Comparison of MALDI-TOF MS and AFLP for strain typing of ESBL-producing *Escherichia coli* isolates collected during an outbreak in a Dutch nursing home

Veenemans J[1], Welker M[2], van Belkum A[2], Saccomani MC[2], Pettersson A[3], Verhulst C[1], Kluytmans-van den Bergh M[1,4], Kluytmans JAJW[1,3]

[1]Laboratory for Microbiology and Infection Control, Amphia Hospital, Breda, The Netherlands. [2]BioMerieux, La Balme Les Grottes, France

[3]Department for Medical Microbiology and Infection Control, VU University Medical Centre, Amsterdam, The Netherlands. [4] Julius Center for Health Sciences and Primary Care, UMC Utrecht, Utrecht, The Netherlands

Objectives

The use of Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) data for organism typing can provide an efficient on-site method to monitor the spread of antibiotic-resistant bacteria and to rapidly detect outbreaks. We compared typing results generated by MALDI-TOF MS to those obtained by Amplified Fragment Length Polymorphism (AFLP) in a set of ESBL-producing *E. coli* isolates collected during an ongoing outbreak of faecal carriage of ST131 *E. coli* in a Dutch nursing home.

Materials and Methods

Setting The nursing home consisted of 4 different departments in 4 (semi-)separate buildings at 2 different locations (NH1 and NH2). Each department (A-D) included 2–3 wards on separate floors. In 2 consecutive cross-sectional surveys in March and May 2013, we collected rectal swabs from all residents, and hand cultures from health care workers (HCW). ESBL-producing *E. coli* were isolated using selective culture methods. The isolate collection (n=52) included every first non-duplicate (based on phenotypic resistance patterns) ESBL *E. coli* isolate from each resident or HCW .

Reference typing methods All ESBL *E. coli* isolates were typed by AFLP [1]. PCRs were used to assign the isolates to one of the *E. coli* phylogroups [2] and to detect ST131-specific sequences [3] for those isolates belonging to phylogroup B2.

MALDI-TOF MS spectra Single colonies of strains grown on Columbia agar at 37°C for 24h were selected, transferred to target plates, and overlaid with CHCA matrix solution. Four replicate samples were prepared for each strain and measured on a VITEK MS RUO system (bioMérieux, Marcy l'Etoile, France), using standard settings as for routine identification. These 4 replicate measurements were used to compute consensus spectra for each strain in SARAMIS, by retaining only those peaks that were found in 3 out of 4 spectra.

Data Analysis Spectral data were initially analyzed with SARAMIS with the in-built single linkage clustering. Further analyses were performed with PAST3 [4] loaded with spectral data (m/z and intensity) aligned with SARAMIS. Data transformations to binary and logarithmic were done in Excel. Multivariate statistical procedures included Principal Component Analysis (PCA), clustering with UPGMA and Ward's method applying different similarity and distance indices, respectively. All analyses were performed without knowledge of results of the other typing methods.

Results

For all strains, spectra with an average of 180 peaks were acquired (median=180) and used to compute consensus spectra (average/median peaks: 157). A share of 61 peaks was recorded for all 52 strains, among them many peaks with high intensity (**Fig.1**). For the data analysis these peaks were removed from the dataset ('purged') to increase the resolution. **Fig. 2** highlights the effect of data purging and deconvolution on the scattering in PCA. Nevertheless, various cluster analyses resulted in a stable clustering, irrespective of the chosen similarity index. A higher resolution is generally given with indices taking into account quantitative data, i.e., peak intensities, (**Fig. 3b**) compared to binary data, i.e., presence/absence (**Fig. 3a**). A comparison to other typing procedures shows a generally good agreement although not full consistency (**Table**).

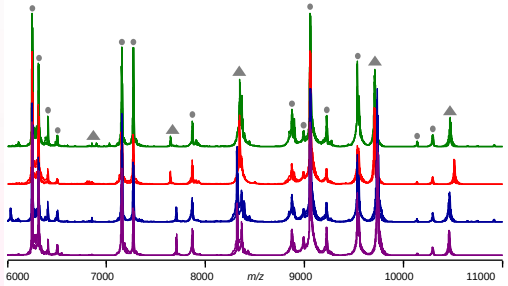

Fig. 1: Partial spectra of four distinct MALDI types. Each color is an overlay of three spectra of three strains. Dots indicate conserved and triangles variable peaks, respectively.

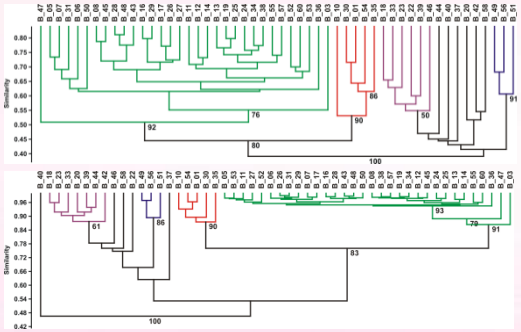

Fig. 3: UPGMA clustering with Jaccard (upper) and cosine (lower) index of similarity, respectively. Clusters were computed from aligned, deconvoluted spectra after purging of common peaks. Bootstrap values >50% (n=500).

