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Welsh Healthcare Associated Infections Programme (WHAIP)
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Clostridium difficile PCR Ribotype Surveillance in Wales

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Summary

- 175 *C. difficile* samples from Health Boards in Wales were PCR ribotyped and tested for susceptibility to 6 antibiotics.
- A total of 26 PCR ribotypes were identified. PCR ribotype 027 was the most commonly isolated strain, accounting for 50% of the sample.
- 027 predominated in all Health Boards, but not in Velindre NHS Trust. The proportion of samples that were 027 varied from 77% in Aneurin Bevan to 21% in Hywel Dda.
- There has been a large increase in the frequency of PCR ribotype 027 and a large decrease in the frequency of PCR ribotype 001 in Wales since the original pilot PCR ribotyping survey in 2005.
- No MIC levels were detected that might equate to clinical resistance to metronidazole; however, the 2 most common PCR-ribotypes (027, 106) did have higher MICs than the less common strains. Types 027 and 106 were resistant to erythromycin and moxifloxacin whereas the majority of other strains were sensitive to both agents.

1. Introduction

Because of increasing concerns regarding the incidence and severity of cases of *Clostridium difficile* across the UK, a *C. difficile* Task Group was set up in Wales by the Welsh Healthcare Associated Infection Sub-Group (WHAISG) of the Welsh Assembly Government. The task group made recommendations regarding changes to the surveillance of *C. difficile* in Wales, which were agreed by the WHAISG in May 2008. One of the changes was to develop a regular survey of the *C. difficile* PCR ribotypes that are causing disease in Wales, coupled with enhanced surveillance of the severity and outcome of disease. This would allow some understanding to be gained on the contribution different PCR ribotypes of *C. difficile* are currently making to the epidemiology of *C. difficile* in Wales.

The first snapshot PCR ribotyping surveillance was carried out in 2008, which also incorporated an enhanced surveillance questionnaire. This report presents the results of the second snapshot surveillance programme which took place at the end of 2009/beginning of 2010. No additional surveillance information was collected for this study.

2. Methods

Between November 2009 and January 2010, Health Boards in Wales submitted a pre-determined number of stool samples positive for *C. difficile* toxins for culture and PCR ribotyping. The number of samples requested from each Health Board was based on the numbers of cases reported in the mandatory *C. difficile* surveillance scheme in the first 6 months of 2009. The overall aim was to process 200 samples from Wales.

Stool samples positive for *C. difficile* toxins were sent for culture at 3 different regional laboratories. The specimens from Cardiff & Vale, Aneurin Bevan and Cwm Taf Health Boards and Velindre NHS Trust were cultured in PHW Microbiology Cardiff; the ones from Abertawe Bro Morgannwg University and Hywel Dda Health Boards were cultured in PHW Microbiology Swansea and the specimens from Betsi Cadwaladr Health Board were cultured in PHW Microbiology Rhyl. Isolates of putative *C. difficile* were then sent to the Anaerobe Reference Laboratory (ARL) in Cardiff for PCR ribotyping and susceptibility testing. Isolates were susceptibility tested against 6 antibiotics (metronidazole, vancomycin, erythromycin, moxifloxacin, co-amoxiclavulanate and piperacillin/tazobactam) using the E test method (AB Biodisk, Solna, Sweden). The following breakpoints were used: metronidazole =>16µg/ml; vancomycin =>4µg/ml; erythromycin=>4µg/ml; moxifloxacin=>32µg/ml; co-amoxiclavulanate =>16µg/ml; piperacillin-tazobactam =>128 µg/ml. PCR ribotyping results were returned to the culture centres, the originating laboratories and WHAIP for analysis.

3. Results

3.1 PCR Ribotyping

- 175 *C. difficile* samples from Health Boards around Wales (Table 1) were PCR ribotyped and tested for susceptibility to 6 antibiotics.

