

Defining criteria to interpret multi-locus variable number tandem repeat analysis to aid *Clostridium difficile* outbreak investigation

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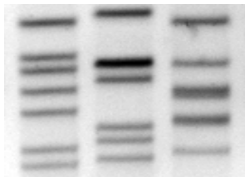
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- *Clostridium difficile* (CD) is a major hospital acquired infection in patients treated with antibiotics
- Currently outbreak investigation of CD in North America is performed using PFGE typing
- PFGE lacks resolution in geographical regions where NAP1 strain is predominant (Quebec, Ontario etc)
- We assess utility of MLVA for hospital outbreak typing and define criteria to interpret the results

Background

- PCR ribotyping – most commonly used in Europe
- PFGE (Pulse Field Gel Analysis) – most commonly used in North America
- REA (Restriction Endonuclease Analysis) – not widely used; research method
- Toxinotyping – provides information about toxins genes but not the genotype of organism
- MLST (multi-locus sequence typing) – no correlation with PFGE
- MLVA (Multilocus Variable-Number Tandem Repeat Analysis) – technically challenging; requires sequencer

Drawbacks of PFGE

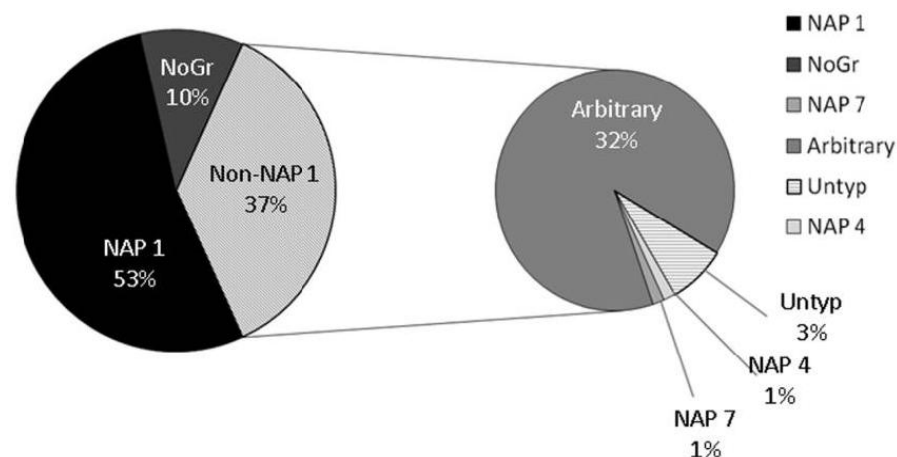


- Labour intensive – takes 4 to 5 days and needs pure culture of CD
- Requires special equipment, casting gels, processing plugs, taking pictures etc.
- Interpretation of banding patterns is subjective (double bands, faint bands, interpretation of band differences etc.)
- Lacks discriminatory power

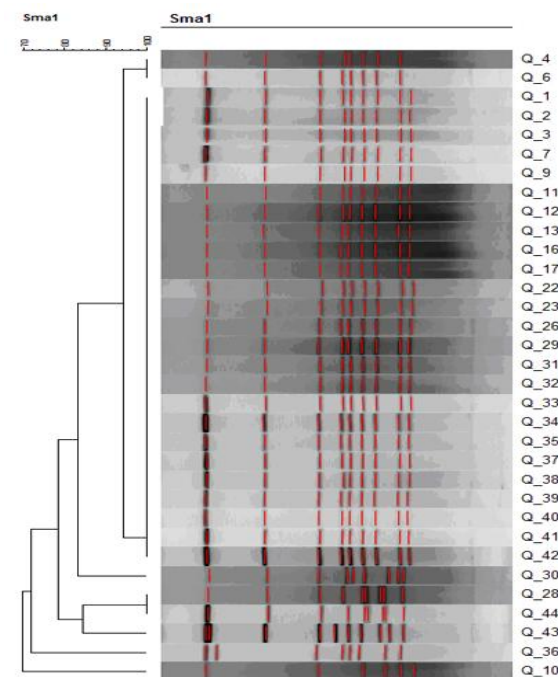
Epidemiology

Investigation of outbreaks

The predominant outbreak isolate in Ontario over 2008-9 was NAP1 strain, with a prevalence of 53%*



