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Characterization of *Clostridium difficile* PCR Ribotype 078 from human and animal origin

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Characterization and MLVA typing of *C. difficile* PCR Ribotype 078

- Isolates originated from human diarrhoeal patients (n=54) from the year 2005, 2006 and 2007
- Diarrhoeal pig isolates(n=11, from 2 pigfarms)
- Toxin gene PCR (*tcdA*, *tcdB*, Binary toxin)
- *ErmB* gene
- Susceptibility pattern (moxifloxacin, ciprofloxacin)
- *tcdC* gene sequencing
- Multi Locus Variable Number Tandem Repeat Analysis (MLVA)

Results characterization type 078

- Human (n=43), pig isolates (n=8)
 - *TcdA* (100%), *TcdB* (100%),
 - Binary toxin (100%),
 - *ErmB* gene (14%): Human isolates (7%), pig isolates (50%)

- Susceptibility (breakpoints CLSI, 2007)
 - Type 078 resistant:
 - moxifloxacin 12% (MIC \geq 4 mg/l)
 - ciprofloxacin 88% (MIC \geq 4 mg/l)
 - “Historic” type 027: moxifloxacin S, ciprofloxacin R
 - “Epidemic” type 027: moxifloxacin R, ciprofloxacin R

TcdC sequencing

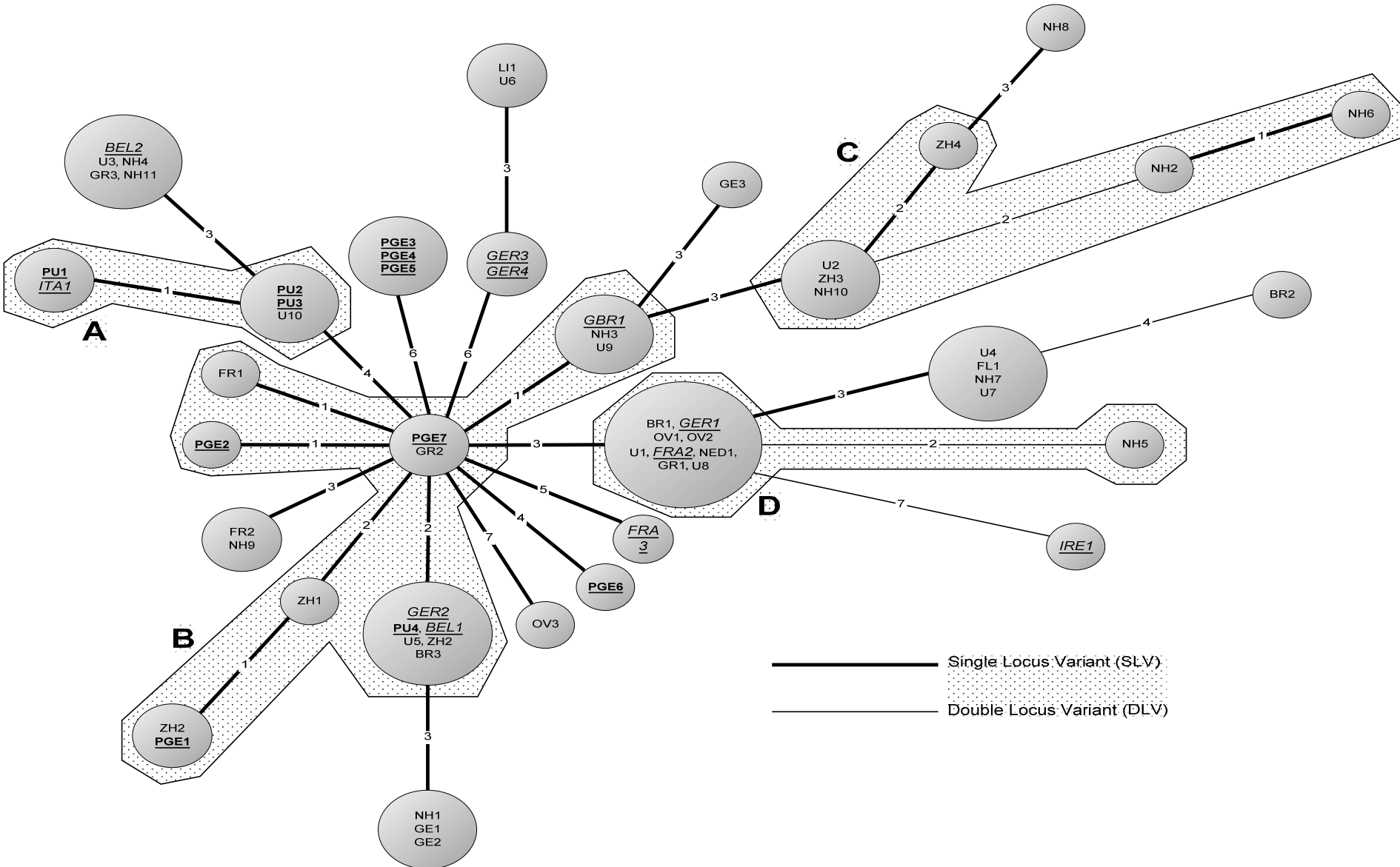
	100	100	110	120	130	140	150	160
630	100	CTACTGGCATT	TATTTT	AGGC	GTGTTTTTT	TGGCAATATAT	CCTCACCAGCTT	GTTCTGAAGA
Ribotype 078	100	CTACTGGCATT	TATTTT	TGGT	GTGTTTTTT	TGGCAATATAT	CCTCACCAGCTT	GTTCTGAAGA
Ribotype 027	100	CTACTGGCATT	TATTTT	-GGT	GTGTTTTTT	TGGCAATATAT	CCTCACCAGCTT	GTTCTGAAGA

	161	160	170	180	190	200	210	220
630	161	ACCATGAGGAGG	TCATTTCTAA	CTAA	CAACATCAGTT	TATAGATTCT	CAAAAAACAGAA	TAGAA
Ribotype 078	161	ACCATGAGGAGG	TCATTTCTAA	TAA	CAACATCAGTT	TATAGATTCT	CAAAAAACAGAA	TAGAA
Ribotype 027	160	ACCATGAGGAGG	TCATTTCTAA	TC	CAACATCAGTT	TATAG	TTCTCAAAAAACAGAA	TAGAA

Results MLVA

- Human isolates (n=54) and pig isolates (n=11)
- Highly discriminatory genotyping method
 - PCR based on tandem repeats on 7 loci
 - Fragment analysis on ABI 3100
 - Data analysis: Bionumerics
- Genetic relationship between strains of the same ribotypes

Results MLVA



Conclusions

- Susceptibility pattern type 078 identical to historic type 027 isolates
- Identical toxin profiles
 - Human and pig isolates
 - Ribotype 078 vs 027
- Point mutations leads to non-functional *TcdC* gene
- Genetic relationships between human and pig isolates
- Ribotype 078 has the same virulence markers as the highly virulent ribotype 027