MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* AT A HOSPITAL IN JAPAN: SHIFTS IN THE PREDOMINANT PCR RIBOTYPE OVER A FIVE-YEAR PERIOD

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Introduction and Purpose

In Europe and North America, large outbreaks due to hypervirulent strains such as *Clostridium difficile* PCR ribotype 027 have been reported. [1,2] The aim of this study was to investigate the prevalent PCR ribotypes at a hospital in Japan and to know nosocomial transmission of specific strains of *C. difficile* including PCR ribotype 027.

Methods

Subjects were 66 patients who suffered from *Clostridium difficile* infection (CDI) from January, 2005 to December, 2009 in Gifu Red Cross hospital, a general hospital with 352 beds(Figure 1., Table 1.). CDI was defined as diarrhea and a stool sample positive for toxic-culture. *C. difficile* isolates were analyzed for the presence of the toxin genes by PCR and analyzed by PCR ribotyping. [3,4,5]

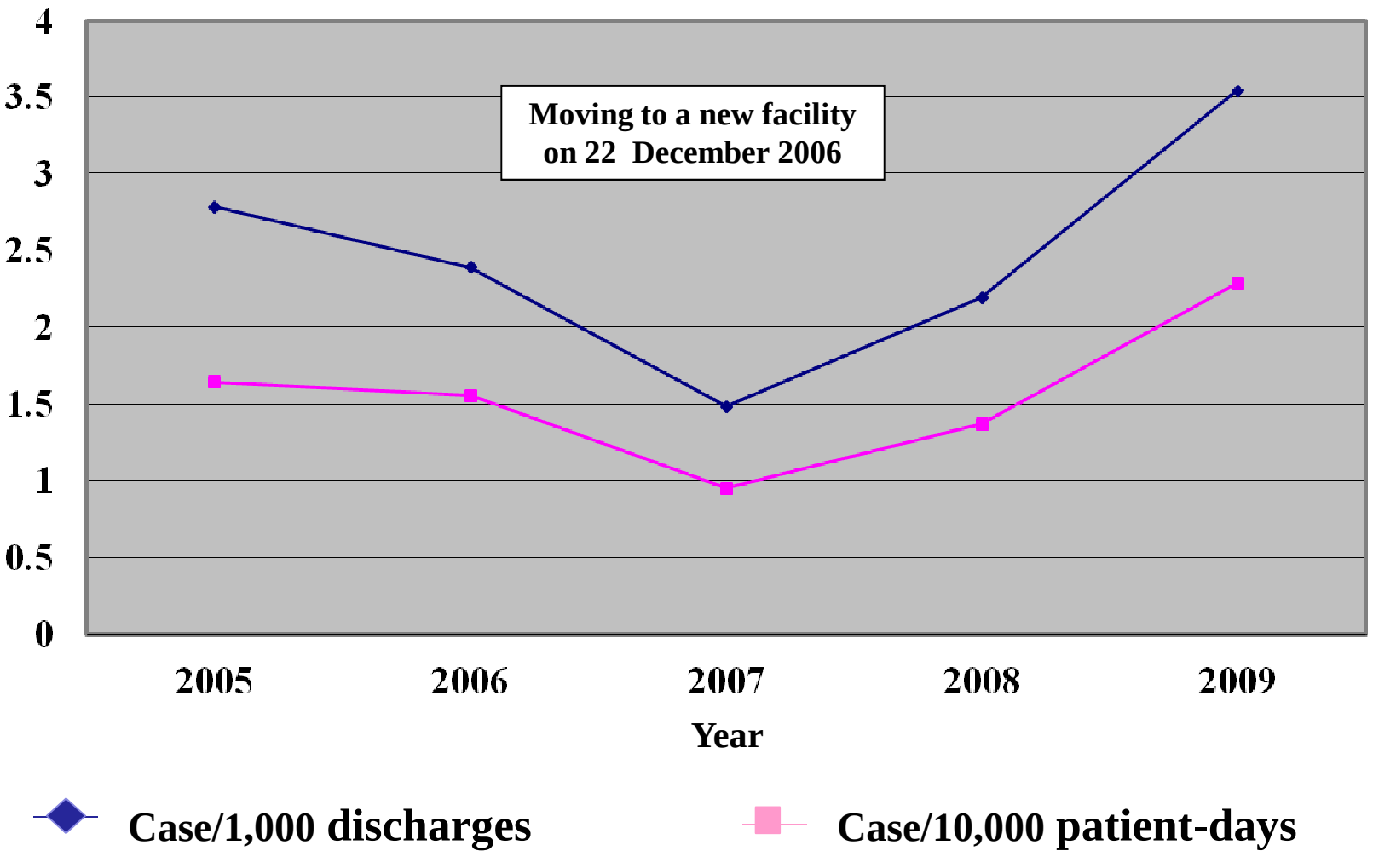


Figure 1. Trends in the incidence of CDI during 5 years from 2005 to 2009.

The number of case during each yearly period was sequentially 13, 12, 8, 12, and 21.

Table 1. Demographics and clinical characteristics in patients with CDI

No. of patients	66
Age (years; mean±SD)	73.5±13.3
Sex (M:F)	33:33
Length of hospital stay (days; mean±SD)	30.8±39.2
Underlying disease	
Digestive diseases	23
Respiratory diseases	19
Diabetes	11
Hematologic malignancy	10
Solid tumor	10
Cerebrovascular diseases	9
Cardiovascular diseases	8