With NAP1 prevalence of 53% PFGE can not distinguish outbreak cluster from sporadic cases



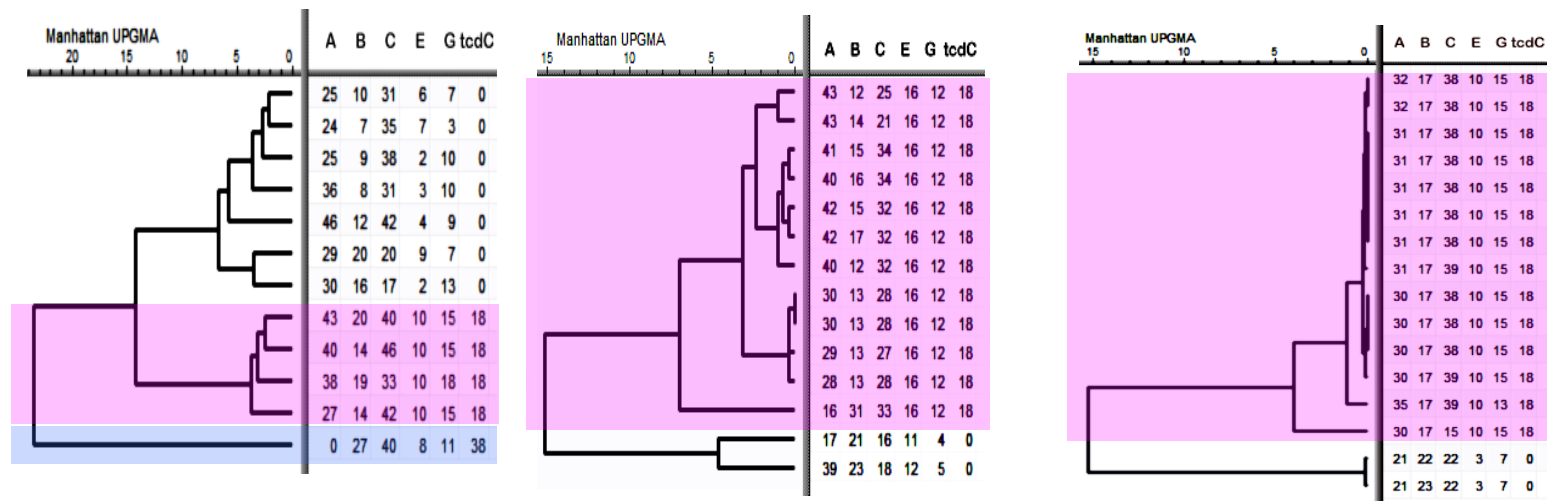
Example of PFGE analysis of *C. difficile* isolated in a hospital

*Pillai DR et al. CID 2010;50:1685–6

Our MLVA method modifications

- F and H regions dropped from analysis
- Primers' sequences modified (to accommodate sequence variation)
- tcdC fragment detection was added to aid typing of NAP1, NAP7 and NAP8 isolates

