27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

Session: OS199 One health perspective on MDR Gram-positives: VRE & MRSA

Category: 3a. Resistance surveillance & epidemiology: MRSA, VRE & other Gram-positives

25 April 2017, 13:42 - 13:52
OS0998

Extensive comparative genome analysis of Enterococcus faecalis and Enterococcus faecium reveals a direct association between absence of CRISPRs system and acquisition of vancomycin-resistance genes in E. faecium

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Background: *Enterococcus* is an ancient bacterial genus that has species highly adapted to live in complex environments and survive to harsh conditions. The success of *E. faecium and E. faecalis* as multidrug-resistant nosocomial pathogens is associated with their ability to acquire resistance genes encoded by mobile genetic elements (MGEs). However, *E. faecium* is intrinsically more frequently resistant to antibiotics especially to vancomycin than *E. faecalis* (*E. faecium* / *E. faecalis* 8.8%/1.0% in Europe, 79.4%/8.5% in US, 22.4%/0.1% in Canada) and *VanA* and *VanB* are the most common mobile genes involved. It has been shown that this is likely due to a difference of presence/absence of CRISPRs between these two species. Here we perform a massive comparative genomics analysis of all genomes of these two species available to date and examine the association between the presence/absence of CRISPRs and acquisition of antimicrobial resistance genes, especially in strains resistant to vancomycin.

Material/methods: Overall 447 and 407 genomes of *E. faecalis* and *E. faecium* were extracted from NCBI database, respectively. Pan-genome analysis was performed using Roary pipeline. Resistance genes were extracted based on orthologous clusters groups and computed using R. We identify single nucleotide polymorphisms (SNPs), recombination loci, suggestive of horizontal transfer of sequences using ClonalFrameML and CRISPR system using Minced software.

Results: Both species present an increasing set of unique and total genes, an evidence of an open pangenome. Maximum-likelihood phylogeny showed that clusters of strains closest to ancestral strain are human-specific. Conversely, those distant to ancestral strain are a mixture of human, animal, food, and environmental strains and the majority were isolated from Europe. Recombination analysis showed that distant strains presented more hotspots of recombination in both species. Interestingly, we found that *E. faecalis* present more CRISPR systems as compared to *E. faecium* (219/447 vs 38/407, respectively; *p*-value<0.0001). Moreover, the presence of CRISPRs in *E. faecalis* was associated with a significant genome size reduction (3.358 Mbs vs 2.932 Mbs in average, *p*-value=0.025) likely suggesting that CRISPRs protect from acquisition of foreign DNA. Finally, *E. faecium* present more vancomycin-resistance operon genes as compared to *E. faecalis* (*E. faecium / E. faecalis*: *VanA* 156/26, *p-value<0.0001*; *VanB* 188/29 *p-value<0.0001*; *VanC 0/82 p-value < 0.0001*) in their genomes than *E. faecalis*. Presence of vancomycin-resistant genes was associated with the absence of CRISPRs. This implies a direct association between absence of CRISPRs in *E. faecium* and acquisition of vancomycin resistance.

Conclusions: Genomic evolution analysis of *E. faecalis* and *E. faecium* genomes revealed that *E. faecium* genomes have significantly less CRISPRs elements and thus are significantly more prone to acquire foreign DNA, especially genes encoding for vancomycin resistance. This likely explains why vancomycin-resistant enterococci are mainly reported worldwide in *E. faecium* and not in *E. faecalis*.