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

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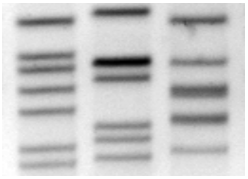
- *Clostridium difficile* (CD) is a major hospital acquired infection in patients treated with antibiotics
- Currently outbreak investigation of CD in North America is performed using PFGE typing
- PFGE lacks resolution in geographical regions where NAP1 strain is predominant (Quebec, Ontario etc)
-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

Background

Current methods used for C. difficile typing

- 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

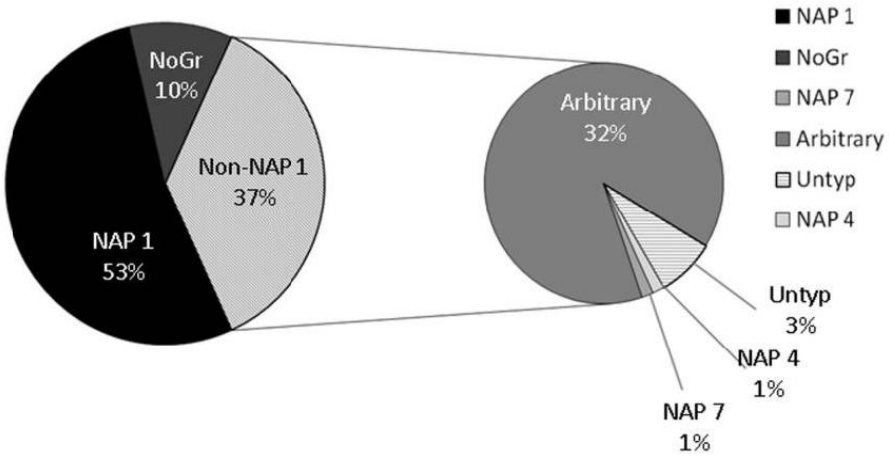


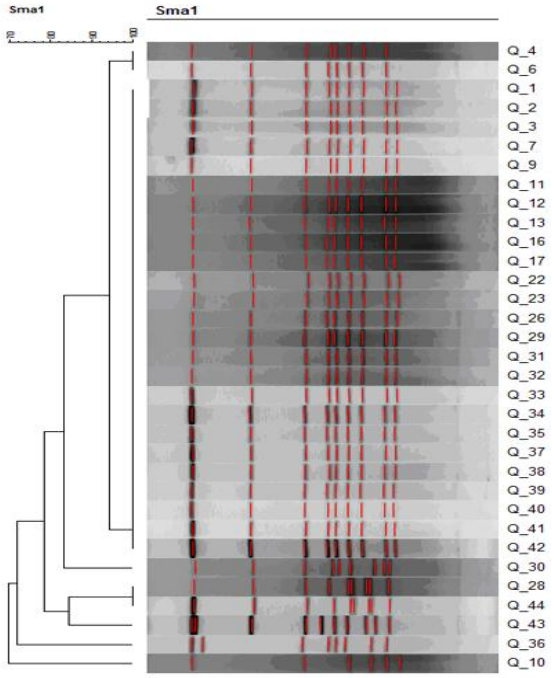
- Labour intensive – takes 4 to 5 days and needs pure culture of CD
- Requires special equipment, casting gels, processing plugs, taking pictures etc.
- Interpretation of banding patterns is subjective (double bands, faint bands, interpretation of band differences etc.)
- Lacks discriminatory power

