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**ePoster Viewing**

**Molecular bacterial typing methods**

**The reproducibility of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) based typing: employment of bioinformatics**

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**Background:** MALDI-TOF holds promise for an easy access typing method compared to conventional approaches. However, the technical, biological and center reproducibility of such data has not yet been explored. The aim of this study is to compare typing data from multiple centers employing bioinformatics.

**Material/methods:** Seven different centers tested 12 extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolates – including two outbreak and one non-related cluster. Every center used a Microflex MALDI-TOF (Bruker, Bremen, Germany) and used the same standard operating procedure. Each center recorded four spectra from each isolate and repeated three times the procedure. Raw data was used to calculate the technical and biological reproducibility in/between each center using the Bionumerics software (Applied Maths, Belgium).

**Results:** Technical reproducibility ranged between 96.8 % and 99.4% for each center calculating the average similarity of each spectrum using the Pearson correlation coefficient (Figure 1A). Biological reproducibility ranged between 47.6 % and 94.4 % for the centers and were calculated by comparing the summary spectra of biological replicates to each other (Figure 1B).

The reproducibility between centers showed in general a comparable clustering among identical isolates, however some important exceptions occurred, which required more complex bioinformatics. First, a principal component analysis showed that samples had a higher tendency to cluster within the same lab. We therefore used a discriminant analysis on the complete dataset, which shows a complete separation of the clusters. Next, we identified separating peaks. For this, we defined a reference lab with specific peaks to allow detection of the clusters from all labs. The peaks were detected after a peak matching was performed on all the spectra from the reference lab followed by an Anova to detect 18 peaks that were significantly different between the clusters.

Finally, a classifier algorithm using including the spectra from the reference lab as reference set and a linear support vector machine on the determined peaks as classifier. An internal validation showed that within the set of the reference lab, the identification of the cluster was 100% correct with a large contrast between the score with the correct cluster and the next best scoring cluster.

**Conclusions:** Detection of specific clusters is possible from spectra obtained from difference centers. Bioinformatics is required to make the analysis robust and reliable. The closer related different clusters are to each other, the less reliable their separation is. Technical reproducibility is a key element to implement MALDI-TOF based typing. Further research is needed to study which elements in the preparation and acquirement of spectra contribute most to variability.

**Figure 1. (A)** Technical reproducibility. In center 6 the experiment was repeated twice. **(B)** Biological reproducibility. Center 4 did not contribute biological replicates.

