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PCR-Dipstick Chromatography for Differential Detection of Carbapenemase Genes Directly in Stool Specimens

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ABSTRACT A PCR-dipstick chromatography technique was designed and evaluated for differential identification of bla_{NDM}, bla_{KPC}, bla_{IMP}, and bla_{OXA-48} carbapenemase genes directly in stool specimens within 2 h. It is a DNA-DNA hybridization-based detection system where PCR products can be easily interpreted by visual observation without electrophoresis. The PCR-dipstick showed high sensitivity (93.3%) and specificity (99.1%) in directly detecting carbapenemase genes in stool specimens compared with multiplex PCR for genomic DNA of the isolates from those stool specimens.

KEYWORDS carbapenemase, *Enterobacteriaceae*, molecular diagnostics

Carbapenem-resistant organisms impede effective treatment options for and increase the mortality of afflicted patients (1). In particular, carbapenemase-producing *Enterobacteriaceae* (CPE) are gaining attention because of their rapid spread and the varied epidemiology of different carbapenemases (2). It is vital to diagnose CPE early to undertake the appropriate measures to prevent transmission (3–5). Thus, a powerful detection system that possesses superior characteristics is needed to detect CPE (6). Here, we introduce the PCR-dipstick as a rapid detection technique for CPE and evaluate its utility for direct clinical specimens.

The PCR-dipstick is a PCR-based detection system where DNA-DNA hybridization of PCR amplicons to their probe in a dipstick strip occurs without denaturation, and the results can be interpreted visually (7, 8). Moreover, the PCR product can be analyzed within 15 min, which is clearly less than the time taken for gel electrophoresis (8). We designed the PCR-dipstick for four of the major carbapenemase genes, i.e., bla_{NDM}, bla_{KPC}, bla_{IMP}, and bla_{OXA-48}, in CPE (9) for future surveillance in Japan, Thailand, and other Southeast Asian countries where these carbapenemase genes are highly prevalent (10, 11). The construction of the PCR-dipstick for carbapenemase genes began with the primer design for multiplex PCR. After optimization, the primers were labeled with a distinct tag-linker sequence and biotin (Tohoku Bio-Array, Sendai, Japan) (see supplemental material). Multiplex PCR for the four carbapenemase genes was first performed using four positive control strains carrying bla_{NDM}, bla_{KPC}, bla_{IMP}, and bla_{OXA-48}. Multiplex PCR products were then mixed with dipstick developing buffer and avidin-coated blue latex beads (Tohoku Bio-Array) (supplemental material, including Fig. S1). If amplification occurred for any of the four carbapenemase genes, the biotin on one

Received 12 January 2017 **Returned for modification** 6 February 2017 **Accepted** 29 March 2017

Accepted manuscript posted online 3 April 2017

Citation Shanmugakani RK, Akeda Y, Yamamoto N, Sakamoto N, Hagiya H, Yoshida H, Takeuchi D, Sugawara Y, Kodera T, Kawase M, Laolerd W, Chaihongsa N, Santanirand P, Ishii Y, Hamada S, Tomono K. 2017. PCR-dipstick chromatography for differential detection of carbapenemase genes directly in stool specimens. Antimicrob Agents Chemother 61:e00067-17. https://doi.org/10.1128/AAC.00067-17.