MLVA analysis of isolates from 3 hospital outbreaks



NAP1



NAP7



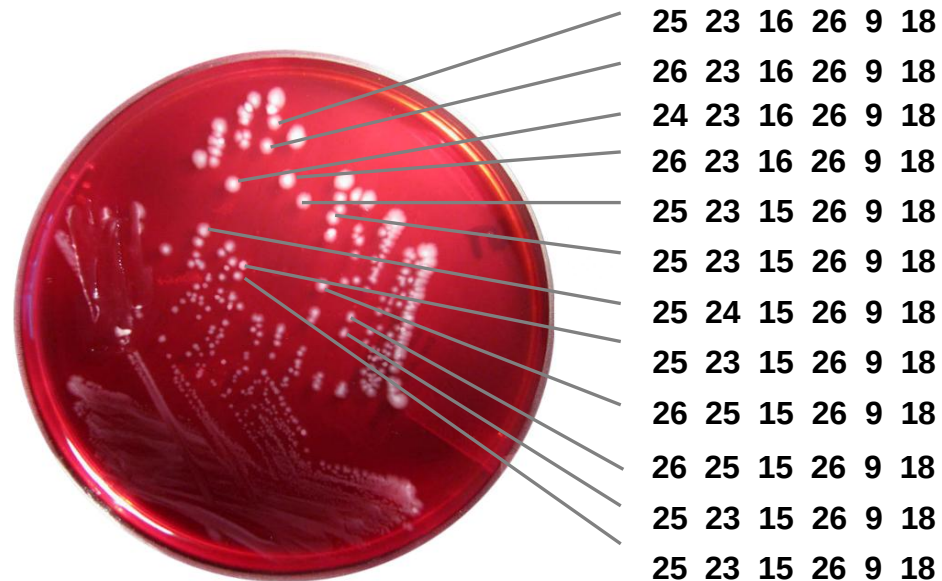
Hospital 1 – mixture of NAP1 and non-NAP1

Hospital 2 – heterogenous NAP1

Hospital 3 – homogenous NAP1

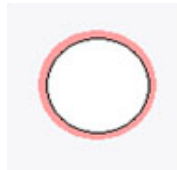
Population analysis

Diversity of MLVA variants isolated from a single specimen



Observed variations in isolates from a single specimen

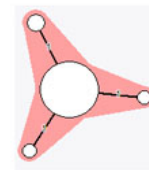
Minimum
spanning
trees



Spcmn 26 VNTR_frags

	A	B	C	D	E	totC	
26	22	16	27	10	18		Q_26.01
26	22	16	27	10	18		Q_26.02
26	22	16	27	10	18		Q_26.03
26	22	16	27	10	18		Q_26.04
26	22	16	27	10	18		Q_26.05
26	22	16	27	10	18		Q_26.06
26	22	16	27	10	18		Q_26.07
26	22	16	27	10	18		Q_26.08
26	22	16	27	10	18		Q_26.09
26	22	16	27	10	18		Q_26.10
26	22	16	27	10	18		Q_26.11
26	22	16	27	10	18		Q_26.12
26	22	16	27	10	18		Q_26.13
26	22	16	27	10	18		Q_26.14
26	22	16	27	10	18		Q_26.15
26	22	16	27	10	18		Q_26.16
26	22	16	27	10	18		Q_26.17
26	22	16	27	10	18		Q_26.18
26	22	16	27	10	18		Q_26.19
26	22	16	27	10	18		Q_26.20
26	22	16	27	10	18		Q_26.21
26	22	16	27	10	18		Q_26.22
26	22	16	27	10	18		Q_26.23
26	22	16	27	10	18		Q_26.24
26	22	16	27	10	18		Q_26_super
26	22	16	27	10	18		Q26_stool

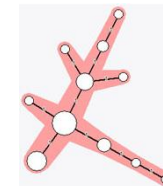
Monoclonal
2%



Spcmn 6 VNTR_frags

	A	B	C	D	E	totC	
30	11	16	41	9	18		Q6.01
30	11	16	41	9	18		Q6.02
30	11	16	41	9	18		Q6.03
30	11	16	41	9	18		Q6.05
30	11	16	41	9	18		Q6.06
30	11	16	41	9	18		Q6.07
30	11	16	41	9	18		Q6.08
30	11	16	41	9	18		Q6.09
30	11	16	41	9	18		Q6.10
30	11	16	41	9	18		Q6.11
30	11	16	41	9	18		Q6.12
30	11	16	41	9	18		Q6.13
30	11	16	41	9	18		Q6.14
30	11	16	41	9	18		Q6.15
30	11	16	41	9	18		Q6.16
30	11	16	41	9	18		Q6.17
30	11	16	41	9	18		Q6.18
30	11	16	41	9	18		Q6.19
30	11	16	41	9	18		Q6.21
30	11	16	41	9	18		Q6.22 A
30	11	16	41	9	18		Q6.24
30	11	16	41	9	18		Q_6_super
30	11	16	41	9	18		Q6_stool
31	11	16	41	9	18		Q6.04
30	11	16	42	9	18		Q6.23
30	11	16	40	9	18		Q6.20
30	11	16	40	9	18		Q6.22 B