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

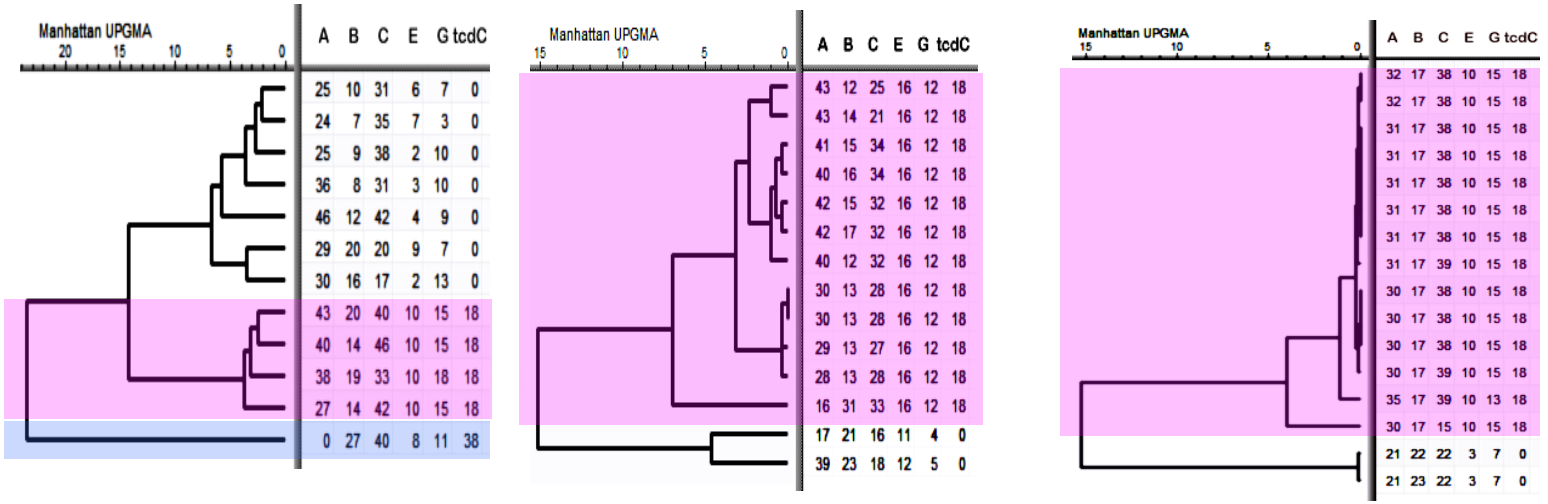
Example of PFGE analysis of *C. difficile* isolated in a hospital

*Pillai DR at 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

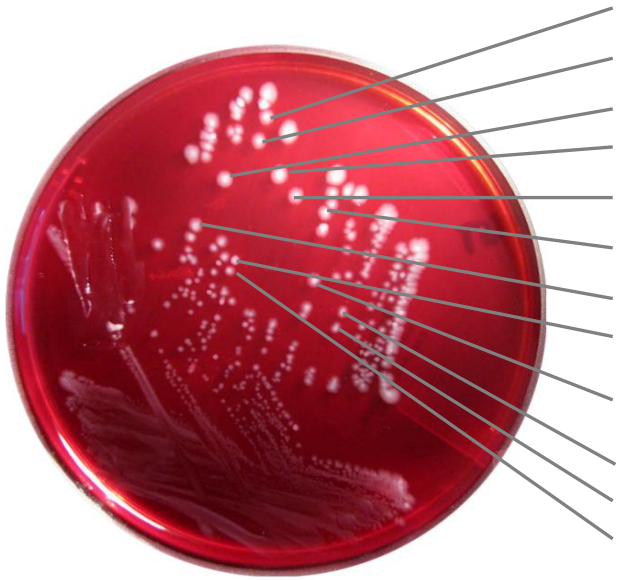
Hospital 1 – mixture of NAP1 and non-NAP1

Hospital 2 – heterogenous NAP1

Hospital 3 – homogenous NAP1

Population analysis

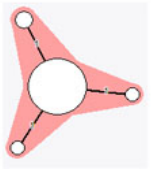
Diversity of MLVA variants isolated from a single specimen