Table 1. Number of *C. difficile* samples PCR ribotyped by site for the PCR ribotyping survey in Wales, 2010

Region (Culture Centre)	Trust	No of Samples
South East Wales (PHW Microbiology Cardiff)	Aneurin Bevan	31
	Cardiff & Vale	43
	Cwm Taf (North)	5
	Cwm Taf (South)	5
	Velindre	3
South West Wales (PHW Microbiology Swansea)	ABMU (East)	10
	ABMU (West)	18
	Hywel Dda (Carmarthenshire)	7
	Hywel Dda (Ceredigion)	2
	Hywel Dda (Pembrokeshire)	5
North Wales (PHW Microbiology Rhyl)	Betsi Cadwaladr (Central)	20
	Betsi Cadwaladr (East)	13
	Betsi Cadwaladr (West)	13

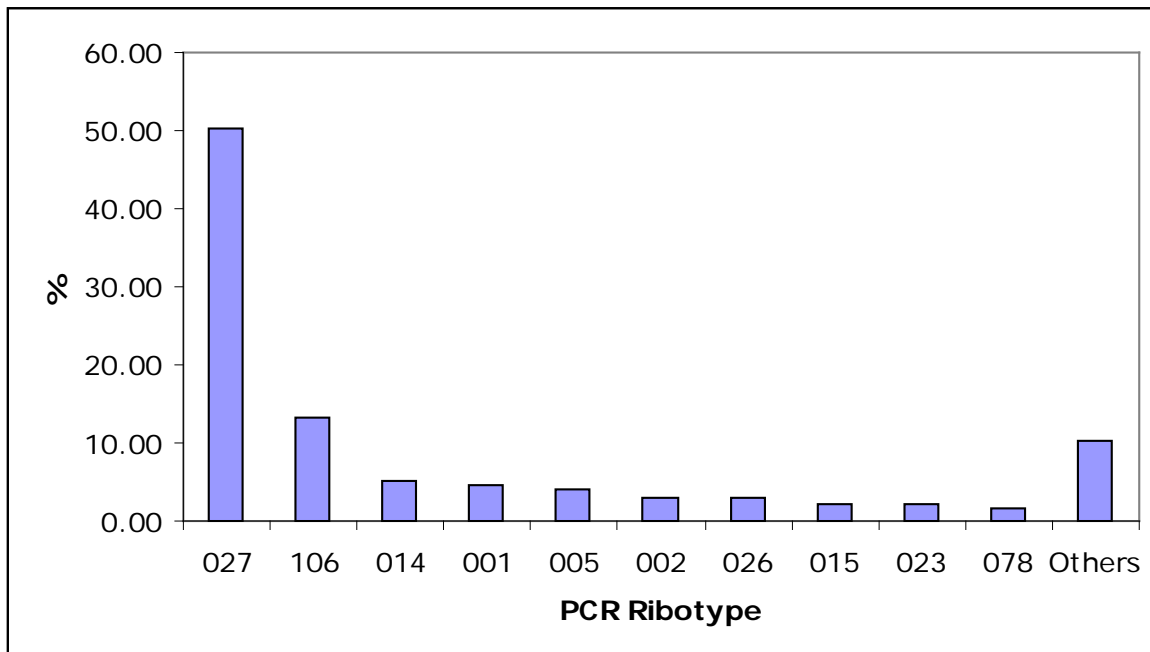
- 82% of samples originated from patients in major acute hospitals within the Health Boards, 11% from other hospitals in the Health Boards and 7% were from GP patients. The proportion of each varied by Health Board (Table 2). The location of the patient when the sample was submitted does not necessarily reflect the location of acquisition of the *C. difficile*.

Table 2. Proportion of Health Board *C. difficile* samples by source location for the PCR ribotyping survey in Wales, 2010

Health Board	% Major Acute Hospital	% Other Hospital	% GP
ABMU	96	4	0
Aneurin Bevan	68	29	3
Betsi Cadwaladr	70	17	13
Cardiff & Vale	100	0	0
Cwm Taf	90	10	0
Hywel Dda	64	0	36
Velindre NHS Trust	100	0	0

- A total of 26 PCR ribotypes were identified. PCR ribotype 027 was the most commonly isolated strain, accounting for 50% of the sample (Figure 1).

