Application of PCR-ribotyping and MultiLocus Variable number tandem repeat Analysis (MLVA) to investigate outbreaks of *Clostridium difficile* infection in Scotland.

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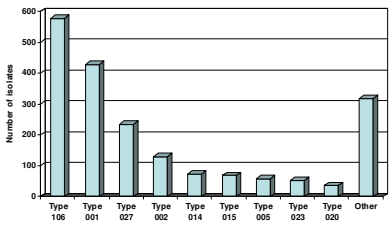
Introduction

In response to a significant increase in the number of laboratory reports of *Clostridium difficile* Infection (CDI) in Scotland between 1995 and 2005, a national mandatory surveillance programme was introduced in all healthcare settings in 2006. Laboratory support, in the form of molecular typing by PCR-ribotyping, has been provided by SSSCDRL since November 2007.

It was apparent from early on that the distribution of ribotypes observed in clinical disease was characterised by the predominance of a small number of ribotypes (Figure 1). This results in reduced discriminatory ability of PCR-ribotyping for epidemiological investigation of clusters of CDI cases.

A recent study of methods for subtyping *C.difficile* [1] concluded that only MLVA and restriction endonuclease analysis had the potential to provide sufficient discriminatory power to distinguish between strains from different outbreaks. The aim of this study was to investigate the ability of MLVA to provide data which could inform the molecular epidemiological investigation of CDI in local outbreaks and in the context of the national population in Scotland.

Figure 1: Distribution of ribotypes submitted to SSSCDRL from cases of CDI in Scotland between November 2007 and May 2010



Methods

Three hundred and fifty isolates of C.difficile from cases of CDI, submitted to SSSCDRL over a period of 30 months from all health board areas, from healthcare associated outbreaks, sporadic cases, and community cases as well as multiple isolates from individual patients, were analysed by the MLVA method according to van den Berg et al [2] with modifications [3]. Ribotypes included 001, 002, 005, 014, 015, 020, 023, 027, 078 & 106. The number of tandem repeats was determined and results for the seven MLVA loci were analysed using eBurst v3.0 [4].

Results and Discussion

DIVERSITY IS NOT EQUAL FOR ALL ALLELES

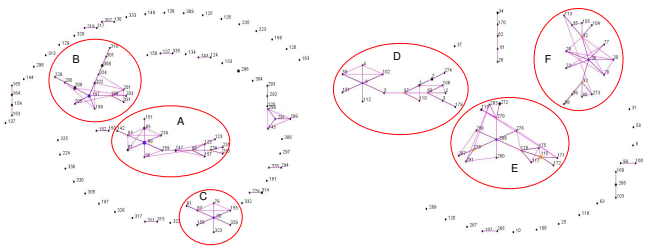
MLVA Allelic Diversity was calculated for unrelated isolates using Simpson's Index of Diversity [5] as described by Hunter & Gaston [6]. Little variation was detected for some alleles (CdF3 & CdH9) in all ribotypes examined while others were highly variable regardless of ribotype (eg. CdB7, CdC6 & CdA6). PCR products were not obtained for some alleles for isolates of ribotype 078 (CdB7 – 4/8 isolates; CdC6 – 8/8 isolates). The published alleles may not be universally applicable to all strains of *C.difficile*.

Simpson's Index of Diversity (D) for MLVA alleles

Ribotype		CdB7	CdF3	CdE7	CdC6	CdA6	CdH9	CdG8	Combined alleles
027	**No. of types**	9	3	7	15	22	2	11	79
n = 97	**D value**	*0.72*	*0.46*	*0.14*	*0.86*	*0.93*	*0.02*	*0.79*	*0.993*
106	**No. of types**	*13*	*2*	*2*	*23*	*16*	*2*	*5*	*106*
n = 128	**D value**	*0.82*	*0.05*	*0.50*	*0.92*	*0.88*	*0.03*	*0.34*	*0.995*
005	**No. of types**	*15*	*2*	*7*	*23*	*19*	*1*	*10*	*47*
n = 48	**D value**	*0.91*	*0.51*	*0.79*	*0.95*	*0.94*	*0.00*	*0.78*	*0.999*

Figure 2

Ribotype 106 **Ribotype 027**



IDENTIFICATION OF CLUSTERS

The use of analytical software such as eBurst allows the visualisation of relationships between isolates depending on the observed differences in tandem repeat numbers at each of the seven alleles. In the above examples, the minimum number of identical loci for group definition was 4, the minimum single locus variant (SLV) count for subgroup definition was 3 and 1000 resamplings were used for bootstrapping. SLV are indicated by pink lines.

A number of major clusters were observed among isolates of ribotype 027 and 106 isolated from all over Scotland between November 2007 and May 2008 (Figure 2).

Cluster A: 20/25 isolates of ribotype 106 from Health Board A, Dec 2007 – Dec 2009

Cluster B: 17/24 isolates of ribotype 106 from Health Board A, Dec 2009 – May 2010

Cluster C: 7/9 isolates of ribotype 106 from Health Board B, Jan 2008 – Mar 2010

Cluster D: 17/20 isolates of ribotype 027 from Health Board C, Feb 2009 – May 2010

Cluster E: 24/27 isolates of ribotype 027 from Health Board D, Aug 2009 – May 2010

Cluster F: 17/19 isolates of ribotype 027 from Health Board A, Mar 2009 – Dec 2009

All clusters confirmed suspected outbreaks but also incorporated isolates separated from the outbreaks by time. This would support a hypothesis of long term circulation of related isolates within local healthcare systems. Changes in these "circulating" clones were also observed as evidenced by a change in subtypes of ribotype 106 isolates from cluster A to cluster B in one Health Board at the end of 2009.

CONCLUSIONS

PCR-ribotyping is a valuable tool for subtyping of C.difficile isolates from cases of CDI but lacks sufficient discriminatory power to distinguish between strains from different outbreaks.

The use of MLVA as a supplemental subtyping method to PCR-ribotyping for C.difficile is highly discriminatory and was able to discern outbreaks through geographical and temporal clustering.

Clear regional clustering was observed for some ribotypes, possibly indicating circulation of related types within areas over extended periods.

Some loci (CdF3 and CdH9) were found to be highly conserved within ribotypes while some MLVA loci were not amplified in all ribotypes. The technique may not, therefore, be universally applicable to all subtypes of *C.difficile*.

MLVA is a valuable genotyping method that can be applied to outbreak detection and epidemiologic investigations of *C.difficile*-associated disease.

The cost effectiveness of MLVA can be maximised by using it as a secondary subtyping method following PCR-ribotyping.

The interpretation of MLVA data will be facilitated by comparison of data with local databases of results from a geographically and temporally diverse collection.

REFERENCES

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