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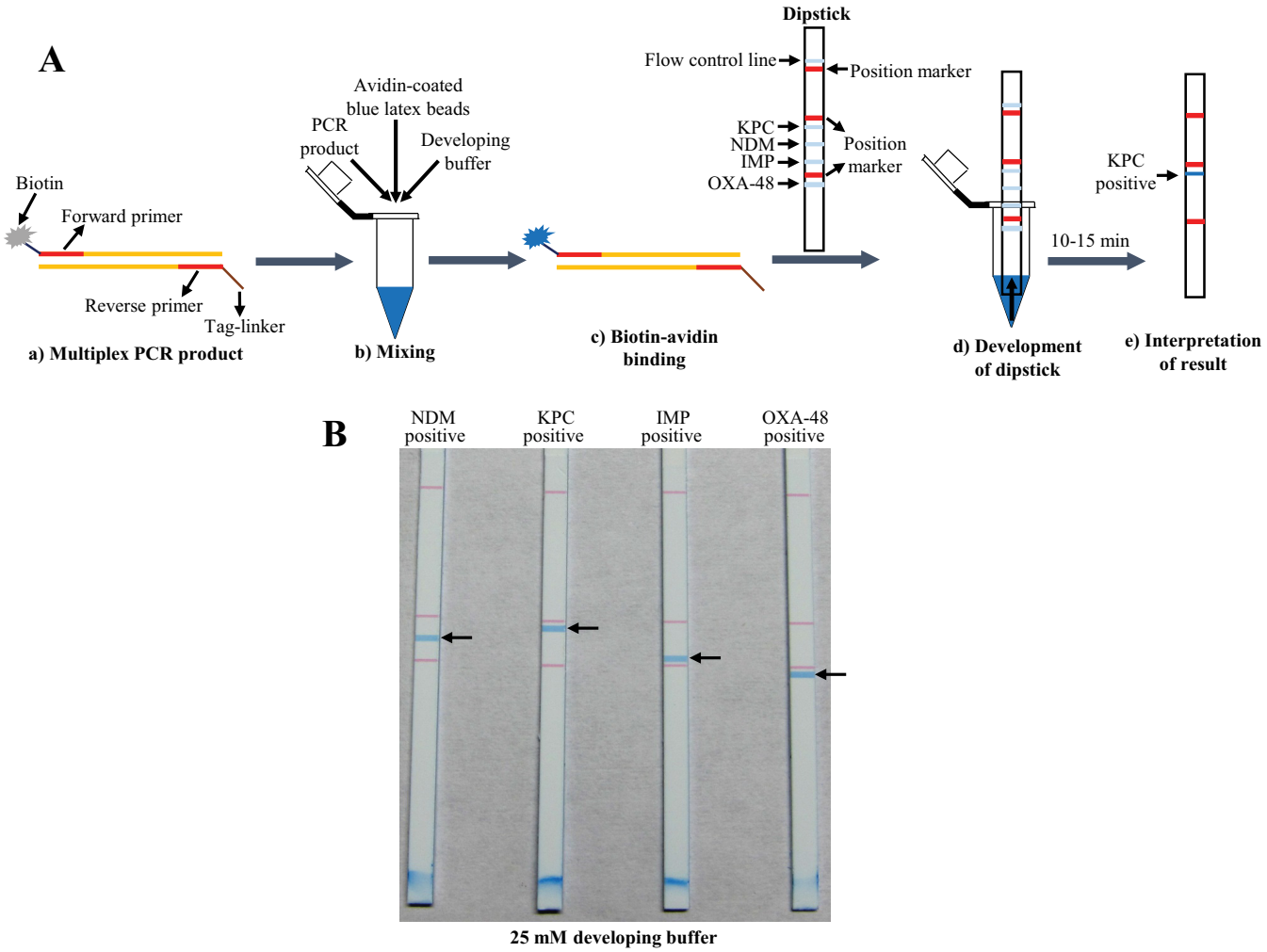

FIG 1 PCR-dipstick technique for the detection of carbapenemase genes. (A) Schematic representation of PCR-dipstick technique with KPC-positive sample. (a) Multiplex PCR product with the labeled primers. (b) PCR product is mixed with blue-dyed latex beads and developing buffer. (c) Avidin immobilized on the blue latex beads binds to biotin on the primer of the PCR product. (d) Dipstick is dipped into the mixture for 10 to 15 min for hybridization; by capillary action, the mixture flows toward the top of the dipstick. (e) Tag-linker sequence in the primer of the PCR product hybridizes to its complementary probe at the respective position in the dipstick forming the blue line. (B) Detection of four different carbapenemase genes, i.e., bla_{NDM}, bla_{KPC}, bla_{IMP}, and bla_{OXA-48}, in the PCR product of positive controls by the PCR-dipstick technique without nonspecific hybridization at 25 mM concentration of developing buffer.

primer of the PCR amplicon bound to latex beads. Then, the dipstick imprinted with the probe for each tag-linker sequence of the primers at different positions was dipped into the reaction mixture. Through capillary action, the reaction mixture was carried upward along the dipstick, during which the tag-linker sequence on the other primer of the PCR product hybridized to its respective probe, forming a blue line within 15 min (Fig. 1).

To evaluate the efficacy of the PCR-dipstick in detecting different variants of the four carbapenemases, genomic DNAs of CPE (n = 57) and non-CPE (n = 10) isolates from Japan (n = 16) and Thailand (n = 51) previously characterized by sequencing carbapenemase genes were used (supplemental material). The sensitivity and specificity of the PCR-dipstick in CPE detection were estimated by comparison with the results of multiplex PCR followed by electrophoresis as the reference comparator. The PCR-dipstick showed 100% sensitivity and specificity in detecting the variants of the carbapenemases bla_{NDM}, bla_{KPC}, bla_{IMP}, and bla_{OXA-48} in different species of CPE (Table 1). Interestingly, two carbapenemases, bla_{NDM} and bla_{OXA-48}, present in the same isolate were differentially identified. Furthermore, the limit of detection of the PCR-dipstick was estimated to be 10^2 CFU/ml (supplemental material).

