COMPARISON OF SENSITIVITY OF EIA AND DISTRIBUTION OF PCR RIBOTYPES IN SOUTH KOREA

Heejung Kim, Heejung Lim, Myungsook Kim, Kyungwon Lee

Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, Seoul, Korea

INTRODUCTION

Enzyme immunoassays (EIAs) for toxin A/B are most commonly used for the laboratory diagnosis of *Clostridium difficile* infection due to their rapidity and ease of use, however, their sensitivity varies greatly (38.0–81.6%). The predominant PCR ribotypes of *C. difficile* vary according to the region or country studied and, recently, it was reported that the sensitivity of EIA was affected by strain type. The objective of this study was to compare the sensitivity of EIAs in relation to PCR ribotype in South Korea.

MATERIALS & METHODS

• Bacterial isolates

A total of 969 toxigenic *C. difficile* recovered from patients with diarrhea in a tertiary teaching hospital from 2006 to 2009, and 2011, were analyzed. Reference strains of *C. difficile* VPI 10463, 3608/03, SE844, 48489 and 1470 were used in both PCR assays and ribotyping.

• 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*, cdtApos-cdtArev for *cdtA,* and cdtBpos-cdtBrev 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 number.

• EIA

Toxin detection was performed by TOX A/B Quick check (TechLab) from 2006 to 2009 and VIDAS *Clostridium difficile* A & B assays (bioMérieux) in 2011.

RESULTS

Table 1. The sensitivity of TOX A/B Quick check and VIDAS *Clostridium difficile* A & B assays for the frequent 5 ribotypes

Ribotypes	EIA positive rate (%)	
	Tox A/B Quick check 2006-2009	VIDAS 2011
AB1 (UK001)	**66.7**	0.0
AB17 (UK018)	**56.6**	**71.3**
aB (UK017)	**48.6**	**54.3**
AB2 (UK014)	25.3	36.4
AB3	13.0	24.6

The prevalence of the predominant 3 ribotypes (AB1, AB17, and aB) which have higher detection rate than other ribotypes by EIA changed in this study period: 41.2% in 2006, 44.8% in 2007, 33.75% in 2008, 25.4% in 2009, 39.4% in 2011. These correlated strongly with the positive rate of EIA for toxigenic *C. difficile*: 47.7% in 2006, 40.0% in 2007, 38.7% in 2008, 29.4% in 2009, 46.3% in 2011.

CONCLUSIONS

1. Although the overall sensitivity of the VIDAS *Clostridium difficile* A & B assays was higher than that of TOX A/B Quick check, the sensitivity of VIDAS *Clostridium difficile* A & B assays was significantly lower than that of the TOX A/B Quick check for detection of ribotype 001.
2. We concluded that the sensitivity of EIA for each country would vary depending on the distribution of common ribotype.

REFERENCES

1. Kato, H., N. Kato, K. Watanabe, N. Iwai, H. Nakamura, T. Yamamoto, K. Suzuki, S. Kim, Y. Chong, and E. B. Wasito. 1998. Identification of toxin A-negative, toxin B-positive *Clostridium difficile* by PCR. J. Clin. Microbiol. 36:2178–2182.
2. Stubbs S, Rupnik M, Gibert M, Brazier J, Duerden B, Popoff M. Production of actin-specific ADP-ribosyltransferase (binary toxin) by strains of *C. difficile*. FEMS Microbiol Lett 2000;186:307-12.
3. Stubbs SL, Brazier JS, O'Neill GL, Duerden BI. PCR targeted to the 16S-23S rRNA gene intergenic spacer region of *Clostridium difficile* and construction of a library consisting of 116 different PCR ribotypes. J Clin Microbiol 1999;37:461-3.