Short Communication

Why cannot a β-lactamase gene be detected using an efficient molecular diagnostic method?

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ABSTRACT

Objective: Fast detection of β-lactamase (bla) genes can minimize the spread of antibiotic resistance. Although several molecular diagnostic methods have been developed to detect limited bla gene types, these methods have significant limitations, such as their failure to detect almost all clinically available bla genes. We have evaluated a further refinement of our fast and accurate molecular method, developed to overcome these limitations, using clinical isolates.

Methods: We have recently developed the efficient large-scale bla detection method (large-scale blaFinder) that can detect bla gene types including almost all clinically available 1,352 bla genes with perfect specificity and sensitivity. Using this method, we have evaluated a further refinement of this method using clinical isolates provided by International Health Management Associates, Inc. (Schaumburg, Illinois, USA). Results were interpreted in a blinded manner by researchers who did not know any information on bla genes harbored by these isolates.

Results: With only one exception, the large-scale blaFinder detected all bla genes identified by the provider using microarray and multiplex PCR. In one of the Escherichia coli test isolates, a bla_DHA-1 gene was detected using the multiplex PCR assay but it was not detected using the large-scale blaFinder.

Conclusion: The truncation of a bla_DHA-1 gene is an important reason for an efficient molecular diagnostic method (large-scaleblaFinder) not to detect the bla gene.

KEY WORDS: β-Lactamase (bla) gene, Large-scale detection, Molecular diagnosis, Minimizing antibiotic resistance.

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INTRODUCTION

The development of fast and accurate diagnostic methods to detect antibiotic resistance genes is needed to minimise antibiotic resistance. β-Lactam antibiotics are some of the most successful drugs used for the treatment of bacterial infections and represent roughly 65% of the total world market for antibiotics. Therefore, resistance to β-lactam antibiotics through the acquisition of genes that encode β-lactamases is one of the most serious problems in Gram-negative pathogenic bacteria. To date several molecular diagnostic methods of bla gene typing have been developed to detect the existence of β-lactamase (bla) gene(s) in clinical isolates. These methods can detect only some
(limited) *bla* genes. Because these methods cannot detect *bla* gene types including almost all clinically available *bla* genes, they cannot perfectly explain the results of the culture-based phenotypic tests.  

This is a big problem in studying β-lactam resistance, as β-lactam resistance can increase due to inappropriate β-lactam use. To solve this problem, we have recently developed the efficient large-scale *bla* detection method (large-scale *bla*Finder) that can detect *bla* gene types including almost all clinically available 1,352 *bla* genes with perfect specificity and sensitivity.  

**METHODS**

We have evaluated a further refinement of this method using clinical isolates provided by International Health Management Associates, Inc. (Schaumburg, Illinois, USA), using the large-scale *bla*Finder method. Results were interpreted in a blinded manner by researchers who did not know any information on *bla* genes harbored by these isolates. With only one exception, the large-scale *bla*Finder detected all *bla* genes identified by the provider using microarray (Check-MDR CT101, Check-Points B.V., Wageningen, the Netherlands) and multiplex PCR. In one of the *Escherichia coli* test isolates, a *bla*<sub>DHA-1</sub> gene was detected using the multiplex PCR assay designed by Perez-Perez and Hanson but it was not detected using the large-scale *bla*Finder (Fig.1A and B).

To resolve this issue, simplex PCR assays were performed for the detection of *bla*DHA-1 gene using the *Escherichia coli* test isolate, *E. coli* E07-10537, and a *bla*DHA-1 negative *Providencia stuartii* isolate.

**RESULTS**

Interestingly, in the *E. coli* test isolate, no band was detected using the reverse primer (DHA (AmpC-2) type-R) used by the large-scale *bla*Finder (Fig.1C and D). The nucleotide position of the primer pair used by Perez-Perez and Hanson is 258-662. However, the nucleotide position of the primer pair used by the large-scale *bla*Finder is 19-899. The results suggest that there is a truncated *bla*DHA-1 (Δ*bla*DHA-1) lacking a 3' (or 5') end sequence in the *E. coli* test isolate.

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[**Fig.1**] PCR assays to detect a truncated *bla*DHA-1 gene using an *Escherichia coli* test isolate (lane 1 of each Figure), *E. coli* E07-10537 (a *bla*DHA-1 positive isolate; lane 2 of each Figure), and a *bla*DHA-1 negative *Providencia stuartii* isolate (lane 3 of each Figure). (A) Simplex PCR assays using a primer pair (DHAMF and DHAMR) used by the method of Perez-Perez and Hanson. Two same bands (405 bp) were detected in the *E. coli* test isolate and *E. coli* E07-10537. (B) Simplex PCR assays using a primer pair (DHA(AmpC-2) type-F and DHA(AmpC-2) type-R) used by the large-scale *bla*Finder. Only one band (881 bp) was shown in *E. coli* E07-10537. (C) Simplex PCR assays using a primer pair (DHAMF and DHA(AmpC-2) type-R). Only one band (642 bp) was detected in *E. coli* E07-10537. (D) Simplex PCR assays using a primer pair (DHA(AmpC-2) type-F and DHAMR). Two same bands (644 bp) were detected in the *E. coli* test isolate and *E. coli* E07-10537. (E) Schematic representation of the DNA sequences surrounding a truncated *bla*DHA-1 gene (Δ*bla*DHA-1) in *E. coli* 271 (Ho et al. (10)) and a newly designed primer pair. Each nucleotide position of Δ*bla*DHA-1 and each primer were shown in parenthesis. 345 bp (position: 796 to 1140) of *bla*DHA-1 sequence were missing at 3' end. (F) Simplex PCR assays using a newly designed primer pair (trpF-F and DHA type-R). Only one band (734 bp) was shown in the *E. coli* test isolate. M1 (size marker), 100 bp DNA ladder (Biosesang, Korea).
DISCUSSION

The previous study showed a ΔblaDHA-1 lacking a 3’ end sequence (Fig.1E). Based on the pNDM-HK sequence (HQ451074), we newly designed a primer pair (trpF-5’-ATGCCCGCGAAAATCAA-3’; and DHA type-R, 5’-CAAAGCCAGTATGCGTACGG-3’) to know the exact truncated blaDHA-1 sequence in the E. coli test isolate (Fig.1E). Using these two primers, one band (734 bp) was detected in the test isolate (Fig.1F). Sequencing data of this band showed that 345 bp (position: 796 to 1140) of blaDHA-1 sequence were missing at 3’ end. The total sizes of ΔblaDHA-1 and blaDHA-1 were 795 bp and 1140 bp, respectively. Therefore, the efficient molecular diagnostic method (large-scale blaFinder) could not detect the ΔblaDHA-1 gene in the E. coli test isolate. Because a truncated bla gene does not show any antibiotic resistance, the large-scale blaFinder has no problem for monitoring the emergence and dissemination of bla genes and minimizing the spread of resistant bacteria. Therefore, the truncation of a bla gene is an important reason for an efficient molecular diagnostic method not to detect the bla gene.

CONCLUSION

The efficient large-scale bla detection method (large-scale blaFinder) is a useful test to detect bla gene types including almost all clinically available genes with perfect specificity and sensitivity, although the method could not detect the ΔblaDHA-1 gene in the E. coli test isolate. That is because a truncated bla gene does not show any antibiotic resistance.

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Declaration of interests: None.

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Authors’ Contributions:

KSP, SHL: Designed the study, did data analysis and prepared the manuscript.
JHL, MP, AMK: Contributed materials/analysis tools.