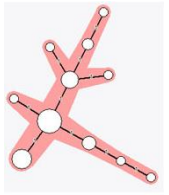


25	23	16	26	9	18
26	23	16	26	9	18
24	23	16	26	9	18
26	23	16	26	9	18
25	23	15	26	9	18
25	23	15	26	9	18
25	24	15	26	9	18
25	23	15	26	9	18
26	25	15	26	9	18
26	25	15	26	9	18
25	23	15	26	9	18
25	23	15	26	9	18

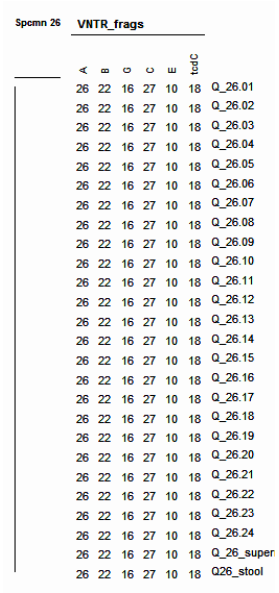
Observed variations in isolates from a single specimen

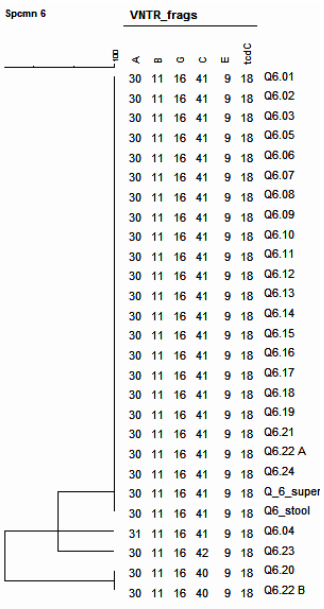
Minimum spanning trees

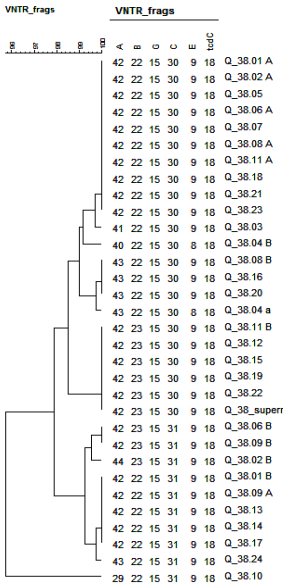




Cluster analysis







Monoclonal 2%

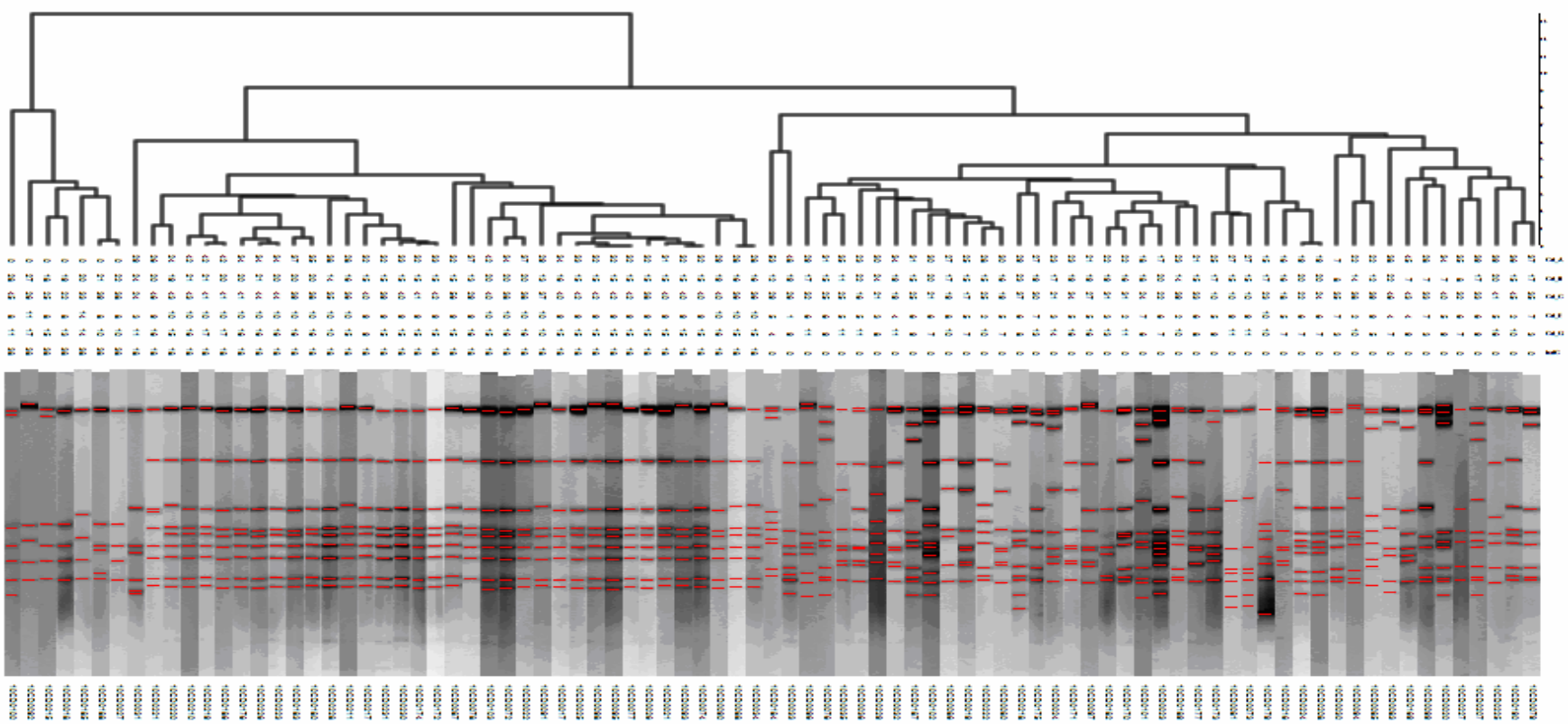
Low diversity <95% 90%

High diversity >95% 8%

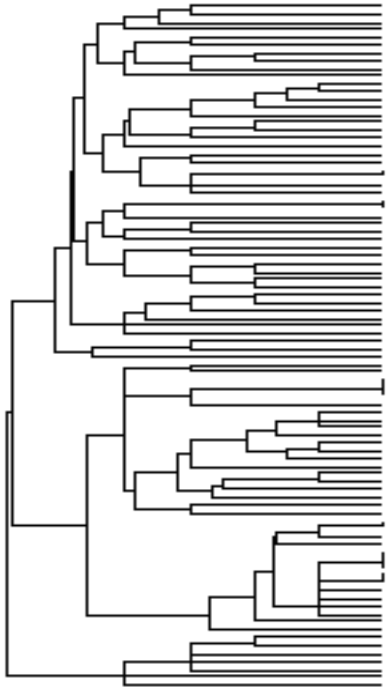
Application of MLVA

Surveillance of CD in a hospital (6 month period)

