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2-hour Oral Session

Identification of *Enterobacter cloacae* virulence genes in a *Galleria mellonella* infection model through a transposon-sequencing approach

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***Objectives*:** *Enterobacter cloacae* is a commensal bacteria of the human gastrointestinal tract that has become a major opportunistic pathogen in hospitalized patients, especially due to its multidrug resistance. The objective of the study was to develop the strategy of "transposon-sequencing" (Tn-seq) in order to determine the genetic determinants involved in bacterial virulence of *E. cloacae*.

***Methods*:** A library of ca. 300,000 mutants was first obtained in *E. cloacae* subsp. *cloacae* ATCC 13047 (genome fully sequenced and annotated) using a Mariner transposon previously used in *Pseudomonas aeruginosa* PA14. The library was then tested in an invertebrate animal model (*Galleria mellonella*) using the Tn-seq approach. Two time-points after *in vivo* passage (T24h and T48h) were compared to a time-point before inoculation (T0). Bioinformatic analysis was performed using the CLC Genomics Workbench software. Changes in frequency of Tn inserts into the *E. cloacae* genome were used to quantify *in vivo* fitness: genes with significant fold-change (FC) decrease or increase corresponded to essential and deleterious genes for pathogenesis, respectively.

***Results*:** Out of 5,166 genes, 518 (10%), 543 (10.5%) and 498 (9.6%) presented less than 1 read at T0, T24 and T48, respectively, of which 262 genes were in common (particularly rRNA and tRNA genes). Noteworthy, the homogeneity of the library was checked with an average of reads by gene of 120, 135 and 184 at T0, T24 and T48H, respectively. There were consistent changes in the recovery of Tn inserts within genes part of operons, and 56 genes (1%) had a FC <-50 and 13 (0.25%) with a FC >50 at T48H. Interestingly, a group of genes implicated in the flagellin synthesis seemed to be essential for infecting *G. mellonella* (mean FC, -47) while genes involved in the pilin synthesis (mean FC -6.5) were also important *in vivo*. Other candidate genes included 18 hypothetical proteins (17 with a FC <-50 and 1 with a FC>50) and 5 regulatory proteins (2 with a FC <-50 and 3 with a FC>50).

***Conclusion*:** The Tn-seq allowed us to highlight several candidate genes involved in *in vivo* fitness. Further investigations are in progress to confirm the role of these different determinants, especially by construction of single deletion mutants and competition assays in the *G. mellonella* model.