

Isolation of the first three cases of *Clostridium difficile* polymerase chain reaction ribotype 027 in Singapore

Lim P L, Ling M L, Lee H Y, Koh T H, Tan A L, Kuijper E J, Goh S S, Low B S, Ang L P, Harmanus C, Lin R T P, Krishnan P, James L, Lee C E

Communicable Disease Division,
Ministry of Health,
16 College Road,
Singapore 169854

Lim P L, MD,
MPH, FAMS
Senior Consultant

James L, MBBS,
MMed, FAMS
Director

Standards & Quality
Improvement Division

Lee H Y, MBBS,
MMed, MA
Deputy Director

Healthcare
Performance Group

Lee C E, MBBS,
MMed, FAMS
Senior Director

Department of
Pathology,
Singapore General
Hospital,
Outram Road,
Singapore 169608

Ling M L, MBBS,
FRCPA, MBA
Senior Consultant

Koh T H, MBBS,
FRCPA, DTM&H
Senior Consultant

Tan A L, MBBS,
FRCPA, FAMS
Senior Consultant

Goh S S, BSc, MSc
Scientific Officer

Low B S, BSc
Senior Medical
Technologist

Ang L P, DipBiomedSc
Laboratory Technologist

Department of
Experimental
Microbiology,
Leiden University
Medical Centre,
Albinusdreef 2,
2333 ZA Leiden,
Netherlands

Kuijper E J, MD,
PhD
Head

Harmanus C
Research Technician

ABSTRACT

Introduction: The incidence of *Clostridium (C.) difficile* infection (CDI) was on the rise from 2001 to 2006 in Singapore. Recent unpublished data suggests that its incidence had remained stable or decreased in most local public hospitals between 2006 and 2010. It is, however, not known if the polymerase chain reaction (PCR) ribotype 027 strains have been circulating, although reports suggest that this strain is emerging in Asia, with the first cases reported from Japan in 2007, as well as in Hong Kong and Australia in 2009. We initiated a culture-based surveillance to detect this epidemic strain in Singapore.

Methods: From September 2008 to December 2009, all non-duplicate toxin-positive stool samples from the three largest public hospitals in Singapore were collected for culture and further analysis.

Results: Out of the 366 samples collected, 272 viable isolates were cultured. Of these, 240 tested toxin-positive and ten tested positive for the binary toxin gene; 35 different PCR ribotypes were found. Three isolates that tested positive for binary toxin contained the same PCR ribotyping pattern as the *C. difficile* 027 control strain. All three had the 18-bp deletion and single nucleotide *tcdC* deletion at position 117. Susceptibility testing was performed, demonstrating susceptibility to erythromycin and moxifloxacin.

Conclusion: We report the first three isolates of *C. difficile* 027 from Singapore. However, their susceptibility patterns are more consistent with the historical 027 strains. Rising CDI incidence may not be associated with the emergence of the epidemic 027 strain at this time.

Keywords: *Clostridium difficile*, epidemiology, ribotype 027, Singapore, surveillance

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INTRODUCTION

Outbreaks of *Clostridium (C.) difficile* infection (CDI), which is marked by higher disease severity and mortality, have occurred in North America since 2001.⁽¹⁾ The epidemic strain has been characterised as toxinotype III, pulsed-field gel electrophoresis type NAP1 and polymerase chain reaction (PCR) ribotype 027.⁽¹⁾ In 2003, the 027 strain was found in the United Kingdom, and in Europe from 2005.⁽²⁾ It is emerging in the Asia-Pacific region, with Japan, as well as Hong Kong and Australia reporting their first cases in 2007 and 2009, respectively.^(3,4,5)

The incidence of hospital CDI in Singapore was 5.4 cases per 10,000 patient days in 2002–2003.⁽⁶⁾ However, another report has indicated that CDI incidence increased sharply in 2001–2006, from 1.49 to 6.64 cases per 10,000 patient days.⁽⁷⁾ Recent unpublished data from the Network for Antibiotic Resistance Surveillance (Singapore) suggests that CDI incidence had remained stable or decreased in most public hospitals in Singapore in 2006–2010. It is not known whether the 027 strains were circulating, as testing was based on toxin detection by enzyme-linked immunoassay (EIA). Since *C. difficile* 027 is not always associated with severe disease, some authorities have recommended culture-based surveillance. This study is a prospective culture-based surveillance project in the three largest public hospitals in Singapore.

METHODS

The participating hospitals were National University Hospital (NUH), Singapore General Hospital (SGH), and Tan Tock Seng Hospital (TTSH), with 900, 1,600 and 1,200 beds, respectively. From September 15, 2008 to December 1, 2009, all non-duplicate stool samples from routine clinical testing that were positive for *Clostridium difficile* toxin (CDT) by EIA (Immunocard Toxins A&B, Meridian Bioscience Inc, Cincinnati, OH, USA) were collected for culture and further analysis. Cultures were performed on both CDC anaerobe 5% sheep blood agar and anaerobe CNA agar with 5% sheep blood (Becton, Dickinson and Co, Sparks, MD, USA) after alcohol

Department
of Laboratory
Medicine,
National University
Health System,
5 Lower Kent Ridge
Road,
Singapore 119074

Lin R T P, MBBS,
FRCPA, FAMS
Senior Consultant
and Head

Department
of Laboratory
Medicine,
Tan Tock Seng
Hospital,
11 Jalan Tan Tock
Seng,
Singapore 308433

