

Abstracts of Outstanding Presentation (2)

Gene Analysis of Nosocomial Infection due to *Clostridium difficile*

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Introduction

Clostridium difficile is a spore-forming toxigenic obligate anaerobe that often causes *C. difficile*-associated diarrhea/disease (CDAD) after the use of an antibacterial agent. Reportedly, *C. difficile* is resistant to heat, dryness, disinfectants, and antibacterial agents and often causes nosocomial infections.

In the present study, we investigated clinical isolates to establish gene analysis (toxin and DNA types) required for epidemiological studies of nosocomial infections.

Materials and Methods

Thirteen *C. difficile* strains were analyzed for their toxin genes (toxins A and B and binary toxin) using polymerase chain reaction (PCR) according to the methods of Katoh, *et al.* and Stubbs, *et al.* DNA types were analyzed with random amplified polymorphic DNA (RAPD)-PCR and pulsed-field gel electrophoresis (PFGE) after *Sma I* digestion.

Results

1. Isolation Status of *C. difficile*

In our hospital, *C. difficile* was isolated from a total of 13 to 19 patients, who had a positive culture or toxin test, monthly for the past 6 months (**Table 1**). Classification of the strains by department revealed that the departments of internal medicine accounted for about 75% of cases and that *C. difficile* was more frequently isolated in departments in which many patients with severe underlying diseases and elderly patients were hospitalized (**Fig. 1**).

Table 1 Number of *C. difficile* carriers per month, January through June 2011

	Jan.	Feb.	Mar.	Apr.	May	June	Total
Culture test (+)	12	13	14	8	10	9	66
Toxin test (+)	9	8	6	8	8	7	46
Culture (+)/Toxin (+)	3	2	1	3	2	2	13
<i>C. difficile</i> carriers	18	19	19	13	16	14	99

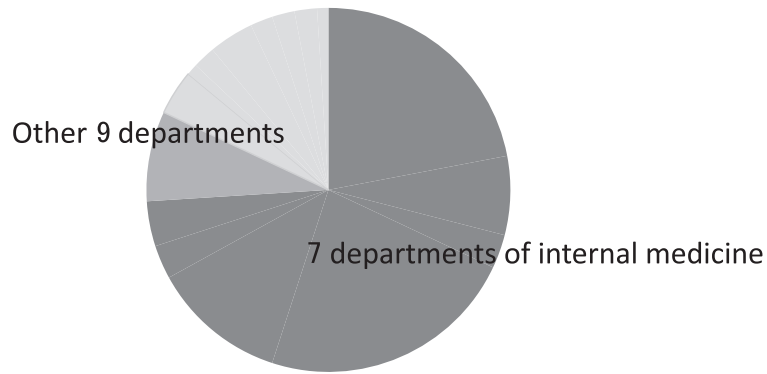


Fig. 1 Number of *C. difficile* carriers detected by department from January through June 2011. Number of *C. difficile* carriers: 99, including 74 (74.7%) in 7 departments of internal medicine and 26 (26.3%) in the other 9 departments.

Note: the upper part of Figure 1 is a table. The pie chart can probably be omitted because it carries very little information, which is already in the text, but I will leave that for the authors to decide.

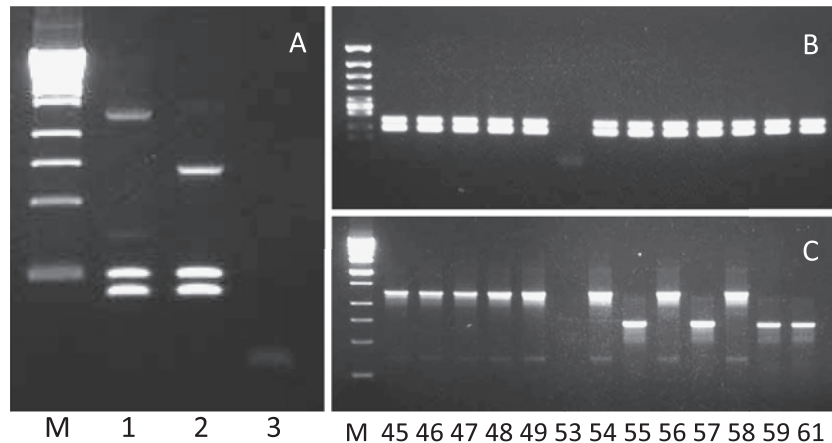


Fig. 2 Agarose gel electrophoresis of PCR products of *C. difficile* strains. PCR with primer set NK3-NK2 for detecting the non-repeating portion of toxin A gene (273 bp), NK104-NK105 for detecting the non-repeating portion of toxin B gene (204 bp), and NKV011-NK11-NK9 for detecting the repeating portions of the toxin A gene (1,266 bp) and the toxin B gene (714 bp). **A:** lane 1, A +B+ type; lane 2, A –B+ type; lane 3, A –B – type; M lane Kb DNA Ladder as a size marker. **B:** PCR products of the non-repeating portions of the toxin A and toxin B genes. **C:** PCR products of the repeating portion of the toxin A gene.

