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Letters to the Editor

Practical Disk-Based Method for Detection of *Escherichia coli* Clinical Isolates Producing the Fluoroquinolone-Modifying Enzyme AAC(6′)-Ib-cr▽

Since a fluoroquinolone-modifying enzyme gene, *aac(6′)-Ib-cr*, was first reported in 2006, it has rapidly spread among *Enterobacteriaceae* clinical isolates worldwide (7). AAC(6′)-Ib-cr differs from AAC(6′)-Ib by two amino acids, Trp102Arg and Asp179Tyr, and these substitutions allow it to reduce the antibacterial activities of norfloxacin and ciprofloxacin through acetylation of their piperazinyl substituent (6). Detection of *aac(6′)-Ib-cr* has so far depended mainly on genotyping, PCR, and sequencing (2). Recently, simultaneous high-resolution melting analysis and pyrosequencing were developed for detection (1, 3). However, these methods are costly and need specialized equipment. Therefore, the availability is limited to highly advanced institutions, such as research laboratories and university hospitals. In the present study, we developed a cost-effective and practical disk-based method to screen for AAC(6′)-Ib-cr producers.

The *Escherichia coli* clinical isolates CR1 [*aac(6′)-Ib-cr* positive], N64 [*aac(6′)-Ib* positive], and BN41 [*aac(6′)-Ib-cr* and *aac(6′)-Ib* negative] were grown in LB broth containing norfloxacin (8 μg/ml), with shaking for 18 h at 35°C. The broth containing the same concentration of norfloxacin as the other tubes, but lacking any bacteria, was used as the control in this study. Ten microliters of each culture medium was applied on the blank disk set on a Mueller-Hinton agar plate inoculated with *E. coli* ATCC 25922 and incubated for 18 h at 35°C. The result is shown in Fig. 1. When the control medium was applied, an 18-millimeter growth-inhibitory zone (corresponding to 80 ng norfloxacin per disk) was observed. The significant decrease of a growth-inhibitory zone was observed when the culture medium of the *aac(6′)-Ib-cr*-positive *E. coli* strain CR1 was applied, while neither of the *aac(6′)-Ib-cr*-negative strains, N64 and BN41, showed a decrease in zone diameter. The decrease in zone diameter is an indicator of AAC(6′)-Ib-cr production and would be attributed to the inactivation of norfloxacin in the culture medium by the AAC(6′)-Ib-cr enzyme produced during growth (4).

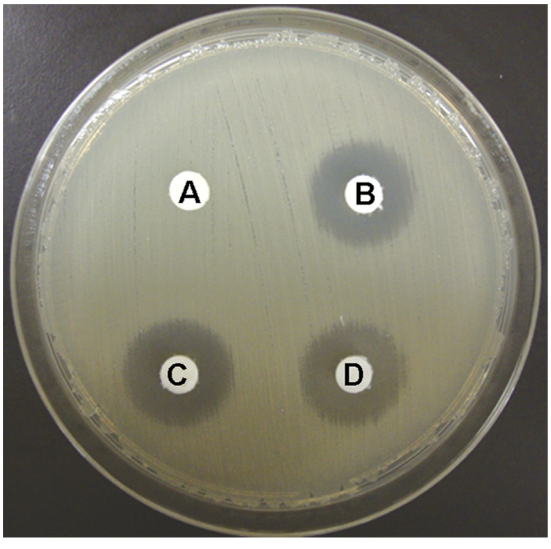

FIG. 1. Result of disk-based detection. (A) *E. coli* strain CR1 [*aac(6′)-Ib-cr* (+)]; (B) *E. coli* strain N64 [*aac(6′)-Ib* (+)]; (C) *E. coli* strain BN41 (negative for both genes); (D) control medium.