Figure 1. Percentage distribution of PCR ribotypes of *C. difficile* in the PCR ribotyping survey in Wales, 2010



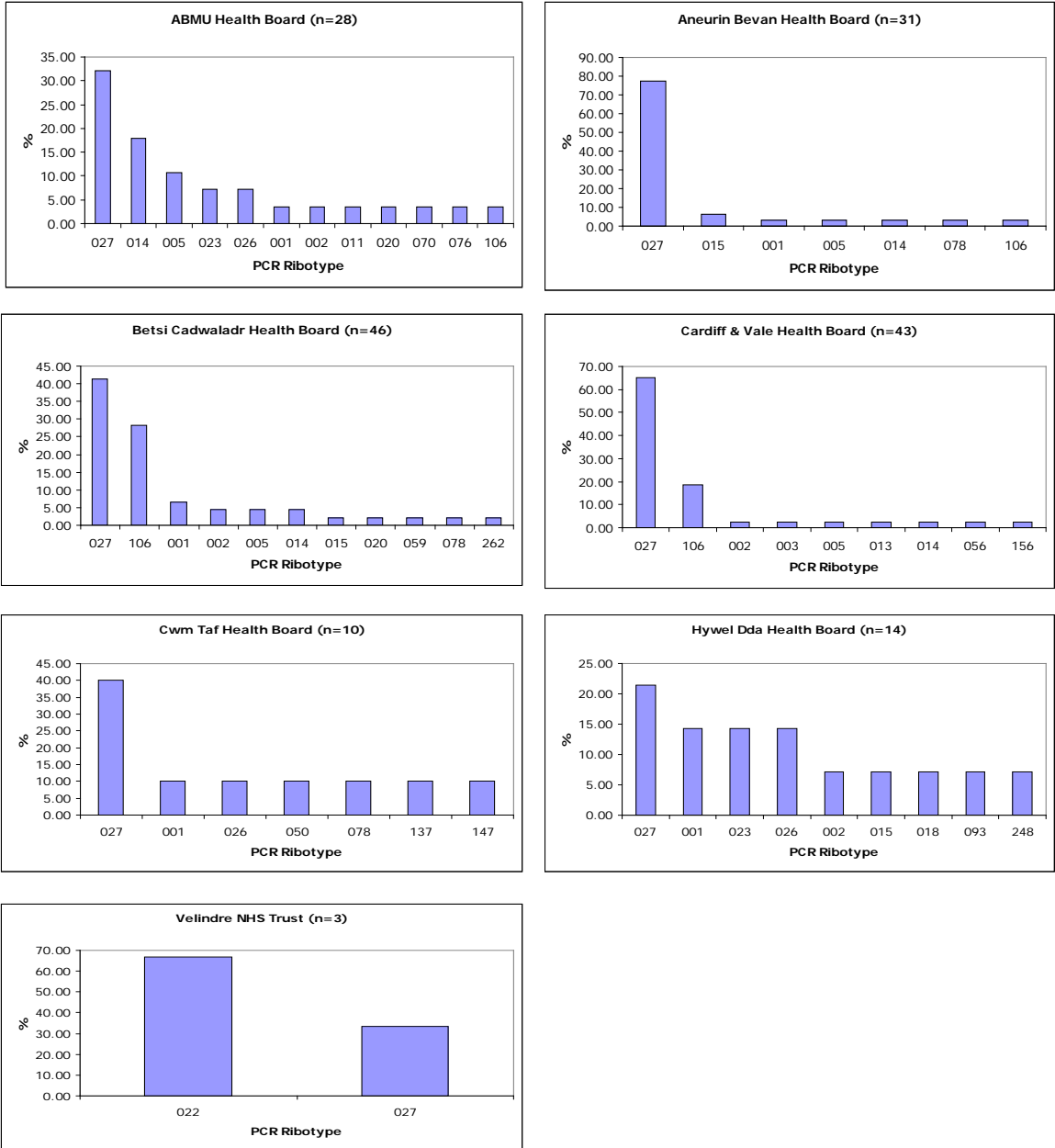
- PCR ribotype 027 predominated in the samples from major acute hospitals, other hospitals and GPs, but represented a much lower proportion of the GP samples (Table 3).