Table 1. Demographics and clinical characteristics in patients with CDI

Origin of CDI	
Healthcare-associated	59
Community-associated	6
Indeterminate	1
Risk factor	
Antimicrobial agents	66
Anticancer drugs	9
Surgery	5
H2RA or PPI	49
Tube feeding	5
Endoscopic findings	
Pseudomembranous colitis	19
Colitis	6
Normal	3
Treatment	
Vancomycin	58
Vancomycin, Metronidazole	4
Reccurence	
Relapse	8
Reinfection	1
Outcome	
Cure of CDI	64
Died of main disease	2
Died of CDI	0

Results

- **A total of 77 isolates of *C. difficile* were recovered from 77 episodes of 66 patients.**
- **The number of isolates obtained during each yearly period was sequentially 20, 12, 8, 14, and 23.**
- **Of the 77 isolates, 46 (59.7%) were toxin A-positive, toxin B-positive, and binary toxin-negative ($A^+B^+CDT^-$), 5 (6.5%) were toxin A-positive, toxin B-positive, and binary toxin-positive ($A^+B^+CDT^+$), and 26 (33.8%) were toxin A-negative, toxin B-positive, and binary toxin-negative ($A^-B^+CDT^-$).**
- **The most predominant type was PCR ribotype trf, the second most frequent type was PCR ribotype smz, and the third frequent type was PCR ribotype 002.**
- **The predominant type shifted from PCR ribotype smz in 2005 to PCR ribotype trf in 2006, and then, to PCR ribotype 002 in 2009.**
- **PCR ribotype 027 was isolated from one patient with pseudomembranous colitis, who had recurrent diarrhea caused by the same PCR ribotype.** [6]
- **Of 9 patients with recurrent CDI, 8 patients relapsed with the original PCR ribotype strain and 1 patient acquired another PCR ribotype strain.**