Low diversity <95%
90%



VNTR_frags VNTR_frags

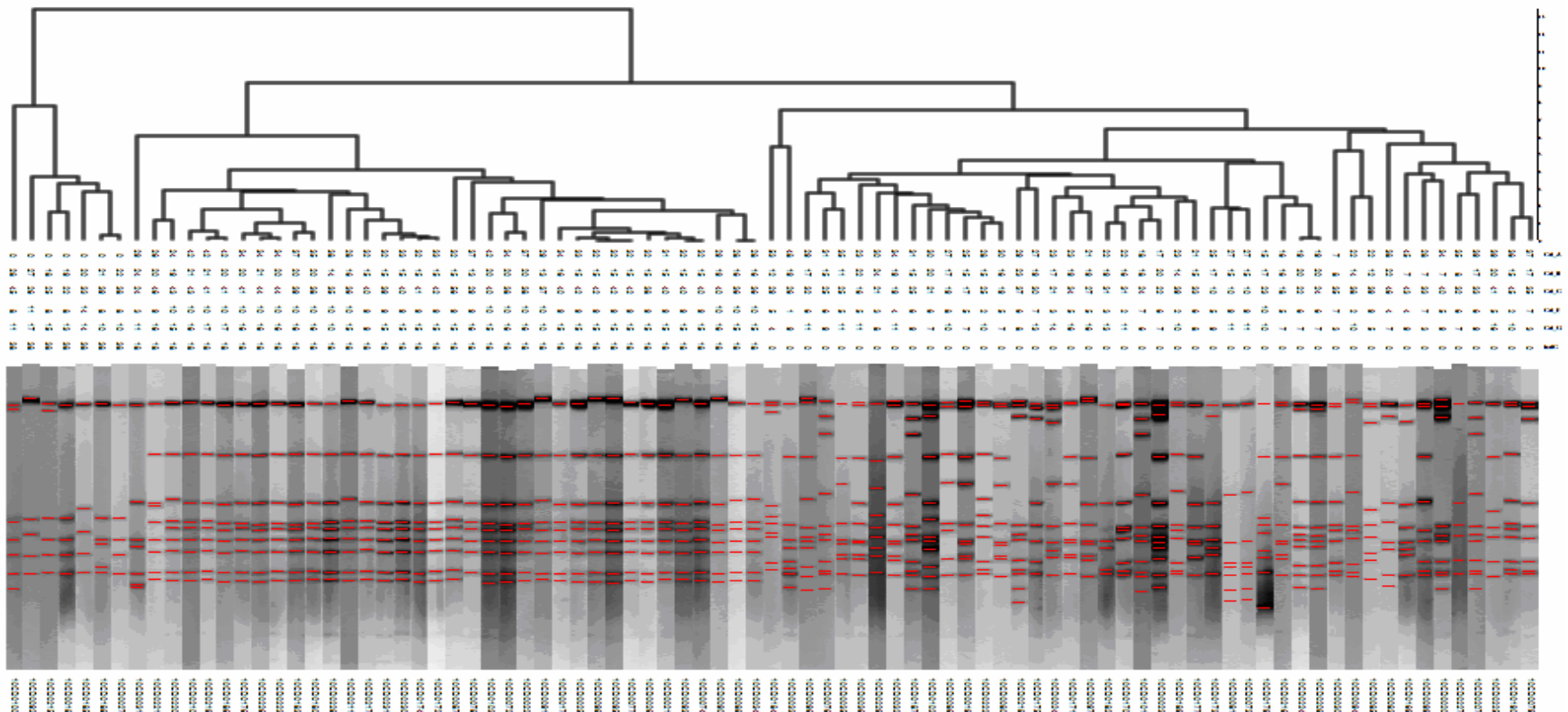
	A	B	C	D	E	totC	
42	22	15	30	9	18		Q_38.01 A
42	22	15	30	9	18		Q_38.02 A
42	22	15	30	9	18		Q_38.05
42	22	15	30	9	18		Q_38.06 A
42	22	15	30	9	18		Q_38.07
42	22	15	30	9	18		Q_38.08 A
42	22	15	30	9	18		Q_38.11 A
42	22	15	30	9	18		Q_38.18
42	22	15	30	9	18		Q_38.21
42	22	15	30	9	18		Q_38.23
41	22	15	30	9	18		Q_38.03
40	22	15	30	8	18		Q_38.04 B
43	22	15	30	9	18		Q_38.08 B
43	22	15	30	9	18		Q_38.16
43	22	15	30	9	18		Q_38.20
43	22	15	30	8	18		Q_38.04 a
42	23	15	30	9	18		Q_38.11 B
42	23	15	30	9	18		Q_38.12
42	23	15	30	9	18		Q_38.15
42	23	15	30	9	18		Q_38.19
42	23	15	30	9	18		Q_38.22
42	23	15	30	9	18		Q_38_supern
42	23	15	31	9	18		Q_38.06 B
42	23	15	31	9	18		Q_38.09 B
44	23	15	31	9	18		Q_38.02 B
42	22	15	31	9	18		Q_38.01 B
42	22	15	31	9	18		Q_38.09 A
42	22	15	31	9	18		Q_38.13
42	22	15	31	9	18		Q_38.14
42	22	15	31	9	18		Q_38.17
42	22	15	31	9	18		Q_38.24
29	22	15	31	9	18		Q_38.10