Table 3. Percentage distribution of the common *C. difficile* PCR ribotypes by location of source patient in the PCR ribotyping survey in Wales, 2010

PCR Ribotype	Major Acute Hospital (n=144)	Other Hospital (n=19)	GP (n=12)
027	51%	63%	25%
106	14%	5%	17%
014	6%	0	0
001	3%	16%	8%
others	26%	16%	50%

- Results were not uniform across Health Boards (Figure 2). PCR ribotype 027 was reported from all Health Boards, but the proportion of the sample varied. The highest proportion of PCR ribotype 027 was in Aneurin Bevan Health Board (77%) and the lowest in Hywel Dda (21%).

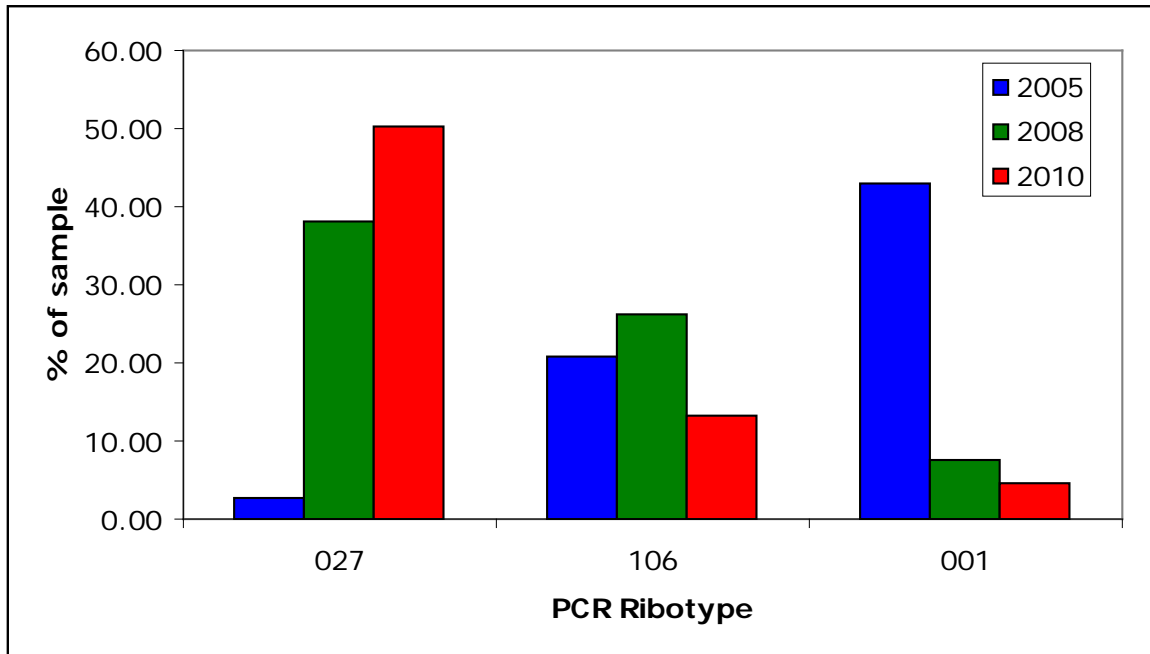
Figure 2. Percentage distribution of PCR ribotypes of *C. difficile* by Health Board in the PCR ribotyping survey in Wales, 2010



