

## Why cannot a $\beta$ -lactamase gene be detected using an efficient molecular diagnostic method?

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### ABSTRACT

**Objective:** Fast detection of  $\beta$ -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 *bla*Finder) 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 *bla*Finder detected all *bla* genes identified by the provider using microarray 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 but it was not detected using the large-scale *bla*Finder.

**Conclusion:** The truncation of a *bla*<sub>DHA-1</sub> gene is an important reason for an efficient molecular diagnostic method (large-scale *bla*Finder) not to detect the *bla* gene.

**KEY WORDS:**  $\beta$ -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.<sup>1</sup>  $\beta$ -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.<sup>1</sup> Therefore, resistance to  $\beta$ -lactam antibiotics through the acquisition of genes that encode  $\beta$ -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  $\beta$ -lactamase (*bla*) gene(s) in clinical isolates.<sup>2-8</sup> 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.<sup>9</sup>

This is a big problem in studying  $\beta$ -lactam resistance, as  $\beta$ -lactam resistance can increase due to inappropriate  $\beta$ -lactam use. To solve this problem, 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.<sup>9</sup>

### 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 blaFinder* method.<sup>9</sup> 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 blaFinder* detected all *bla* genes identified by the

provider using microarray (Check-MDR CT101, Check-Points B.V., Wageningen, the Netherlands) and multiplex PCR.<sup>2</sup> 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<sup>1</sup> but it was not detected using the *large-scale blaFinder* (Fig.1A and B).

To resolve this issue, simplex PCR assays<sup>9</sup> were performed for the detection of *bla*<sub>DHA-1</sub> gene using the *Escherichia coli* test isolate, *E. coli* E07-10537,<sup>9</sup> and a *bla*<sub>DHA-1</sub> 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)<sup>9</sup> used by the *large-scale blaFinder* (Fig.1C and D). The nucleotide position of the primer pair used by Perez-Perez and Hanson<sup>2</sup> is 258-662. However, the nucleotide position of the primer pair used by the *large-scale blaFinder* is 19-899. The results suggest that there is a truncated *bla*<sub>DHA-1</sub> ( $\Delta$ *bla*<sub>DHA-1</sub>) lacking a 3' (or 5') end sequence in the *E. coli* test isolate.

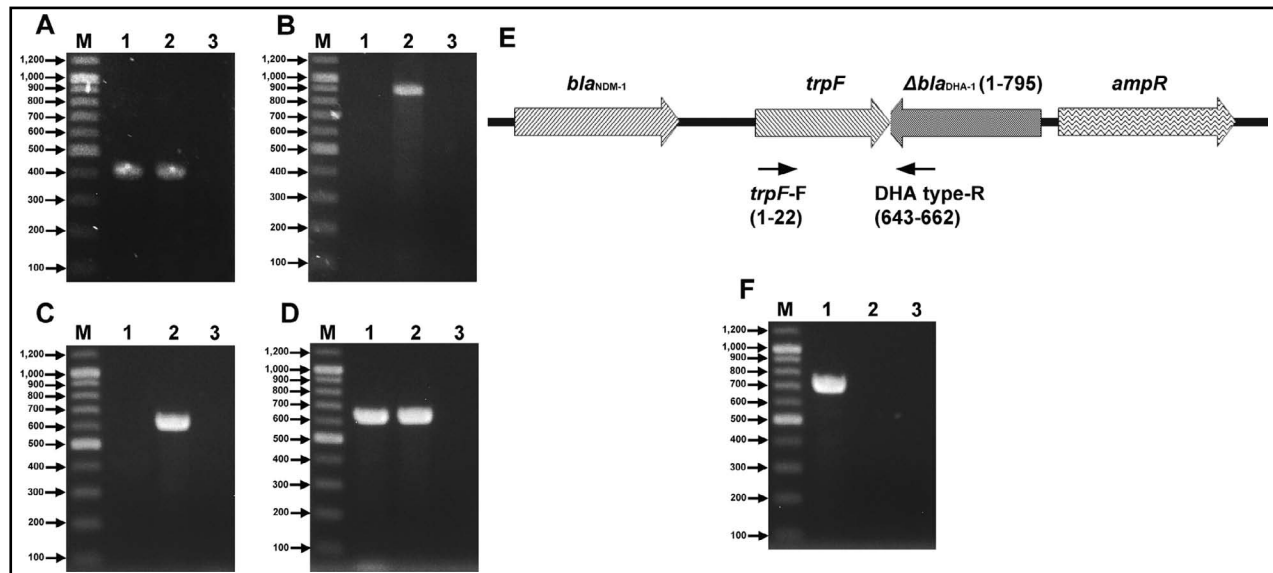


Fig.1: PCR assays to detect a truncated *bla*<sub>DHA-1</sub> gene using an *Escherichia coli* test isolate (lane 1 of each Figure), *E. coli* E07-10537 (a *bla*<sub>DHA-1</sub> positive isolate; lane 2 of each Figure), and a *bla*<sub>DHA-1</sub> negative *Providencia stuartii* isolate (lane 3 of each Figure). (A) Simplex PCR assays using a primer pair (DHAMF and DHAMR) used by the method of Perez-Perez and Hanson (1). 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 blaFinder*. 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*<sub>DHA-1</sub> gene ( $\Delta$ *bla*<sub>DHA-1</sub>) in *E. coli* 271 (Ho et al. (10)) and a newly designed primer pair. Each nucleotide position of  $\Delta$ *bla*<sub>DHA-1</sub> and each primer were shown in parenthesis. 345 bp (position: 796 to 1140) of *bla*<sub>DHA-1</sub> 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  $\Delta bla_{DHA-1}$  lacking a 3' end sequence (Fig.1E).<sup>10</sup> Based on the pNDM-HK sequence (HQ451074), we newly designed a primer pair (*trpF*-F, 5'-ATGCCCGCGAAAATCAA-GATTTG-3'; and DHA type-R, 5'-CAAAGCCAG-TATGCGTACGG-3') to know the exact truncated  $bla_{DHA-1}$  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  $bla_{DHA-1}$  sequence were missing at 3' end. The total sizes of  $\Delta bla_{DHA-1}$  and  $bla_{DHA-1}$  were 795 bp and 1140 bp, respectively.<sup>9,10</sup> Therefore, the efficient molecular diagnostic method ( $_{large-scale} bla$ Finder) could not detect the  $\Delta bla_{DHA-1}$  gene in the *E. coli* test isolate. Because a truncated *bla* gene does not show any antibiotic resistance, the  $_{large-scale} bla$ Finder 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 ( $_{large-scale} bla$ Finder) 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  $\Delta bla_{DHA-1}$  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.