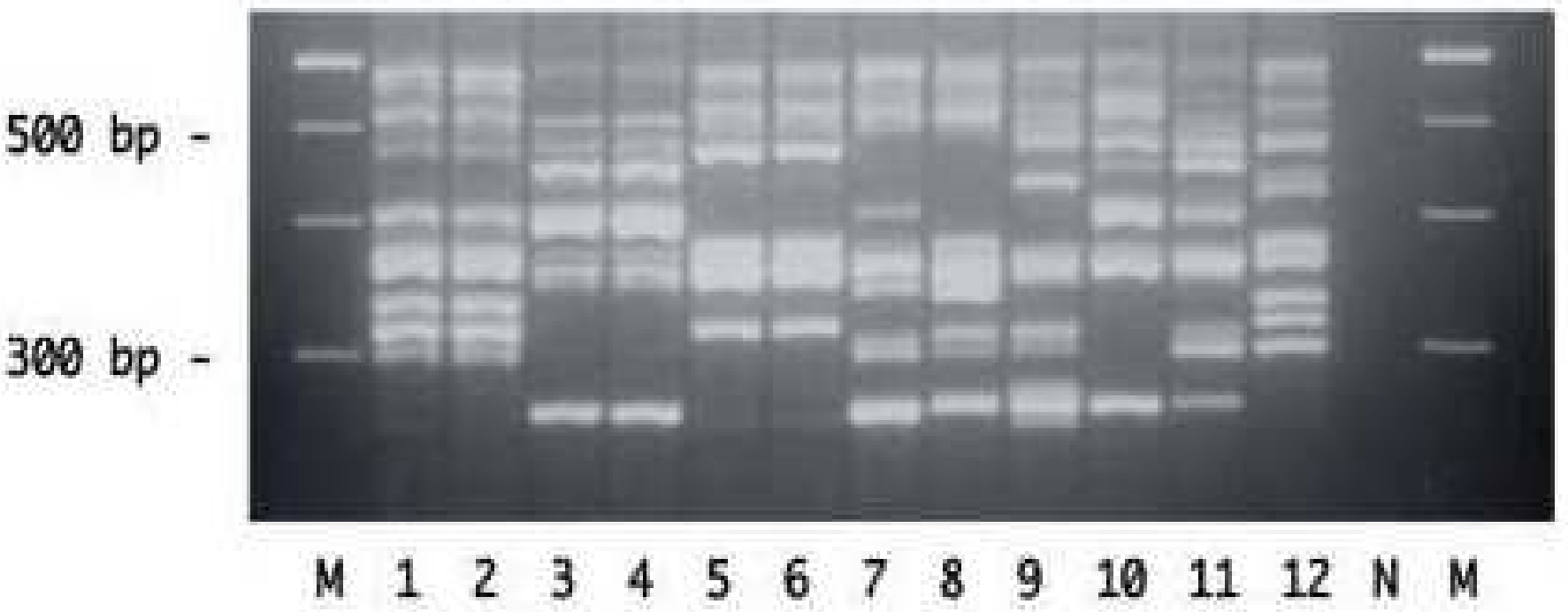


Figure 2. Representative PCR ribotype patterns of *C. difficile* isolates

Lanes 1 and 2, type smz; Lanes 3 and 4, type trf; Lanes 5 and 6, type 002; Lane 7, type 001; Lane 8, type 014; Lane 9, type gc0577; Lane 10, type g9376; Lane 11, type 027; Lane 12, type gf811;Lane M, 100 bp ladder ; Lane N, negative control

The 77 isolates were classified into 20 types by PCR ribotyping.

Table 2. Toxin production and PCR ribotype of isolates

Period (year)	Toxin production	$A^+B^+CDT^-$					$A^+B^+CDT^+$		$A^-B^+CDT^-$
	PCR ribotype	smz	002	001	014	others	027	others	trf
2005		7*	0	3	2	5	2	1	0
2006		1	0	0	1	0	0	0	10
2007		1	0	0	0	1	0	0	6
2008		3	3	0	0	2	0	1	5
2009		2	8	2	1	4	0	1	5
2005~2009		14	11	5	4	12	2	3	26

*Number of isolates

The most predominant type was PCR ribotype trf (26 isolates, 33.8%), the second most frequent type was PCR ribotype smz (14 isolates, 18.2%), and the third frequent type was PCR ribotype 002 (11 isolates, 14.3%).

