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K-mer analysis of *bla*$_{ACT}$ genes for fast and reliable delineation of members of the *Enterobacter cloacae* complex

Poster P2678

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Background

For the last decade, *Enterobacter cloacae* complex (ECC) has been divided in 13 genetic clusters (C-1 to C-13) on the basis of *hsp60* sequencing (1). Distinction of each cluster is of clinical relevance since ECC is a complex cluster of species and subspecies having different abilities to give hospital-acquired infections and outbreaks.

More recently, this classification has been extended by using a phylogenetic approach using whole-genome sequencing data (groups A to R) (2).

The aim of this study was to investigate a one-gene-based K-mer approach for the fast identification of the different ECC clusters.

Material and methods

- ✓ All the available ECC genomes (n = 1,307) in the patric database were downloaded and included in the *in silico* analysis.
- ✓ The correct species identification and the absence of contaminant were checked using the rMLST data base provided by PubMLST.
- ✓ The *hsp60, rpoB* and *bla*$_{ACT}$ genes were extracted from all the genomes and clustered using cd-hit with a 99% identical cutoff. A distance matrix between all the genomes based on the *K-mer* content was calculated using the Mash software, and a neighbor-joining tree (NJT) was constructed using the neighbor software available in the PHYLIP package.
- ✓ Tree and molecular typing for all the strains were represented using the iTOL website (https://itol.embl.de).

Results

- ✓ The K-mer approach provided a distance matrix of >1,000 genomes in <1 hour on a personal laptop. Clusters recovered in the NJT were overall in *ad equation* with the *hsp60* clustering method for C-11, C-2, C-5 and C-7. Furthermore, *hsp60* and *rpoB* clustering method provided classification errors owing to its weak diversity and especially in C-4, C-6, C-8, C-9 and C-13.
- ✓ Consequently, *hsp60*- and *rpoB*-based clustering methods were not discriminant enough and led to cluster misidentification. By contrast, the diversity in *bla*$_{ACT}$ sequences significantly improved the discrimination between the clusters and avoided missclusterization (**Figure 1**).
- ✓ So, we identified more than 86 specific typical sequences of every cluster or group defined by *hsp60* method or NJT (PPV 100%). Furthermore it allowed identifying some additional subgroups.

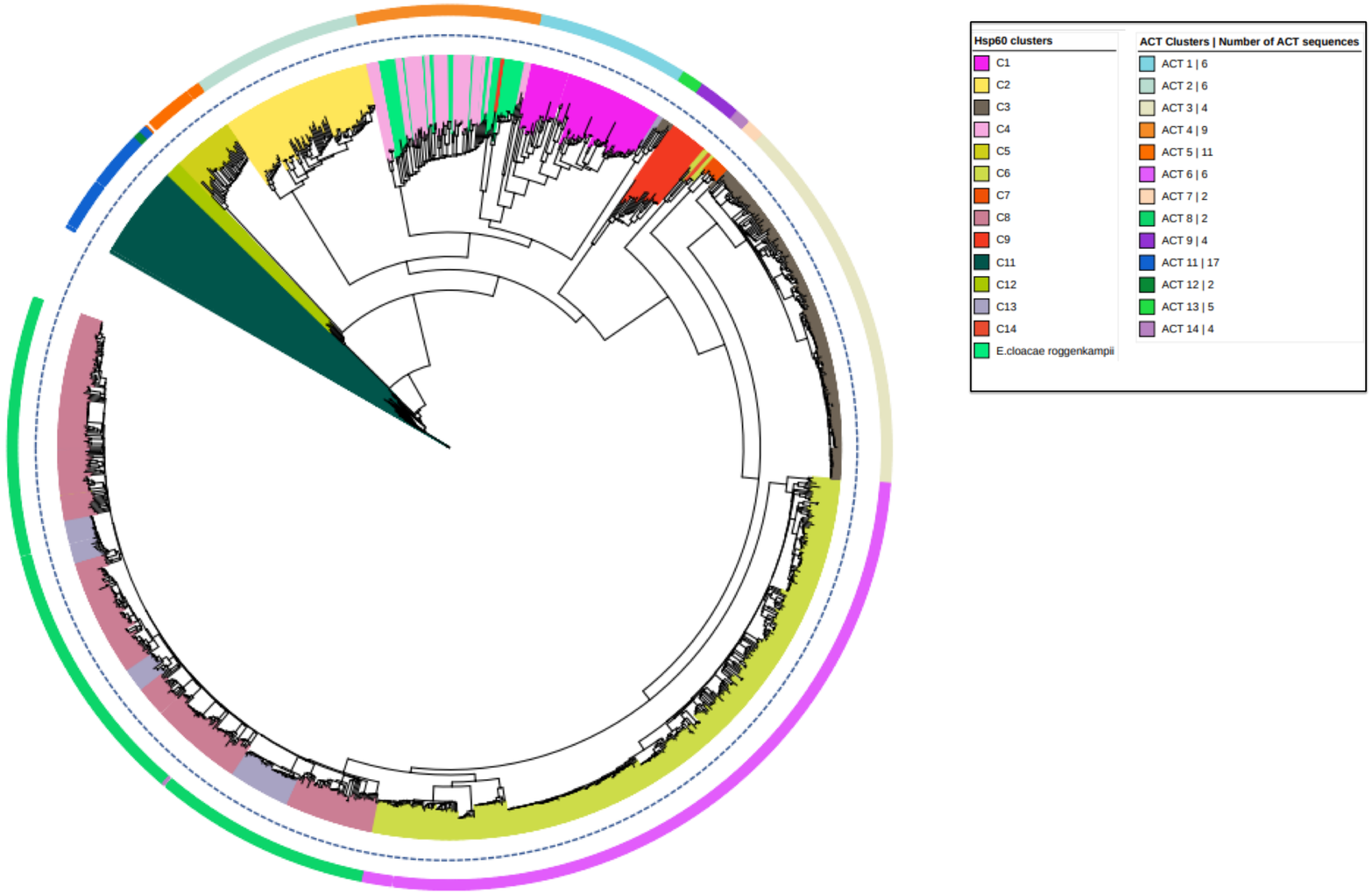


Figure 1 : Neighbour-joining tree based on K-mer composition determined using Mash software then created using PHYLIP package. Each clusters (1 to 14) is represented in a distinct colour in inner circle and ACT clusters (1 to 14) are also plotted in outer concentric circles.

Conclusions

- ➢ ***bla*$_{ACT}$ K-mer analysis provides a fast alternative approach for a precise and high-throughput identification of ECC clusters. Other genes have been tested for further clustering analysis.**

References:

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