- PCR ribotype 106 was the second most common ribotype overall. It was reported from 4 Health Boards, but more than 90% was reported from Betsi Cadwaladr (13/23) and Cardiff & Vale (8/23) combined.
- PCR ribotype 014 was the third most common ribotype overall. It was reported from 4 Health Boards, but more than 50% (5/9) of samples were from ABMU Health Board.
- There has been a large increase in the frequency of PCR ribotype 027 and a large decrease in the frequency of PCR ribotype 001 in Wales since the original pilot PCR ribotyping survey in 2005. PCR

ribotype 106 increased in frequency between 2005 and 2008, but the proportion of this ribotype has reduced in the current survey (Figure 3).

Figure 3. Changes in the percentage distribution of *C. difficile* PCR ribotypes 027, 106 and 001 in the PCR ribotyping surveys in Wales, 2005, 2008 and 2010



3.2 Antibiotic Susceptibility Results

- No MIC levels were detected that might equate to clinical resistance to metronidazole; however, the 2 most common PCR-ribotypes (027, 106) did have higher MICs than the less common strains. Types 027 and 106 were resistant to erythromycin and moxifloxacin whereas the majority of other strains were sensitive to both agents. More detailed results are provided in Appendix A.

4. Acknowledgements

We would like to acknowledge the staff of the ARL, WHAIP and the microbiology laboratories in the Health Boards in Wales, for their contributions to this report.

5. Appendix A

Antibiotic Susceptibility Testing Results

5.1. Metronidazole

The MICs of all the *C. difficile* isolates to metronidazole are shown in Figure 4. No MIC levels that might equate to clinical resistance to metronidazole were detected. However, the MIC₅₀ and MIC₉₀ levels for metronidazole against the 2 most common PCR ribotypes (027, 106) are several dilutions higher, and their MIC ranges larger, compared to the less common strains (See Table 4).

Figure 4. MICs of *C. difficile* to metronidazole in the enhanced PCR ribotyping survey in Wales, 2010