2. Toxin Types

C. difficile produces toxins A (enterotoxin) and B (cytotoxin) and binary toxin (actin-specific ADP-ribosyltransferase), which are closely involved in the virulence of *C. difficile*. However, the toxin types cannot be distinguished with the toxin test used in our hospital. The 13 strains were analyzed for their toxin types to demonstrate 8 toxin A-positive and toxin B-positive strains, 8 toxin A-negative and toxin B-positive strains, and 1 nonpathogenic strain (**Fig. 2**). No binary toxin was detected (data not shown).

3. DNA Type Analysis

PFGE is commonly used to analyze DNA types. However, PFGE reportedly cannot distinguish *C. difficile*

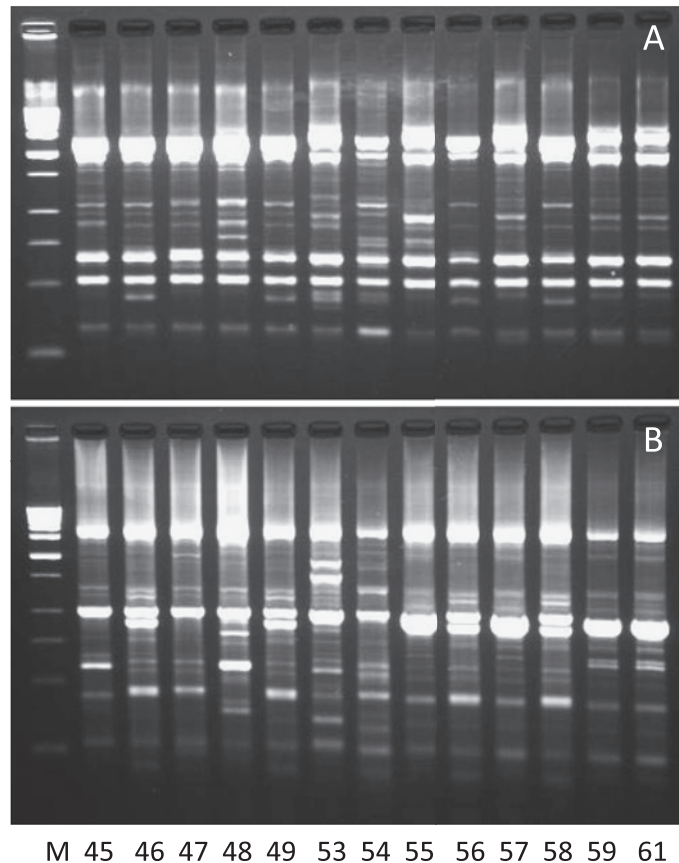


Fig. 3 Agarose gel electrophoresis of RAPD-PCR products of 13 *C.difficile* isolates. Primers whit Eric-2 (A) and 1254 (B).

strains. Thiourea, added to electrophoretic buffer, allows strain distinction. Thus, we added thiourea but still could not distinguish some strains (data not shown). Subsequently, DNA types were analyzed with RAPD-PCR using Eric-2 (A) and 1254 (B) primers. A comparison of PCR products has allowed strain distinction (**Fig. 3**).

Discussion

Spore-forming *C. difficile* is a highly-resistant microorganism because of spore formation, often causing repeated exacerbation and relapse of CDAD. *C. difficile* is attracting attention as a causative bacteria of nosocomial infections.

The toxin and culture tests should be combined because the detection rate of *C. difficile* with the toxin test is 50% lower than that with the culture test. Strain isolation allows the distinction of toxin types and epidemiological studies of suspected nosocomial infection.

PFGE could not distinguish strains during nosocomial infections, as previously reported. Thus, further investigation is needed. On the other hand, poorly reproducible RAPD-PCR could be used to distinguish strains under the same conditions, thus providing a useful analytical method.