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REFERENCES

1. Sexton DJ, Chen LF, Anderson DJ. Current definitions of central line–associated bloodstream infection: is the emperor wearing clothes? *Infect Control Hosp Epidemiol* 2010;31(12):1286–1289.

Toxin A–Negative, Toxin B–Positive *Clostridium difficile* Infection Diagnosed by Polymerase Chain Reaction

To the Editor—Clostridium difficile, the major cause of nosocomially acquired diarrhea and colitis, may produce 2 major virulence factors: toxin A and toxin B. The genes *TcdA* and *TcdB* are located in the same pathogenicity locus (PaLoc) and transcribed as 1 mRNA, so detection of toxin B alone by enzyme immunoassay is often used for diagnosis of *C. difficile* infection (CDI) regardless of its lower sensitivity and greater cost.[1] However, this approach fails to provide information as to whether toxin A is present or not. The presence of toxin A is important because toxin A^-B^+ *C. difficile* strains, such as ribotype 017, have been reported more frequently in Asia and Latin America than anywhere else.[2] Toxin A^-B^+ strains have been associated with higher rates of antibiotic resistance and may pose a great risk to patients.[3] The use of polymerase chain reaction (PCR) for the diagnosis of CDI is increasing because of its increased sensitivity and speed. For these reasons, the use of PCR methods that include the detection of *TcdA* genes is important for epidemiological surveys.

Recently, PCR methods have been used in some countries to conduct surveys of CDI.[2,5-7] In these surveys, we found that the rate of toxin A^-B^+ *C. difficile* strains differs significantly from region to region, ranging from 2.5% through 75%.[2,5,6] To make sure that the same PCR methods were used, we compared widely used primers, such as NK1–NK2, NK2–NK3, NK104–NK105, and A1C–A2N, used to conduct epidemic surveys in Japan, France, Argentina, and other countries. We found that isolation rates of toxin A^-B^+ *C. difficile* differ widely between Europe and Asia, that the PCR primers for *TcdA* from different countries are not uniform, and that the molecular criteria for *TcdA*-negative *TcdB*-positive strains need to be improved. These findings may explain why there are differences in the isolation rates of toxin A^-B^+ strains among different geographic regions.

We performed bioinformatic and comparative genome analysis on the primer regions of 7 published *C. difficile* sequences while looking for genomic sequence variations. In our analysis, primers M68 and CF5 belong to toxin A^-B^+, and others belong to the toxin A^+B^+ group (Figure 1). The PaLoc sequences of CF5 exhibit only 1 base pair difference from those of M68. However, the intergroup of toxin A^+B^+ and the intragroup between toxin A^+B^+ and toxin A^-B^+ show more diversity in the PaLoc region. These diversities, or single-nucleotide polymorphisms (SNPs), that occur in primers may weaken our signal when we use PCR methods to detect *TcdA/B* regions. For *TcdB*, NK104 and NK105, designed by Kato,[6] are widely used for detection. Their amplicons are located between the 2 highly variable regions of the 7 strains sequenced via SNP analysis and may explain their worldwide utility. However, we still found 3 sites (NK104, T17C, C26T; NK105, C20T) with apparent mutations among the 7 genomes (Figure 1).

Although the *TcdA* gene is conserved in the toxin A^-B^+ group, some papers have still found that these isolates have structural variation in the 3′ end of the gene, with the deletion of 600–1,700 base pairs.[5,8] Two or 3 pairs of primers (NK1, NK2, NK3, NK9, NK11, A1C, A2C, A3C, A2N, A3N, A4N) for each have been reported across this deletion region that can identify *TcdA* variation by the presence and size of their amplicons.[9] Among these primers, we found 7 sites (Figure 1*B*) with mutations in NK2–NK3 and A4N in the public genome that may cause a false negative result for toxin A^-B^+ strains when these regions are used for primers. We did not find mutations in any other primer regions. Thus, if we wish to simplify the process to detect toxin A, then primers A3C and A4N might be recommended to improve primer design.[5] We also advise using the downstream A4N position, which is closer to or beside the *TcdC* region, for lower SNP frequency. From SNP analysis of 7 known sequences, we can assert that the SNP in *TcdA* shows less diversity than that in *TcdB*, in addition to structural variation, and that primer design in different regions is based on the diversity of actual geographic strains. Proper primer design for *TcdA* should make the epidemiological data more accurate.

In conclusion, our group compares the primers for detection of toxins A and B from different regions via genome comparison and bioinformatics analysis. We found two gaps in current toxin A/B PCR detection, the toxin A^-B^+ strain isolation rate in Asia and North America and the different toxin A/B loci in different regions. According to these findings, we would advocate the establishment of a unified primer and method for toxin detection in different geographical regions. A unified approach should allow appropriate interpretation of the distinctly higher toxin A^-B^+ rates in Asia and Latin America. We also propose that the diverse *C. difficile* genomes found in different parts of the world be used to design primers for epidemiological surveys instead of existing primers that can give false negative results. Finally, we provide advice on the design of *TcdA* primers that we will apply to epidemiological surveys in Chinese hospitals in the near future.