ID	MALDI	AFLP type	PT	ST131	Ward
B_18	3	AT13066	A		NH1-A2
B_33	3	AT13066	A		NH1-A2
B_44	3	AT13066	A		NH1-A2
B_23	3	AT13083	A		NH1-A2
B_39	3	AT13085	A		NH1-A3
B_20	3	AT13089	B1		NH2-D1
B_42	3	AT13090	B1		NH2-D1
B_22		AT13082	A		NH1-A1
B_58		AT13091	D		NH2-D1
B_40		AT13092	A		NH2-D2
B_46		AT13120	A		NH1-A2
B_49	4	AT13087	A		NH1-A3
B_56	4	AT13087	A		NH1-A3
B_51	4	AT13087	A		NH1-A3
B_37		AT13119	A		NH1-C3
B_35	2	AT13084	B2	non-ST131	NH1-A2
B_01	2	AT13086	B2	non-ST131	NH1-A3
B_30	2	AT13086	B2	non-ST131	NH1-A3
B_54	2	AT13086	B2	non-ST131	NH1-A3
B_10	2	AT13088	B2	non-ST131	NH1-C1
B_05	1	AT13093	B2	ST131	NH2-D2
B_27	1	AT13093	B2	ST131	NH2-D2
B_52	1	AT13093	B2	ST131	NH2-D2
B_03	1	AT13081	B2	ST131	NH1-B1
B_07	1	AT13081	B2	ST131	NH1-B1
B_08	1	AT13081	B2	ST131	NH1-B1
B_12	1	AT13081	B2	ST131	NH1-B1
B_14	1	AT13081	B2	ST131	NH1-B1
B_25	1	AT13081	B2	ST131	NH1-B1
B_34	1	AT13081	B2	ST131	NH1-B1
B_38	1	AT13081	B2	ST131	NH1-B1
B_06	1	AT13081	B2	ST131	NH1-B2
B_13	1	AT13081	B2	ST131	NH1-B2
B_16	1	AT13081	B2	ST131	NH1-B2
B_24	1	AT13081	B2	ST131	NH1-B2
B_29	1	AT13081	B2	ST131	NH1-B2
B_36	1	AT13081	B2	ST131	NH1-B2
B_45	1	AT13081	B2	ST131	NH1-B2
B_55	1	AT13081	B2	ST131	NH1-B2
B_57	1	AT13081	B2	ST131	NH1-B2
B_60	1	AT13081	B2	ST131	NH1-B2
B_17	1	AT13081	B2	ST131	NH1-C2
B_19	1	AT13081	B2	ST131	NH1-C2
B_26	1	AT13081	B2	ST131	NH1-C2
B_28	1	AT13081	B2	ST131	NH1-C2
B_31	1	AT13081	B2	ST131	NH1-C2
B_43	1	AT13081	B2	ST131	NH1-C2
B_47	1	AT13081	B2	ST131	NH1-C2
B_48	1	AT13081	B2	ST131	NH1-C2
B_50	1	AT13081	B2	ST131	NH1-C2
B_11	1	AT13081	B2	ST131	NH1-C3
B_53	1	AT13081	B2	ST131	NH1-C3

Table: Summary of different typing approaches and origins of strains.

MALDI: Four strain types were assigned based on cluster analyses with different similarity indices; only stable clusters with bootstrap >50% were considered.

Five clusters were identified by **AFLP**: the largest AFLP cluster consisted of 31 indistinguishable isolates (in green) whereas the other 4 clusters included only few indistinguishable isolates (yellow, blue, pink and red), and 11 isolates were considered unique (in white). All isolates from the green AFLP cluster belonged to phylogroup ('**PT**') B2, and were found to be ST**131** by PCR. (For one isolate of this cluster, PCR sequence type results were confirmed by MLST). Isolates from the green cluster were exclusively found in buildings B and C in NH1 (as shown in the far right column '**Ward**': the location of residence).

A smaller cluster of 3 *E. coli* ST131 was found in NH2 (appr 45 kms distance from NH1), and although these isolates were considered as a separate cluster by AFLP, they were assigned to the largest cluster based on MALDI-MS spectral data, suggesting regional spread. Six of the unique isolates (AFLP) were considered part of a cluster by MALDI MS spectral typing.

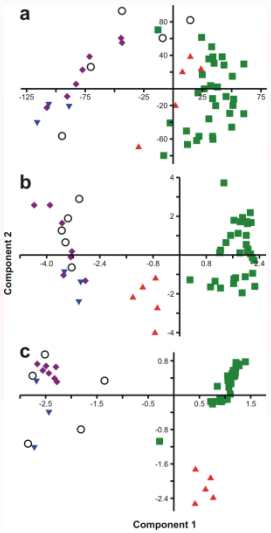

Fig. 2: PCA scatter plots computed based aligned spectra with relative intensities in percent (a), binary transformed data with common peaks purged and deconvoluted (b), and with log-transformed intensities with common peaks purged and deconvoluted (c). Color codes correspond to MALDI types (see table and figures).

Discussion

The analysis of MALDI spectra revealed the importance of rational data treatment to improve the resolution of this typing approach. Especially the purge of background similarity had a marked effect on the separation of strains into MALDI-types. Which transformation steps and statistical procedures are the best for a given set of strains remains to be established. In a local setting, MALDI-typing could be used to efficiently trace the spread of (patho)types among wards and hospitals, respectively, with similar resolution as AFLP.

Conclusion The performance of MALDI-TOF MS for typing of *E. coli* is promising, showing good agreement with typing results obtained by AFLP and other typing methods. In particular, the short time to result and low cost per analysis may allow applications in epidemiological surveys.

References.[1] Mohammadi T et al. Br. J. Haematol. 2005;131:403-409. [2] Doumith M et al, . J. Clin. Microbiol. 2005;50:3108-10. [3] Dhanji H et al, Int. J. Antimicrob. Agents. 2010:36:355-8. [4]Hammer et al., Paleo. Elect. 2001:4