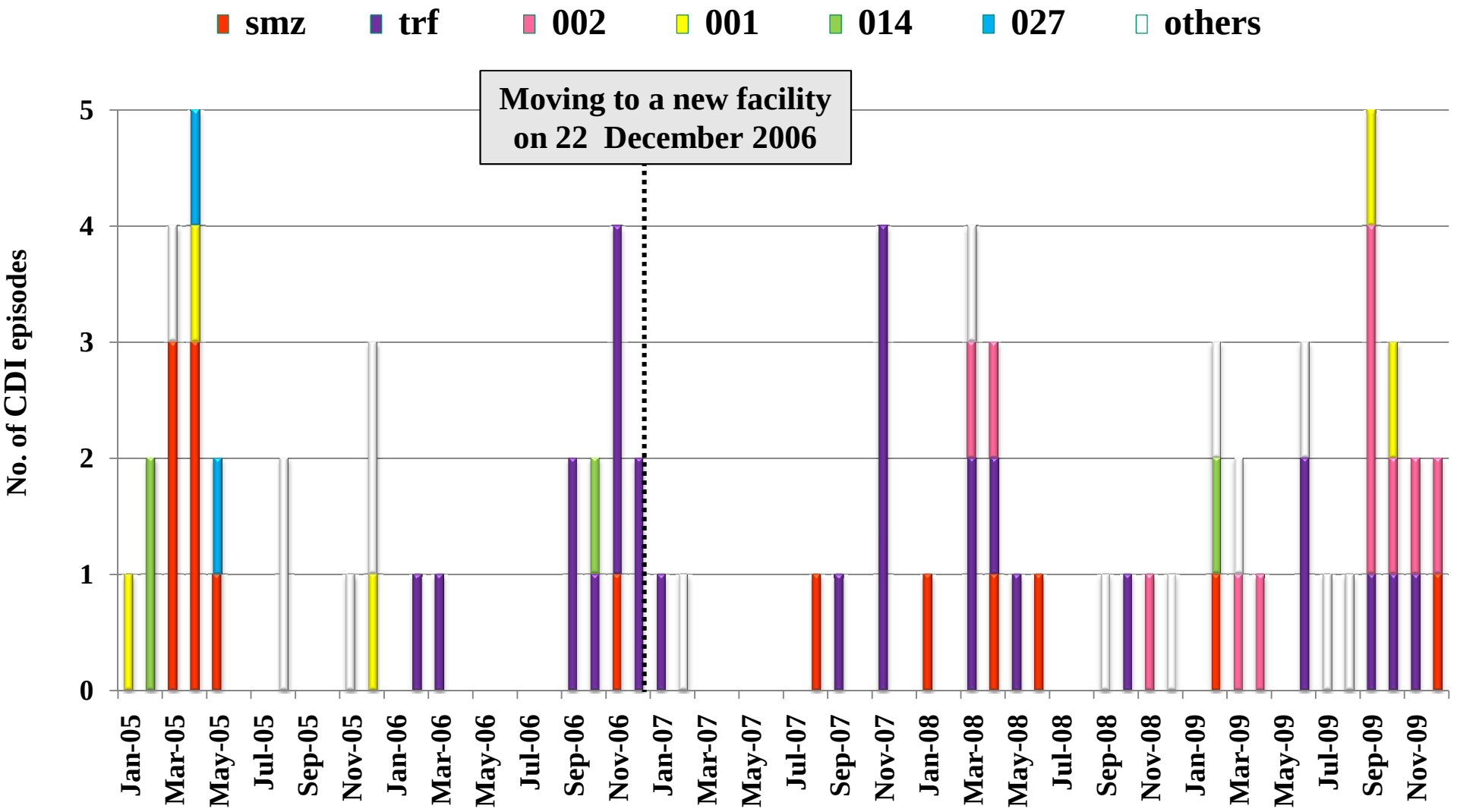