High diversity >95%
8%

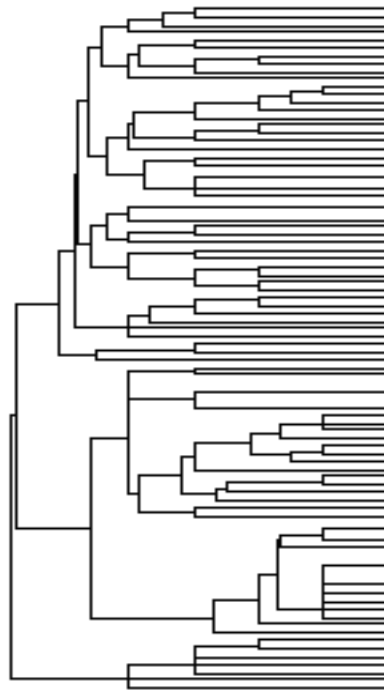
Application of MLVA

Surveillance of CD in a hospital (6 month period)

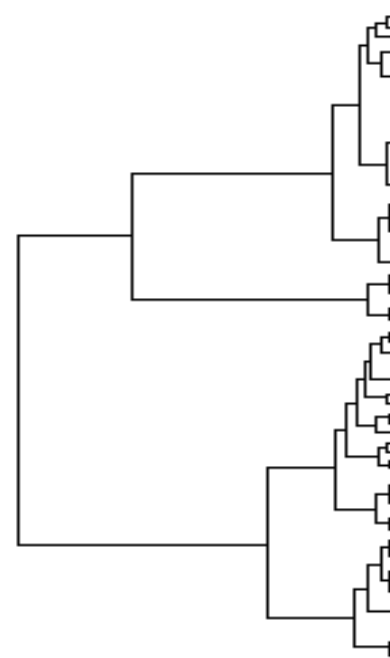
MLVA correlates with PFGE