To assess the utility of the PCR-dipstick for direct stool specimens, 151 nonduplicated stool samples from patients in the tertiary hospitals in Osaka, Japan (n = 88) and

TABLE 1 PCR-dipstick chromatography compared with multiplex PCR for clinical isolates

Isolate type	Carbapenemase gene	Carbapenemase variant	Species	PCR-dipstick results (no. positive/total isolates)
CPE	bla_{KPC}	KPC-2	*K. pneumoniae*	3/3
			E. aerogenes	3/3
			E. cloacae	2/2
			E. coli	2/2
	bla_{NDM}	NDM-1	*E. coli*	6/6
			K. pneumoniae	5/5
			E. cloacae	3/3
		NDM-5	*E. coli*	2/2
	bla_{IMP}	IMP-6	*E. coli*	7/7
			K. pneumoniae	8/8
			E. cloacae	1/1
	bla_{OXA-48}	OXA-48	*E. coli*	1/1
			K. pneumoniae	2/2
		OXA-181	*K. pneumoniae*	4/4
		OXA-232	*K. pneumoniae*	3/3
	bla_{NDM} & bla_{OXA-48}	NDM-1 & OXA-232	*K. pneumoniae*	5/5
Non-CPE			*E. coli*	0/1
			K. pneumoniae	0/3
			E. cloacae	0/5
			E. aerogenes	0/1

Bangkok, Thailand ($n = 63$) were used. Bacterial characterization and antibiotic susceptibility testing showed that 29 of the specimens carried carbapenem-resistant *Enterobacteriaceae* whose carbapenemase genes were confirmed with multiplex PCR (supplemental material). Since we did not detect *Klebsiella pneumoniae* carbapenemase (KPC)-positive CPE in the stool specimens examined, a KPC-producing *K. pneumoniae* ATCC BAA-1705 isolate was spiked into a CPE-negative stool suspension at a final concentration of 10^2 CFU/ml (limit of detection of PCR-dipstick) as a KPC-positive specimen. A total of 152 stool samples, comprising 30 CPE-positive and 122 CPE-negative samples, were subjected to the PCR-dipstick system. The PCR-dipstick showed a sensitivity of 93.3% and specificity of 99.1% compared with the reference comparator standard, i.e., multiplex PCR followed by gel electrophoresis (Table 2; supplemental material). Of the 30 CPE-positive specimens, 2 carrying bla_{IMP} were not detected by the PCR-dipstick. Of the 122 CPE-negative specimens, 1 was positive for bla_{OXA-48} by the PCR-dipstick. The false-negative/false-positive results were presumed to be due to the presence of inhibitory contaminants in the stool or to DNA in the specimen that is below the detection limit of the PCR-dipstick, respectively.

We then examined the PCR-dipstick system for detection of carbapenemase genes in pooled stool specimens ($n = 10$), and the result was matched with the presence of the corresponding carbapenemase gene. When the pooled specimens were applied onto the M-ECC selective medium for carbapenem-resistant *Enterobacteriaceae* (12), it was difficult to isolate/identify the CPE (see Fig. S2 in the supplemental material), which seemed to be due to the huge bacterial load of carbapenem-resistant non-*Enterobacteriaceae*, such as *Pseudomonas* spp. If the PCR-dipstick showed a positive result with any of the pooled specimens, the technique would be performed for each of the samples in that pool to confirm the exact CPE-positive specimen. Thus, the

TABLE 2 Sensitivity and specificity of PCR-dipstick chromatography versus multiplex PCR for 152 clinical specimens

PCR-dipstick	Multiplex PCR		Sensitivity (95% CI[a])	Specificity (95% CI)
	Positive	Negative		
Positive	28	1	93.3 (77.9–99.1)	99.1 (95.5–99.9)
Negative	2	121		

[a]CI, confidence interval.

PCR-dipstick can act as a simple and inexpensive CPE surveillance tool (approximately $4/reaction). The PCR-dipstick takes less time than other DNA hybridization techniques and detects multiple genes without the denaturation step for hybridization. Moreover, the PCR-dipstick technique costs much less than other molecular detection systems, such as real-time PCR. In less-facilitated hospitals where no sophisticated equipment is available, the PCR-dipstick can be an easy alternative diagnostic test for CPE. Because the PCR-dipstick can be used for CPE detection in pooled specimens, it can be a quick point-of-care testing tool at an affordable price. In this study, we analyzed the PCR-dipstick technique for a few variants of carbapenemase genes due to the limited availability of variants; however, the primers can detect all the variants mentioned above, as confirmed by their conserved DNA sequences. Because the PCR-dipstick system can be customized for any carbapenemase genes, such as bla_{VIM}, surveillance of CPE in regions of unknown epidemiology can be performed with a customized PCR-dipstick detecting various carbapenemases.

In conclusion, the PCR-dipstick can be a valuable CPE surveillance tool in regions of high CPE prevalence or in cases of outbreak where expensive/sophisticated equipment is not affordable/applicable to undertake appropriate infection control measures.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00067-17.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

This work was supported by the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) from the Ministry of Education, Culture, Sport, Science and Technology in Japan and the Japan Agency for Medical Research and Development (AMED).

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