In this study, a total of 89 *E. coli* clinical isolates from our own strain collection, which were obtained from 49 facilities throughout Japan between 2002 and 2010, were subjected to the developed disk-based method. The MICs of norfloxacin for these strains were ≥16 μg/ml, and they showed visible growth in liquid broth containing 8 μg/ml of norfloxacin. The presence and absence of the *aac(6′)-Ib-cr* and *aac(6′)-Ib* genes in these isolates were preliminarily determined by PCR and nucleotide sequencing (5) but were not examined for the presence of other fluoroquinolone resistance mechanisms, such as *qnr* and mutations in DNA gyrase and topoisomerase IV, in the present study. As a result, all of the 19 *aac(6′)-Ib-cr*-positive strains showed a decrease in zone diameter of >10 mm, while all of the 70 *aac(6′)-Ib-cr*-negative strains, including 10 *aac(6′)-Ib*-positive ones, showed a growth-inhibitory zone that was the same size as that of the control medium. These results indicated that the new disk-based method developed here might have a specificity and sensitivity equivalent to those of genotyping using PCR and nucleotide sequencing. Therefore, the method seems to be effective for screening of AAC(6′)-Ib-cr producers, although application of the method is limited to bacterial strains that can grow in medium including norfloxacin.

We applied the method to *aac(6′)-Ib-cr*-positive clinical isolates of *Klebsiella pneumoniae* ($n = 3$), *Serratia marcescens* ($n = 1$), and *Citrobacter freundii* ($n = 1$), and we confirmed the >10-mm reduction in zone diameter seen in *aac(6′)-Ib-cr*-positive *E. coli*. This result indicates the possibility that the method could be applied not only to *E. coli* but also to the other bacterial strains belonging to the family *Enterobacteriaceae*. Extension of this method to members of the *Enterobacteriaceae* family other than *E. coli* would, however, require substantial additional experimentation aimed at demonstrating that the method is more broadly applicable to these other species.

We conclude that our method is simple and highly sensitive and specific for identification of AAC(6′)-Ib-cr producers and that it would be of great assistance in screening for AAC(6′)-Ib-cr producers and their epidemiology in clinical microbiology laboratories.

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REFERENCES

1. **Bell, J. M., J. D. Turnidge, and P. Andersson.** 2010. *aac(6′)-Ib-cr* genotyping by simultaneous high-resolution melting analyses of an unlabeled probe and full-length amplicon. Antimicrob. Agents Chemother. **54:**1378–1380.
2. **Cano, M. E., et al.** 2009. Detection of plasmid-mediated quinolone resistance genes in clinical isolates of *Enterobacter* spp. in Spain. J. Clin. Microbiol. **47:**2033–2039.
3. **Guillard, T., et al.** 2010. Rapid detection of *aac(6′)-Ib-cr* quinolone resistance gene by pyrosequencing. J. Clin. Microbiol. **48:**286–289.
4. **Jung, C. M., T. M. Heinze, R. Strakosha, C. A. Elkins, and J. B. Sutherland.** 2009. Acetylation of fluoroquinolone antimicrobial agents by an *Escherichia*

coli strain isolated from a municipal wastewater treatment plant. J. Appl. Microbiol. **106:**564–571.

5. **Park, C. H., A. Robicsek, G. A. Jacoby, D. Sahm, and D. C. Hooper.** 2006. Prevalence in the United States of *aac(6′)-Ib-cr* encoding a ciprofloxacin-modifying enzyme. Antimicrob. Agents Chemother. **50:**3953–3955.
6. **Robicsek, A., et al.** 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat. Med. **12:**83–88.
7. **Strahilevitz, J., G. A. Jacoby, D. C. Hooper, and A. Robicsek.** 2009. Plasmid-mediated quinolone resistance: a multifaceted threat. Clin. Microbiol. Rev. **22:**664–689.

Jun-ichi Wachino*
Kunikazu Yamane
Yoshichika Arakawa
Department of Bacteriology II
National Institute of Infectious Diseases
4-7-1 Gakuen, Musashi-Murayama
Tokyo 208-0011, Japan

*Phone: 81-42-561-0771, ext.539
Fax: 81-42-561-7173
E-mail: wachino@nih.go.jp

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