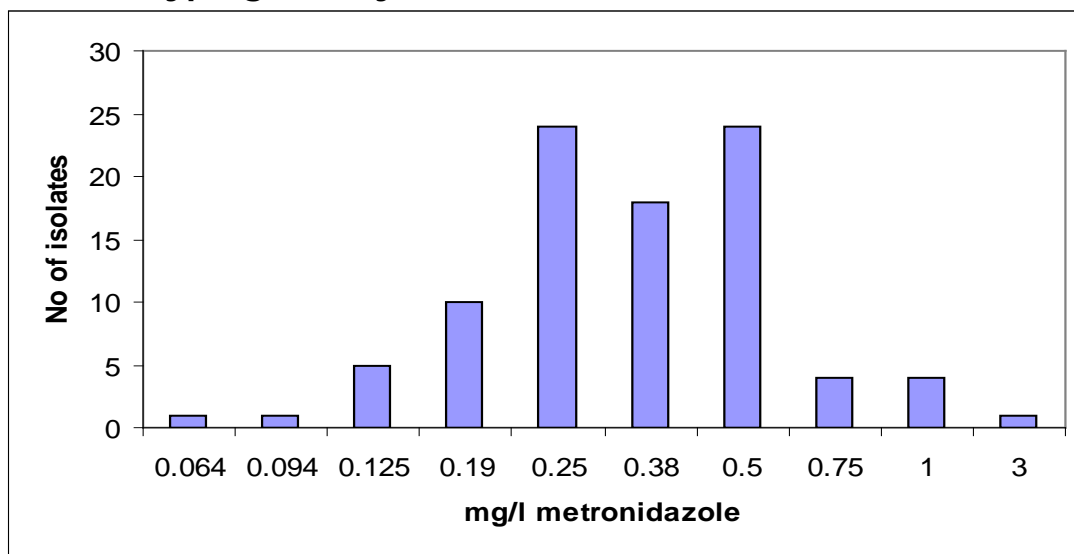


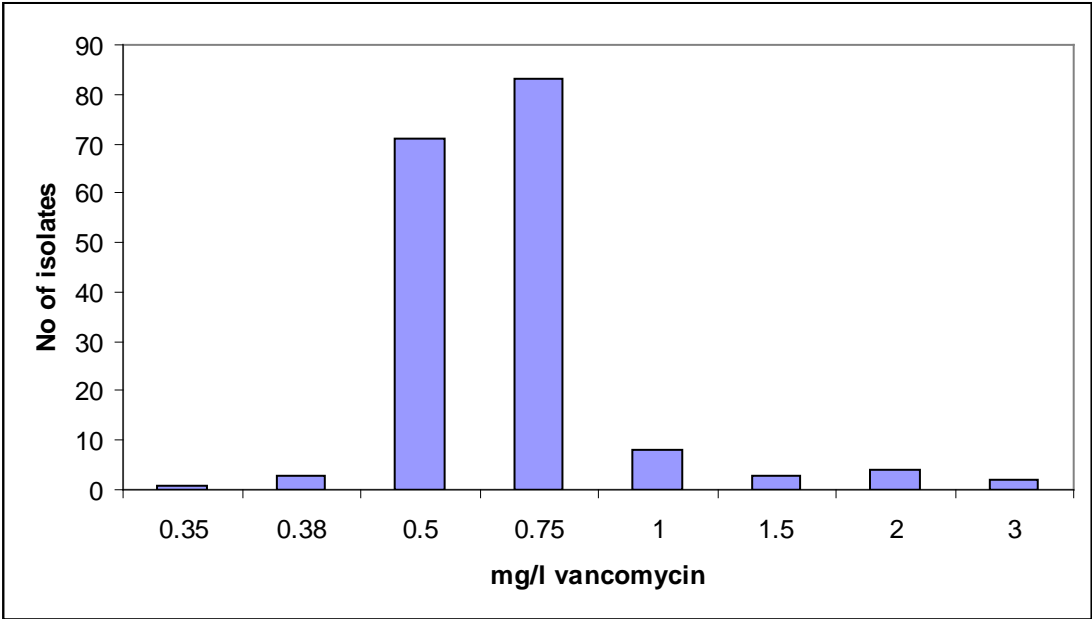
Table 4. MICs of *C. difficile* isolates to metronidazole by PCR ribotype in the enhanced PCR ribotyping survey in Wales, 2010

PCR Ribotype	N	Mean MIC	Median MIC	Range
027	88	0.38	0.38	0.125 - 1
106	23	0.35	0.38	0.094 - 1
Others combined	64	0.21	0.19	0.032 - 0.5

5.2. Vancomycin

The MICs of all the *C. difficile* isolates to vancomycin are shown in Figure 5. There is no evidence of raised MICs to vancomycin, with MICs for all PCR ribotypes ranging from 0.35 to 3.0 mg/l.

Figure 5. MICs of *C. difficile* to vancomycin in the enhanced PCR ribotyping survey in Wales, 2010



5.3. Erythromycin and Moxifloxacin

The MICs of all the *C. difficile* isolates to erythromycin and moxifloxacin are shown in Figures 6 and 7. All isolates of PCR-ribotypes 027 and 106 were resistant to erythromycin and moxifloxacin (see Table 5) whereas the majority of other strains were sensitive to both agents. Combined resistance to these agents is a good indicator of a common PCR-ribotype.