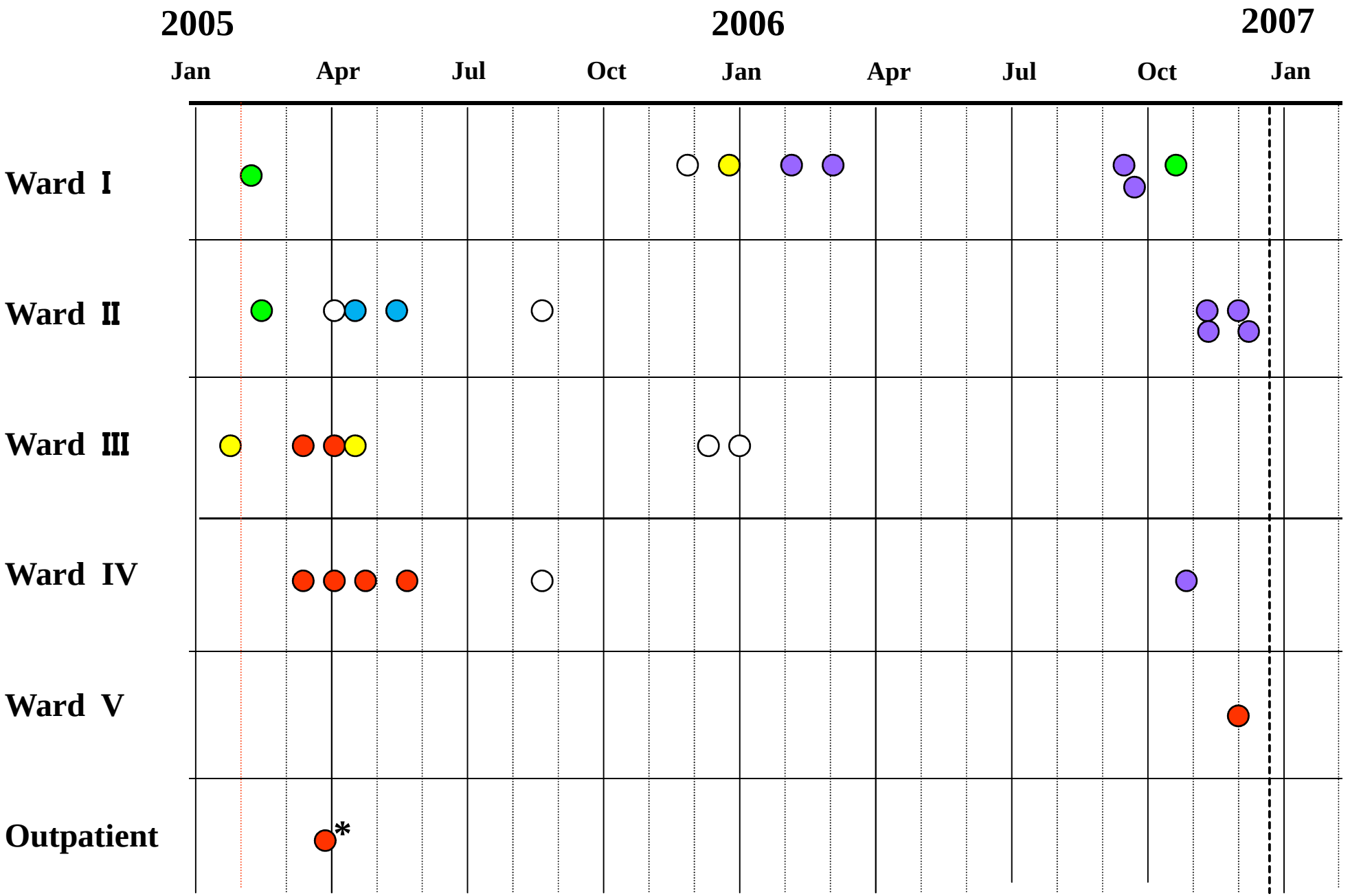
