen our understanding of the spread of antibiotic resistance genes and its impact on the recovery process of critically ill patients.

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