CHANGES IN PREVALENT PCR RIBOTYPES OF *CLOSTRIDIUM DIFFICILE* AND THEIR ANTIMICROBIAL RESISTANCE IN A TERTIARY CARE KOREAN HOSPITAL OVER 10 YEARS

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INTRODUCTION

Resistance to antimicrobial agents in *Clostridium difficile* may have played a part in its selection in the hospital environment. The aim of this study was to evaluate the relevance of changes in prevalence of various PCR ribotypes of *C. difficile* over a 10-year period and to investigate the antimicrobial resistance of *C. difficile* within predominant ribotypes.

MATERIALS & METHODS

· Bacterial isolates A total of 1407 unduplicated clinical isolates of *C. difficile* recovered from patients with diarrhea in a tertiary care hospital in Korea from 2000 to 2009 was analyzed. Reference strains of *C. difficile* VPI 10463, 3608/03, SE844, 48489 and 1470 were used in both PCR assays and ribotyping. Quality control strains used for susceptibility testing included *Bacteroides thetaiotaomicron* ATCC 29741 and *B. fragilis* ATCC 25285.

· Toxin analysis by PCR *C. difficile* toxin genes were detected by PCR as described previously [1, 2]. The primer pairs used were NK9-NK11 for the repetitive domain of *tcdA*, NK104-NK105 for *tcdB*, cdtA pos-cdtA rev for *cdtA*, and cdtB pos-cdtB rev for *cdtB*.

· PCR ribotyping PCR ribotyping was performed as previously described with the primers 5′-CTGGGGTGAAGTCGTAACAAGG-3′ (position 1445 to 1466 of the 16S rRNA gene) and 5′-GCGCCCTTTGTAGCTTGACC-3′ (position 20 to 1 of the 23S rRNA gene) [3]. Comparison of PCR ribotyping patterns was performed visually. Strains with ribotype patterns that differed by at least one band were assigned to different types. Ribotype group were designated by upper and lower-case letters combined with a number.

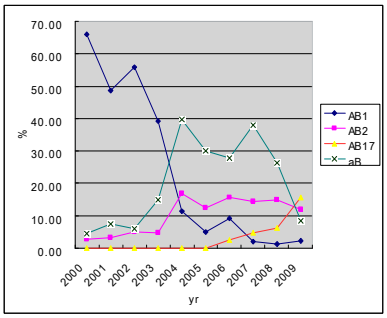
· Antimicrobial susceptibility test Antimicrobial susceptibility tests were performed with a randomly selected 120 *C. difficile* of four ribotypes-showed definite change of prevalence during the study period-using the agar dilution method on Brucella blood agar, according to the recommendations of the CLSI.

· Molecular analysis of the gyrase A and B Genomic regions including the quinolone resistance-determining-region (QRDR) of gyrA and gyrB were amplified and sequenced in the 120 *C. difficile* isolates as previously described methods [4].

RESULTS

1. A total of 74 different ribotype patterns were found. There were 33 patterns within A^+B^+ CDT^- strains, 11 within A^+B^+ CDT^+, 1 A^-B^+ CDT^- and 29 within A^-B^-.
2. Four ribotypes [Ribotype AB1, AB2, AB17 ($A^+B^+CDT^-$) and aB (A^-B^+ CDT^-)] showed definite change of prevalence during the study period. The ribotypes AB1, AB2 and aB are equivalent to UK001, 014 and 017, respectively.

Fig. Changes of prevalence of 4 ribotypes over 10 years.



3. Antimicrobial resistances of 120 *C. difficile* isolates are summarized in Table 1. Antimicrobial resistance was significantly higher for AB17 and lower for AB2 than for the other ribotypes.

Table 1. Antimicrobial resistance (%) of 120 *C. difficile* isolates with indicated ribotype

Ribotypes	CC	E	CTT	CIP	MXF	IPM
AB1	100	100	13	100	22	8
AB2	12	8	0	100	31	0
AB17	100	100	100	100	100	63
aB	100	100	44	100	85	12

Abbreviations: CC, clindamycin; E, erythromycin; CTT, cefotetan; CIP, ciprofloxacin, MXF, moxifloxacin; IPM, imipenem.

4. All isolates with moxifloxacin resistance had a mutation resulting in the amino acid substitution in gyrA or gyrB.

Table 2. Amino acid substitutions in gyrase A & B of moxifloxacin resistant *C. difficile* isolates

Ribotypes	Amino acid substitution		% (MIC)
	Gyrase A	Gyrase B	
AB1	The82→Val		78 (4)/22 (8)
AB2	The82→Ile		4 (16)
		Asp426→Asn	8 (8)
		Asp426→ Val	19 (8)
AB17	The82→Ile		100 (16)
aB	The82→Ile	Asp426→Asn	20 (128-128<)
	The82→Ile		65 (16-32)

CONCLUSIONS

1. Four of 74 ribotypes showed a definite change of prevalence over 10 years.
2. Significant differences of antimicrobial resistance were seen in different ribotypes.
3. Moxifloxacin resistance was associated with gyrase A or gyrase B mutation which is stable and related to ribotypes.

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