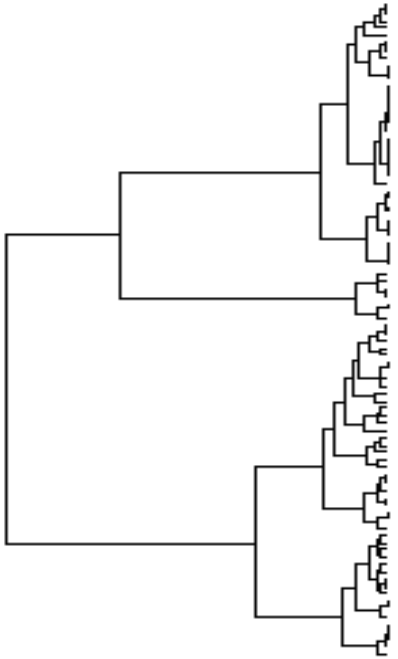
MLVA correlates with PFGE



Interpretation of MLVA differs based on similarity method used



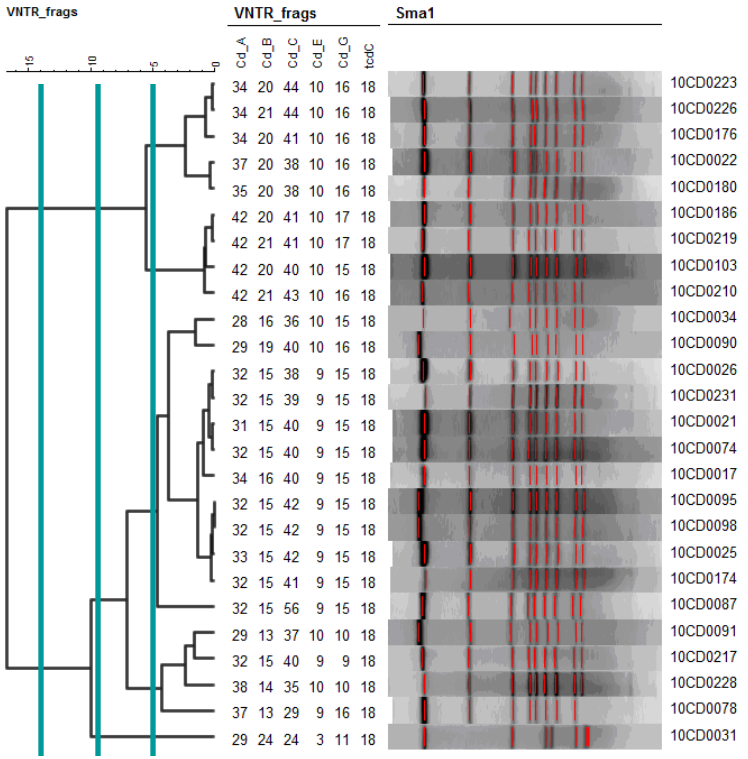
Categorical



Distance-based

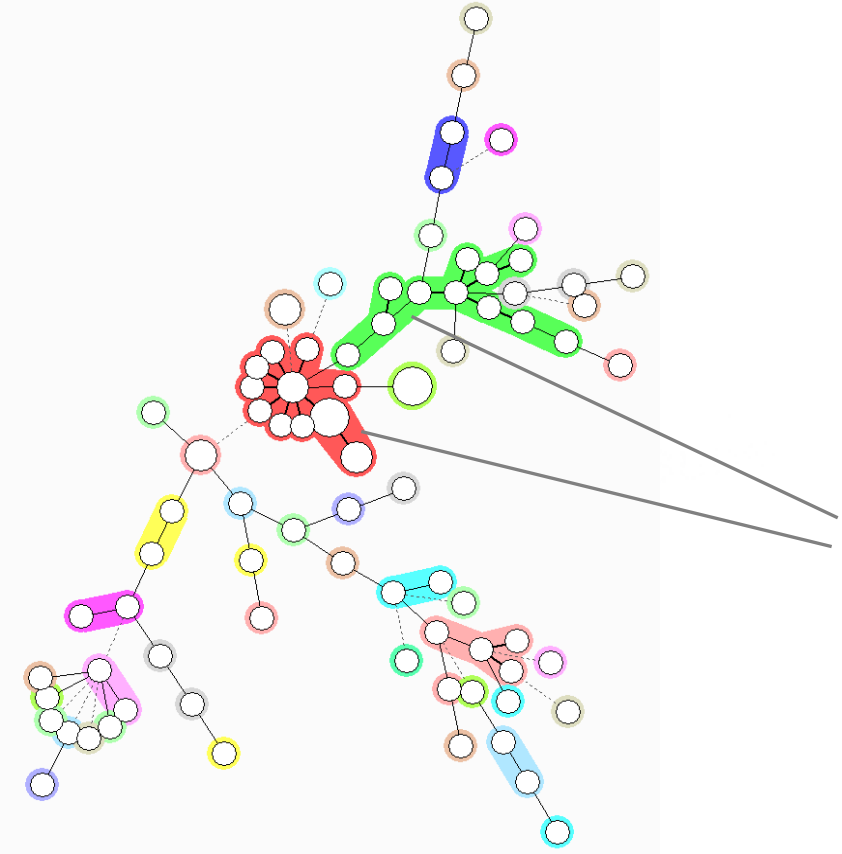
Comparison of PFGE and MLVA for isolates with tcdC 18 bp deletion (NAP1)