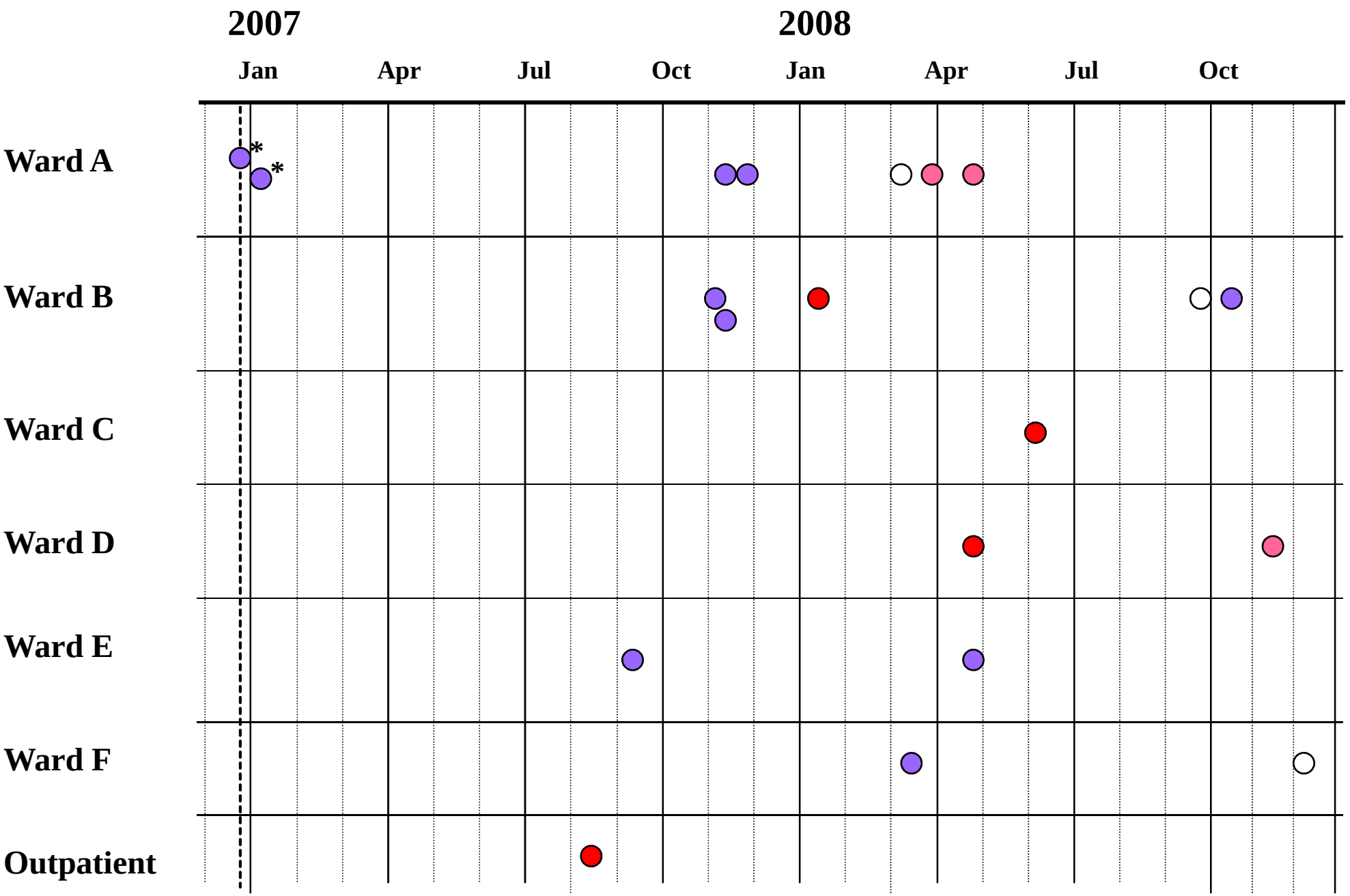


