

Outbreak analysis with MALDI-TOF Mass Spectrometry – potentials and limits – an outlook

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Introduction

Strains of *Klebsiella pneumoniae* producing a carbapenemase and therefore showing resistance to carbapenems were not isolated in Germany until 2008.

Between January and December 2008 we could isolate this bacterium from 9 patients. We used these isolates to evaluate the utility of MALDI-TOF MS analysis for outbreak investigations.

Materials and Methods

Ten isolates from the outbreak patients (KP1-9, 11) – one patient was colonised with two phenotypically different isolates - and two unrelated isolates underwent typing with PFGE and were analysed with MALDI-TOF MS. Strains were cultivated on blood agar (36°C, 18h), extracted with a formic-acid/ethanol method and a series of 24 spectra for each isolate was generated. Spectra were evaluated, internally calibrated and (standard) MSPs (mass spectra) were calculated. Using the Bruker Daltonics microflex mass spectrometer and the standard software package (MALDI Biotyper 2.0) three types of analysis were performed: dendrogram generation, subtyping and composite correlation index (CCI). For the dendrogram generation standard MSPs were used. For subtyping special subtyping MSPs were generated using the spectra of isolates KP 4, 5, 13, 14. (KP4 and KP5: the first two KPC carrying isolates, KP13 and 14: unrelated controls). All other isolates were subtyped against these 4 subtyping MSPs. For CCI

Results

PFGE



Fig1: PFGE of 2 control strains (Co) and 10 outbreak isolates (1-9, 11)

Results

Dendrogram generation (KP:= *Klebsiella pneumoniae*)

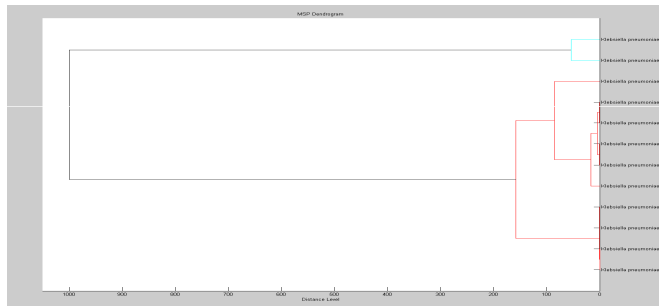


Fig2: Dendrogram, KP1-9, 11: outbreak strains, KP13, 14: unrelated controls

Subtyping: subtyping MSP for isolates KP4, 5, 13, 14 were generated, the other isolates were subtyped against these four subtyping spectra; the higher the score the better the match, 3:= absolute identity

isolate	best match	score	2. best	score	3. best	score	4. best	score
KP1	KP4	2,787	KP5	2,767	KP14	2,192	KP13	1,902
KP2	KP4	2,511	KP5	2,314	KP14	1,792	KP13	1,362
KP3	KP4	2,644	KP5	2,378	KP14	1,937	KP13	1,688
KP4	KP4	3,000						
KP5	KP5	3,000						
KP6	KP4	2,852	KP5	2,803	KP14	2,182	KP13	1,766
KP7	KP4	2,622	KP5	2,521	KP14	2,239	KP13	1,817
KP8	KP4	2,907	KP5	2,798	KP14	2,182	KP13	2,006
KP9	KP4	2,665	KP5	2,581	KP14	1,852	KP13	1,445
KP11	KP5	2,634	KP4	2,577	KP14	2,229	KP13	1,877
KP13	KP13	3,000						
KP14	KP14	3,000						

Table1: subtyping scores, KP:= *K. pneumoniae*, KP1-9, 11: outbreak strains, KP13, 14: unrelated controls

Composite correlation index (CCI): a 4x4 matrix was generated using isolate KP4, 5, 13, 14, all other isolates were matched against this matrix, the higher the score the better the match, 1:=exact match

isolate	best match	score	2. best	score	3. best	score	4. best	score
KP1	KP5	0,964	KP4	0,955	KP13	0,648	KP14	0,552
KP2	KP5	0,825	KP4	0,800	KP13	0,578	KP14	0,482
KP3	KP5	0,909	KP4	0,885	KP13	0,642	KP14	0,551
KP4	KP4	0,966	KP5	0,948	KP13	0,656	KP14	0,559
KP5	KP5	0,954	KP4	0,950	KP13	0,640	KP14	0,560
KP6	KP5	0,976	KP4	0,936	KP13	0,678	KP14	0,582
KP7	KP5	0,825	KP4	0,761	KP13	0,669	KP14	0,590
KP8	KP4	0,918	KP5	0,902	KP13	0,737	KP14	0,633
KP9	KP5	0,903	KP4	0,880	KP13	0,609	KP14	0,519
KP11	KP5	0,885	KP4	0,884	KP13	0,518	KP14	0,453
KP13	KP13	0,969	KP14	0,759	KP4	0,695	KP5	0,662
KP14	KP14	0,920	KP13	0,771	KP4	0,602	KP5	0,602

Table2: CCI scores, KP:= *K. pneumoniae*, KP1-9, 11: outbreak strains, KP13, 14: unrelated controls

Conclusions

1. all three MALDI-TOF MS analysis methods correctly grouped the outbreak isolates together
2. these results warrant a prospective usage of MALDI-MS in a future outbreak situation