Figure 6. MICs of *C. difficile* to erythromycin in the enhanced PCR ribotyping survey in Wales, 2010

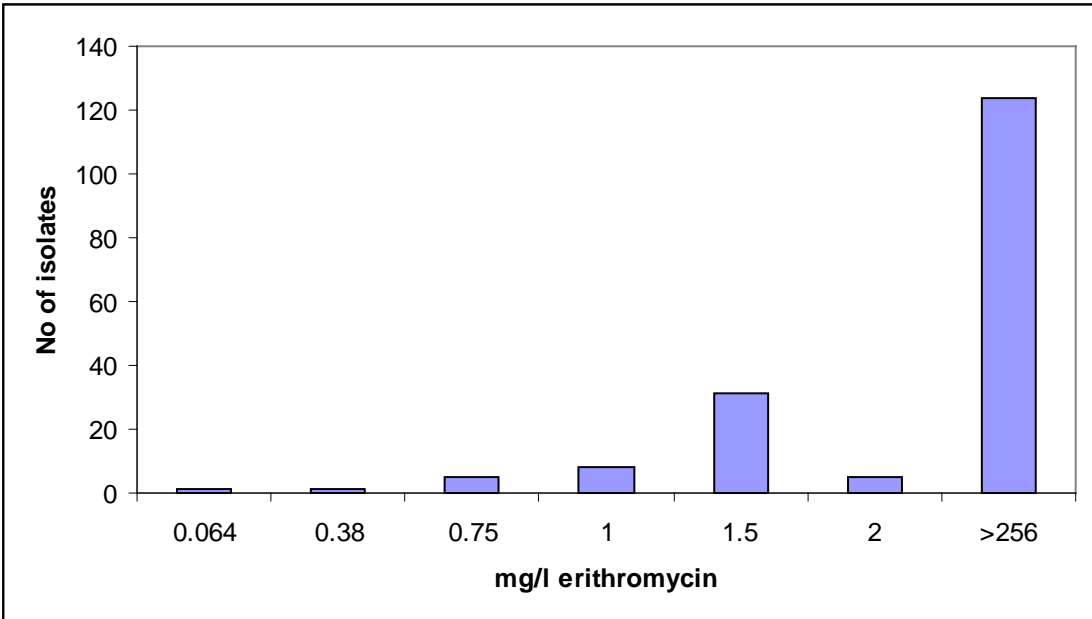


Figure 7. MICs of *C. difficile* to moxifloxacin in the enhanced PCR ribotyping survey in Wales, 2010