Figure 3. *C.difficile* PCR ribotypes identified during 5 years

The predominant type shifted from PCR ribotype smz in 2005 (7/20, 35.0%) to PCR ribotype trf in 2006 (10/12, 83.3%), and then, to PCR ribotype 002 (8/23, 34.8%) in 2009.


2005
2006
2007
Jan
Apr
Jul
Oct
Jan
Apr
Jul
Oct
Jan
Ward I
Ward II
Ward III
Ward IV
Ward V
Outpatient
*


2007
2008
Jan
Apr
Jul
Oct
Jan
Apr
Jul
Oct
Ward A
Ward B
Ward C
Ward D
Ward E
Ward F
Outpatient

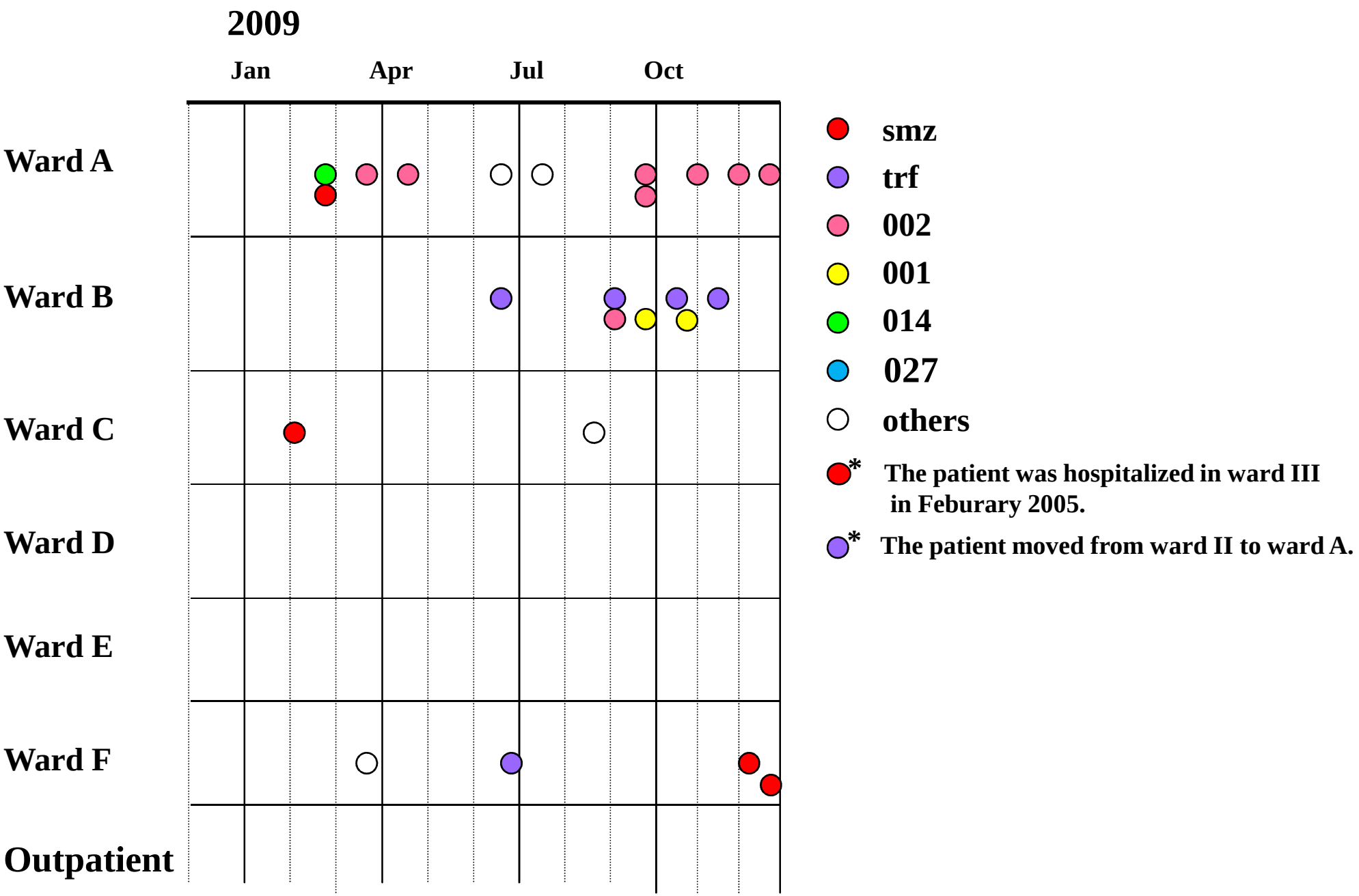

2009
Jan
Apr
Jul
Oct
Ward A
Ward B
Ward C
Ward D
Ward E
Ward F
Outpatient
smz
trf
002
001
014
027
others
* The patient was hospitalized in ward III in Feburary 2005.
* The patient moved from ward II to ward A.

Figure 4. Spatial and temporal analyses of *C. difficile* PCR ribotypes from January 2005 to December 2009
The time and ward regarding *C. difficile* isolation are plotted.

The cluster formation of PCR ribotypes smz and trf was recognized in one or two wards in 2005 and 2006, respectively. PCR ribotypes 002 in addition to those two ribotypes appeared to be predominant in the entire hospital at the end of 2009.

Conclusion

The molecular epidemiology of CDI in our hospital was characterized by changing twice the dominance of specific *C. difficile* PCR ribotypes over a five-year period.

References

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