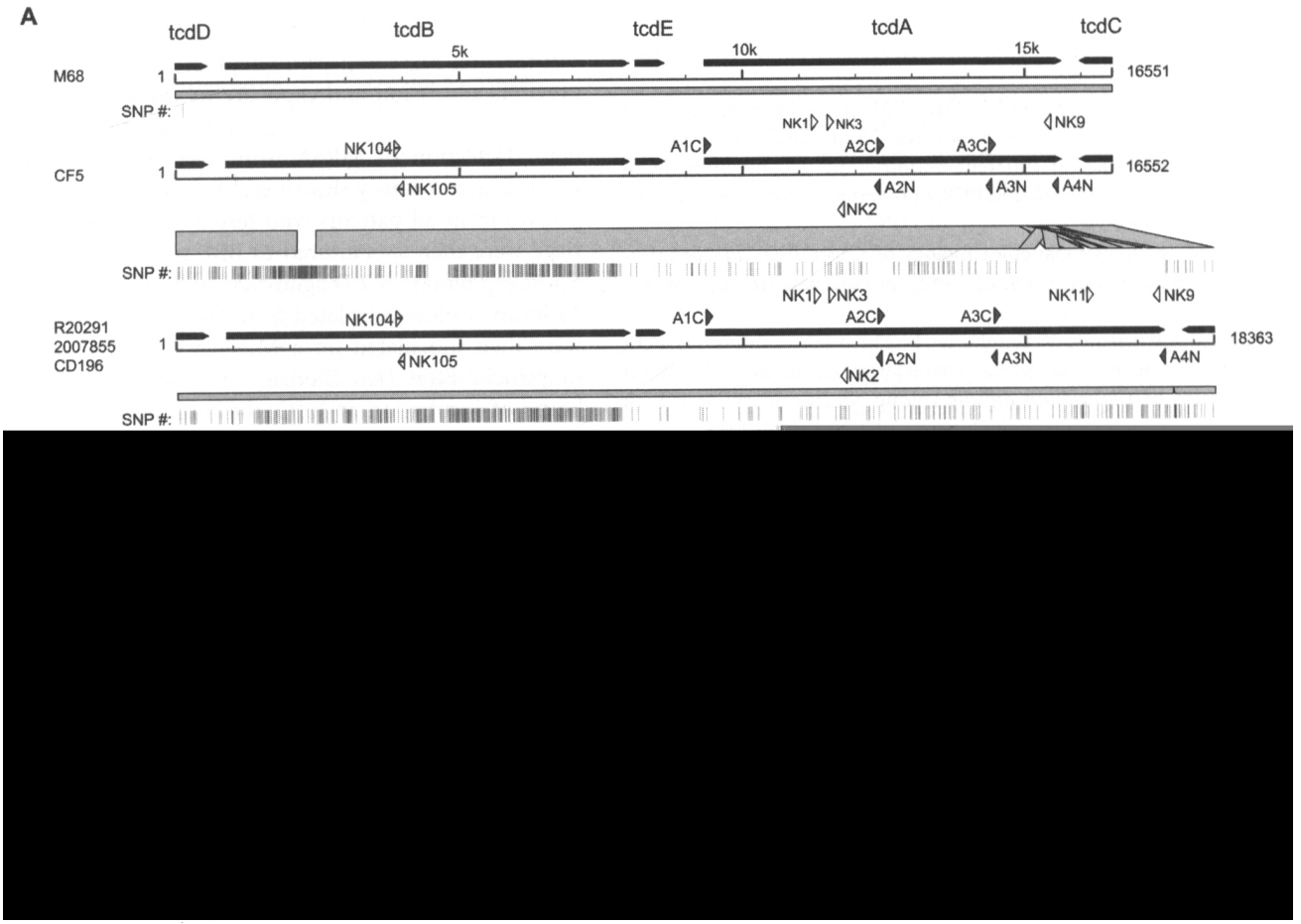

FIGURE 1. Identification of primer similarity in different pathogenicity locus (PaLoc) orthologous groups. *A*, Distribution of primers in 7 public PaLoc genomes. Genetic comparison structure and single-nucleotide polymorphisms (SNPs) have been presented between each orthologue by using light gray bars (*e*-value, $1e-10$) and light gray lines. Three different sets of primers are marked with different types of arrows along the genome. *B*, Primers with SNPs among 7 PaLoc regions. At right is shown the primer pair for the major experiment.

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REFERENCES

1. Crobach MJ, Dekkers OM, Wilcox MH, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing *Clostridium difficile* infection (CDI). *Clin Microbiol Infect* 2009;15(12):1053–1066.
2. Goorhuis A, Legaria MC, van den Berg RJ, et al. Application of

