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Paper Poster Session

The MDR enterococcus

Fitness cost of Tn1546-*vanA* carrying plasmids in different enterococcal clonal backgrounds

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Background: Vancomycin resistance (VR) *Enterococcus faecium* (Efm) has increasingly been reported worldwide since 1986. Most VREfm belong to a specialized population, clade “A”, associated with clinical isolates and enriched in mobile genetic elements (MGE). Clade “B” mostly comprises isolates from healthy humans. Tn1546-*vanA*, the predominant VR genotype, is mostly located on plasmids (PI) of RepA_N and Inc18 families. The intrinsic fitness of Efm susceptible/resistant natural isolates of clades A and B was comprehensively analyzed as well as the fitness cost imposed, in different backgrounds, by the acquisition of Tn1546-PI.

Material/methods: Sixty-five clade “A” (n=58) and “B” (n=7) strain were studied (16 VRE; 48 VSE). PIs analyzed include: i) globally spread mosaic PI carrying Tn1546 containing RepA_N (3 pRUM, 2 pLG1) and/or Inc18 (n=4) and/or pMG1 (n=1) replication modules (rep); and ii) prototype PI RepA_N (pRUM) and Inc18 (pRE25, pIP501). PI transferability and fitness cost were assessed using Efm (GE1, 64/3) and *Enterococcus faecalis* (Efc, JH2-2, FA202, UV202) receptor strains. PI stability was analyzed after 300 generations. Growth curves were performed using Bioscreen C and Relative Growth Rates (RGR) were calculated in presence/absence of vancomycin in non-evolved and evolved strains. Whole genome sequencing (WGS, Illumina MiSeq) of both non-evolved and evolved strains (GE1 and 64/3, n=50) was performed. SNP calling was analyzed by breseq software using non-evolved strains for comparison.

Results: Isolates of clade “B” isolates present a better RGR (reference strain: GE1) than those of clade “A”. Significant differences in fitness were observed between AmpS (1.1520±0.1451) and AmpR (0.9392±0.1235) isolates, but not between VREfm (1.0665±0.1793) and VSEfm (0.9962±0.1379). All PIs were transferred into different Efm backgrounds but only PI carrying replnc18 were transferred into Efc, indicating the broader host range of Inc18-PI compared to RepA_N-PI. Tn1546-PI and prototype-Pis slightly improve or reduce Efm-GE1 and Efm-64/3 fitness (-2%-16%). Fitness cost of Tn1546 expression varies according with the Tn variant and background (5%-49%). Stability of Tn1546-Pis and pRUM was verified in all cases, sometimes with loss of phenotypic resistance and PI modules (replication, conjugation or stability). Contrarily, pRE25 and pIP501 were lost at variable rates (20-95%). Mutation analysis revealed mutations and/or indels in plasmids and/or chromosome of evolved strains associated with essential bacterial functions (DNA replication and repair, carbohydrate and amino acid metabolism and environmental information processing).

Conclusions: The low cost, stability and narrow host of predominant Tn1546-Pis contribute to the confinement and successful spread of Tn1546 in Efm. The enhanced relative fitness of Efm isolates of clade “B” over those of clade “A” deserves further analysis. Differences in accessory genome (MGE as PIs or genetic islands) might explain “individual” fitness values observed here but also contribute to “inclusive fitness” of heterogeneous human Efm populations that determine its success as nosocomial pathogen.