Interpretation of MLVA differs based on similarity method used



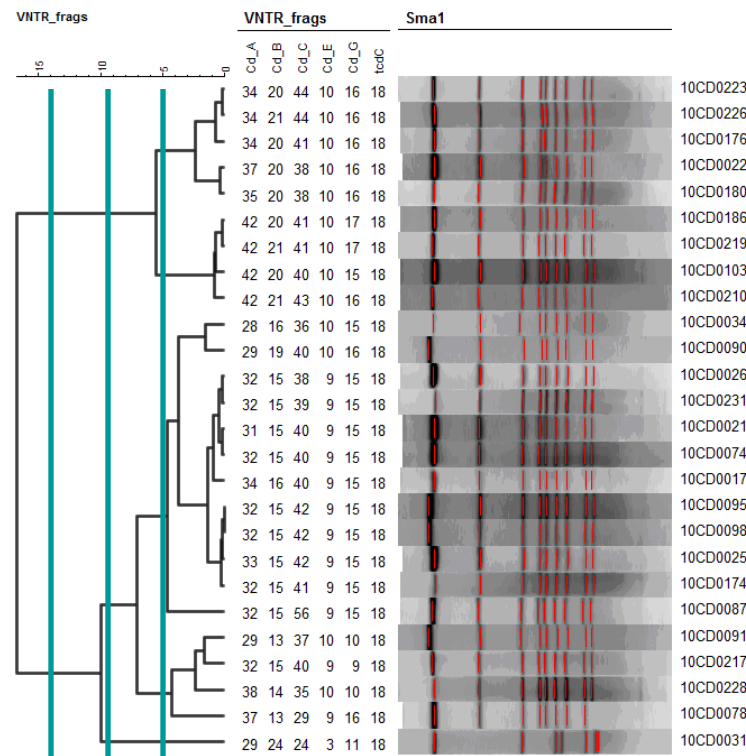
Categorical



Distance-based

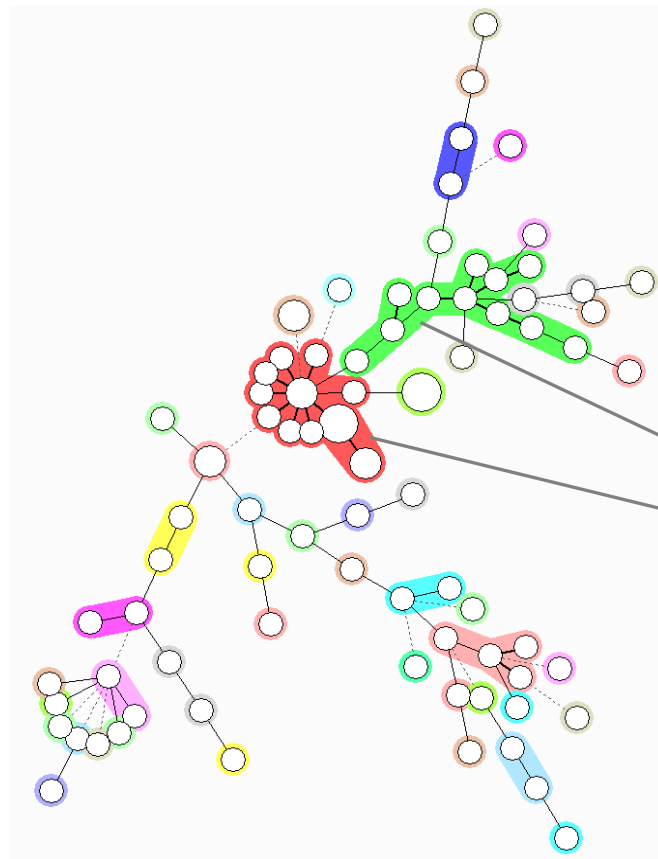
Comparison of PFGE and MLVA for isolates with tcdC 18 bp deletion (NAP1)