multiple-locus variable-number tandem-repeat analysis to determine clonal spread of toxin A–negative *Clostridium difficile* in a general hospital in Buenos Aires, Argentina. *Clin Microbiol Infect* 2009;15:1080–1086.
3. van den Berg RJ, Claas EC, Oyib DH, et al. Characterization of toxin A–negative, toxin B–positive *Clostridium difficile* isolates from outbreaks in different countries by amplified fragment length polymorphism and PCR ribotyping. *J Clin Microbiol* 2004; 42(3):1035–1041.
4. Gerding DN. Global epidemiology of *Clostridium difficile* infection in 2010. *Infect Control Hosp Epidemiol* 2010;31(suppl 1):S32–S34.
5. Barbut F, Lalande V, Burghoffer B, Thien HV, Grimprel E, Petit JC. Prevalence and genetic characterization of toxin A variant strains of *Clostridium difficile* among adults and children with diarrhea in France. *J Clin Microbiol* 2002;40:2079–2083.
6. Kato H, Kato N, Watanabe K, et al. Identification of toxin A–negative, toxin B–positive *Clostridium difficile* by PCR. *J Clin Microbiol* 1998;36:2178–2182.
7. Geric B, Rupnik M, Gerding DN, Grabnar M, Johnson S. Distribution of *Clostridium difficile* variant toxinotypes and strains with binary toxin genes among clinical isolates in an American hospital. *J Med Microbiol* 2004;53(9):887–894.
8. Rupnik M, Kato N, Grabnar M, Kato H. New types of toxin A–negative, toxin B–positive strains among *Clostridium difficile* isolates from Asia. *J Clin Microbiol* 2003;41(3):1118–1125.
9. Kato H, Kato N, Katow S, Maegawa T, Nakamura S, Lyerly DM. Deletions in the repeating sequences of the toxin A gene of toxin A–negative, toxin B–positive *Clostridium difficile* strains. *FEMS Microbiol Lett* 1999;175:197–203.

Prevalence of Nasal Carriage of Mupirocin-Resistant *Staphylococcus aureus* among Hospitalized Patients in Thailand

To the Editor—In various parts of the world, mupirocin has been used as a component of a "search and destroy" strategy to prevent methicillin-resistant *Staphylococcus aureus* (MRSA) infection prior to a surgical procedure.[1-3] Increased use of mupirocin has been associated with an increased prevalence of mupirocin-resistant MRSA.[4,5] In Thailand, there is neither routine surveillance for nasal carriage of MRSA nor attempts to eradicate carriage among hospitalized inpatients. Given that mupirocin and antibiotics can be purchased at outpatient pharmacies in Thailand without a prescription,[6] it is expected that the prevalence of mupirocin-resistant MRSA nasal carriage is high. We performed a point prevalence study to evaluate the prevalence of MRSA nasal carriage and to estimate the proportion of individuals with mupirocin-resistant MRSA nasal carriage among admitted inpatients at Thammasat University Hospital.

From January 1 to January 7, 2010, we cultured anterior nares swab specimens to detect MRSA carriage. Consecutive hospitalized patients who consented to the study participation on day 1 of hospitalization provided swab samples from both anterior nares that were then cultured for presence of MRSA. Information regarding demographics and clinical history of previous admission and exposure to antibiotics (in both inpatient and outpatient settings) was collected from each patient. Detection of MRSA from swab specimens was performed as previously described.[4] Samples containing MRSA isolates from all patients who tested positive for MRSA carriage were then subcultured to BBL trypticase soy agar with 5% sheep blood (BD Diagnostics) and incubated at 35°C for 24 hours. Colonies isolated from the BBL trypticase soy agar were then inoculated on a Mueller Hinton II plate, and a mupirocin Etest (AB Biodisk) strip was applied. After 24 hours of incubation at 35°C, the minimum inhibitory concentration (MIC) was read. Isolates were classified as susceptible (MIC, <8 μg/mL), low-level resistant (MIC, 8–256 μg/mL), or high-level resistant (MIC, 512 μg/mL).

During the study period, 250 (86%) of 290 adult patients who were admitted to all units at Thammasat University Hospital provided consent and were enrolled in the study; 149 (60%) were male, and the median age was 45 years (range, 20–89 years). Nasal carriage of MRSA was detected in 9 patients (3.6%). Four (44%) of these 9 patients were admitted to medical units, 3 (33%) were admitted to surgical units, and 2 (23%) were admitted to an orthopedic unit. All patients with nasal carriage of MRSA had a history of hospital admission within the past 12 months. Low-level mupirocin-resistant MRSA was detected in 2 patients (22%). These 2 patients had a history of skin abrasions and had self-purchased mupirocin and fluoroquinolones from a local pharmacy within 3 months of admission to the hospital.

Our data suggest that MRSA nasal carriage occurred among patients who had a history of contact with the Thai health care system, and that injudicious use of mupirocin in the community may be associated with carriage of low-level mupirocin-resistant MRSA isolates. The relatively low prevalence of MRSA nasal carriage (3.6%) among hospitalized patients in our study suggests that a search and destroy strategy may not be cost-effective to implement in this middle-income country, while the relatively high prevalence of mupirocin-resistant MRSA (22%) among patients with nasal carriage of MRSA suggests the need to employ an antimicrobial stewardship program at the community level to help limit the unnecessary use of mupirocin and other antibiotics. Further studies are needed to evaluate the relationship between the unnecessary use of mupirocin in the community and the emergence of mupirocin-resistant MRSA among hospitalized inpatients and to evaluate the cost-effectiveness of a search and destroy strategy for MRSA in Thailand.

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