Manhattan Distance based clustering



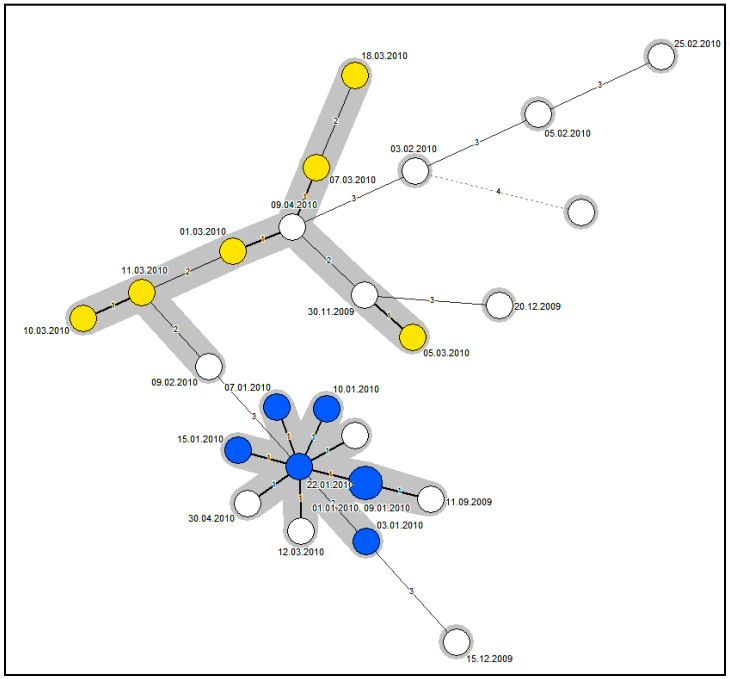
MLVA minimum spanning tree

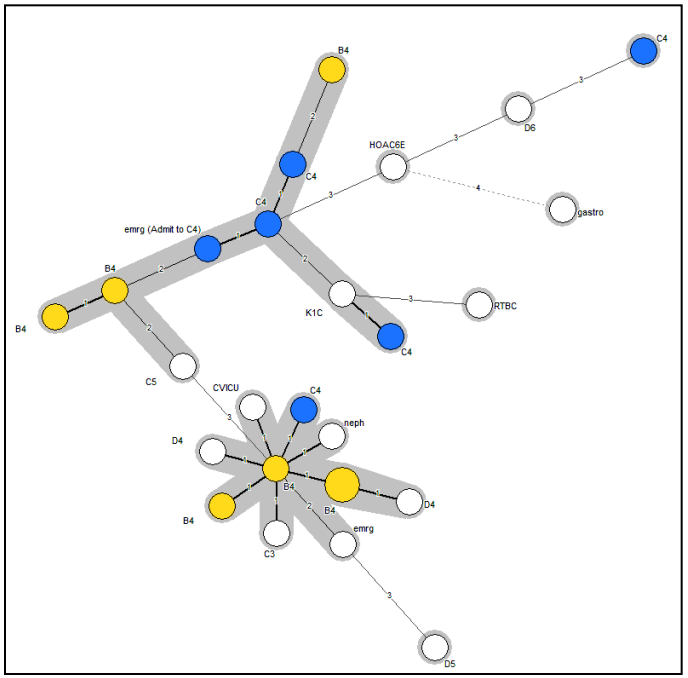
Advanced clustering analysis of outbreak



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