**Manhattan
Distance based
clustering**



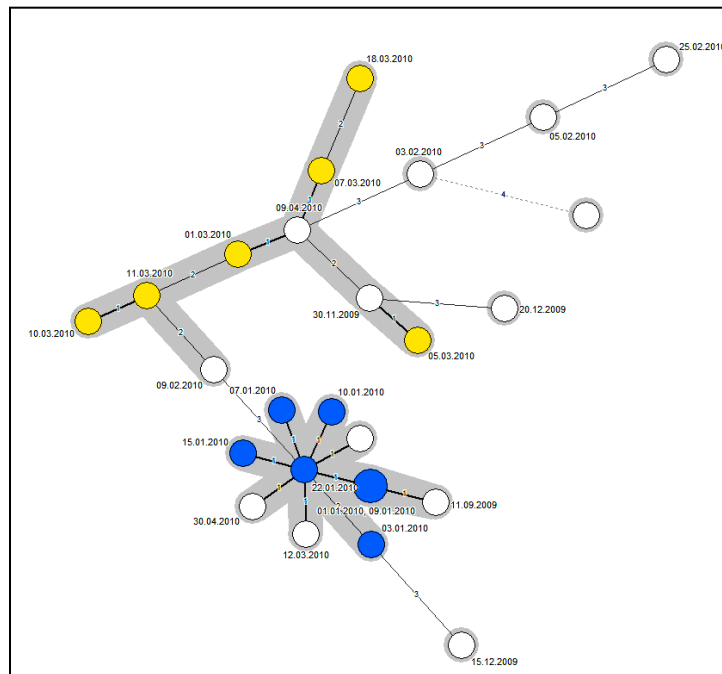
MLVA minimum spanning tree

Advanced clustering analysis of outbreak



Two clusters of
NAP1 isolates

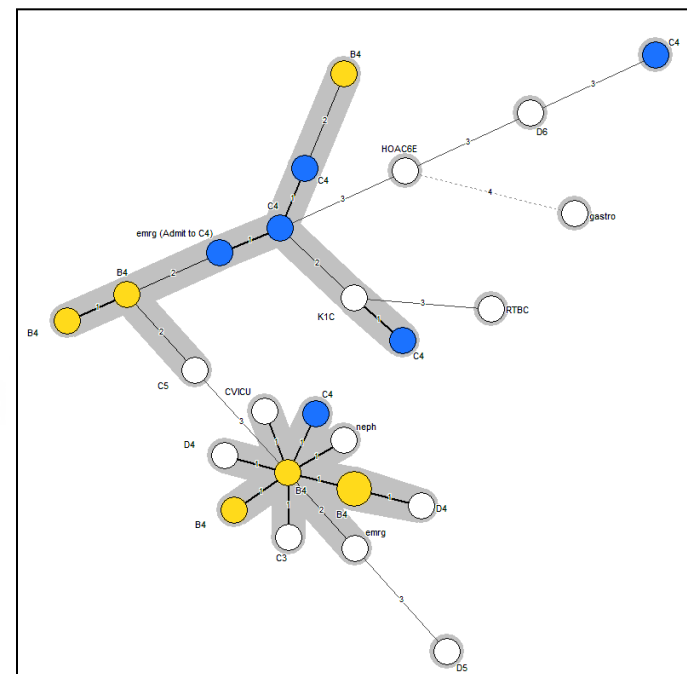
Minimum spanning tree of NAP 1 isolates coupled to temporospatial data



Date of admission

Blue nodes – January

Yellow nodes – March



Location

Blue nodes – C4

Yellow nodes – B4

Conclusions

- MLVA is a discriminatory typing method capable of defining clusters of NAP1 isolates
- The challenge is to determine cut-off values for clusters
- Population analysis suggests that a cut-off value of <1% is overly discriminatory, but a cut-off of 3-5% should capture true clusters
- Hospital surveillance using clinical and epidemiological data reinforces that a cut-off of ~5% identifies true clusters of NAP1
- Further multicentre studies relying on advanced clustering methods which combine both MLVA data and clinical epidemiological information are required

Points for discussion

- Consensus criteria for interpretation of MLVA clustering is required
- Number of isolates per specimen to type for reliable identification of all strains present
- What other targets, if any, should be included in the test to increase usefulness of MLVA?
- Direct MLVA typing from stool specimens may be beneficial for rapid assistance in outbreak investigation