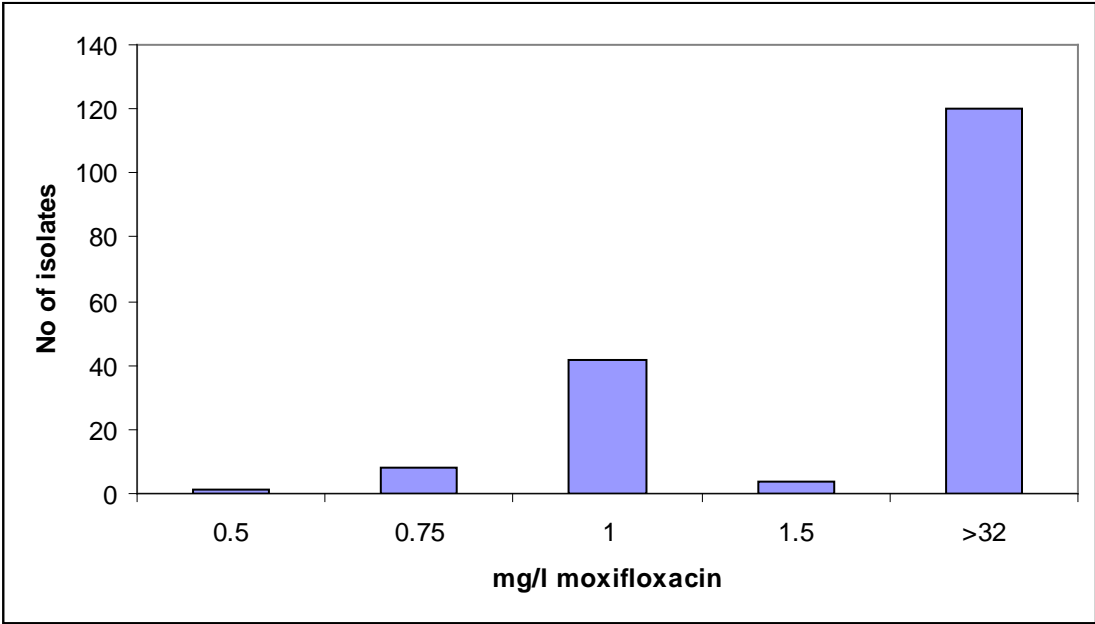


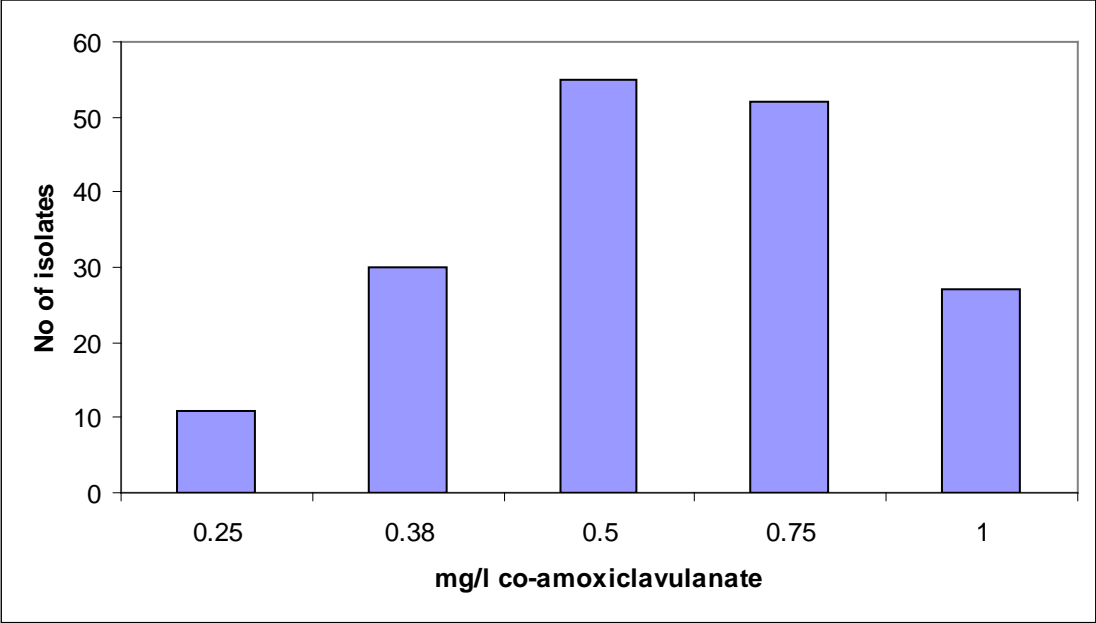
Table 5. MICs of *C. difficile* isolates to erythromycin and moxifloxacin by PCR ribotype in the enhanced PCR ribotyping survey in Wales, 2010

PCR Ribotype (N)	Antibiotic	Mode MIC	Range
027 (N=88)	erythromycin	>256	>256 only
	moxifloxacin	>32	>32 only
106 (N=23)	erythromycin	>256	>256 only
	moxifloxacin	>32	>32 only
Others combined (N=64)	erythromycin	1.5	0.064 ->256
	moxifloxacin	1	0.5 - >32

5.4. Co-amoxiclavulanate

The MICs of all the *C. difficile* isolates to co-amoxiclavulanate are shown in Figure 8. Co-amoxiclavulanate has a high degree of activity against all ribotypes, MIC range 0.25 – 1.0mg/L.

Figure 8. MICs of *C. difficile* to co-amoxiclavulanate in the enhanced PCR ribotyping survey in Wales, 2010



5.5. Piperacillin-Tazobactam

The MICs of all the *C. difficile* isolates to piperacillin-tazobactam are shown in Figure 9. This agent has high activity against *C. difficile*.

Figure 9. MICs of *C. difficile* to piperacillin-tazobactam in the enhanced PCR ribotyping survey in Wales, 2010