Krishnan P, MBBS,
FRCPA, DTM&H
Senior Consultant
and Head

Correspondence to:
Dr Lim Poh Lian
Tel: (65) 6357 7919
Fax: (65) 6252 4056
Email: pllim@post.harvard.edu

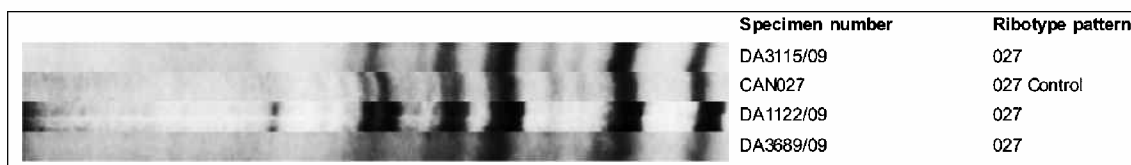


Fig.1 Molecular strain type analysis of 3 PCR ribotype 027 isolates (DA3115/09, DA1122/09 and DA3689/09) shows the same band patterns as the PCR ribotype 027 control (CAN027).

shock using 95% ethanol solution. *C. difficile* was identified by colony morphology and API® 20A strip (bioMérieux, Marcy L'Etoile, France). Separate cultures were performed in cooked meat broth at 35°C under anaerobic conditions for 24 hours before toxin detection. The binary toxin gene was detected in toxin A and B positive cultures by PCR. Ribotyping was performed⁽⁸⁾ and compared to a control *C. difficile* 027 strain, and the putative negative regulator of toxin production gene (*tcdC*) was sequenced. Clinical data on the positive cases was collected by medical record review with prior ethics consent from the relevant institutional review boards (NHG DSRB E/08/610 and Singhealth IRB 2008/246/E).

RESULTS

We collected 366 samples, from which 272 viable isolates were cultured. Of these, 240 were toxin-positive, yielding ten isolates that were positive for the binary toxin gene; 35 different PCR ribotypes were found. One particular PCR ribotype was predominant (comprising 24% of the isolates), and was found in all three hospitals. Three isolates positive for binary toxin had the same PCR ribotyping pattern as the *C. difficile* 027 control strain (Fig. 1). All three had the 18-bp deletion and the *tcdC* position 117 deletion previously described.⁽⁹⁾ Amplification and sequencing of the *tcdC* gene for the first two isolates were performed using primers C1 and C2. The third isolate failed to amplify using these primers, so the C1 primer was replaced by the CD1 primer. Both the 18-bp and position 117 deletions were detected through sequencing. The first two isolates were confirmed by the Reference Laboratory for *C. difficile*, Leiden University Medical Centre, The Netherlands. Susceptibility testing was performed on supplemented brucella agar (BioMedia Laboratories, Melaka, Malaysia) inoculated with 027 strains that were suspended in thioglycolate broth to 1 McFarland turbidity Etest® gradient strips (AB bioMérieux, Marcy L'Etoile, France) were used (Table I).

Case 1 was a 46-year-old woman with breast cancer who was admitted in November 2008 to NUH for pneumonia, bacteraemia and *Escherichia* (*E.*) *coli* urinary tract infection, and was treated with three

different broad-spectrum antibiotics. During her second week of hospitalisation, the patient developed diarrhoea and tested positive for CDT, but recovered with oral metronidazole. Case 2 was a 66-year-old man with widely metastatic rectal cancer who was receiving palliative chemotherapy. He was re-admitted to SGH in March 2009 with fever and profuse diarrhoea, one month after receiving clindamycin from his previous hospitalisation. The diagnosis of CDI was confirmed and the patient was treated with oral metronidazole for two weeks, followed by oral vancomycin for one week due to persistent diarrhoea. He was discharged in stable condition and subsequently passed away at home. Case 3 was an 84-year-old woman with lymphoma who had received ceftriaxone in May 2009 for urinary tract infection. She was re-admitted to NUH in August 2009 with neutropenia and *E. coli* bacteraemia, which required treatment with ceftazidime and later, ciprofloxacin. During her August admission, the patient developed diarrhoea and was treated with oral metronidazole for CDI before being discharged home.

All three cases were unlinked; there was no history of travel related to these infections, and they appeared to have arisen as sporadic cases. Standard CDI infection control measures were applied, including contact precautions (gowns, gloves, strict hand hygiene, individual isolation rooms, if available) and environmental cleaning with hypochlorite bleach. No associated secondary cases or outbreaks were detected.

DISCUSSION

We report the first three cases of *C. difficile* PCR ribotype 027 isolated in Singapore. The strains were found in an endemic situation of CDI, corresponding with 1.62, 2.96 and 3.0 CDI cases per 10,000 patient-days in the three participating hospitals in 2009. This is an important finding, as *C. difficile* PCR ribotype 027 has been associated with increased virulence, clinically severe disease and mortality, as well as with outbreaks in hospitals in Canada and nursing homes in the United Kingdom. These cases would have gone undetected without the enhanced culture-based surveillance enabled by this prospective study. Relying only on signals of

Table I. Minimum inhibitory concentrations (MICs) of antibiotics for *Clostridium difficile* ribotype 027 strains using Etest strips.

	MIC ($\mu\text{g/mL}$) for 027 strains			
	DA3689-08	DA1122-09	DA3115-09	CAN027-111 (027 control)
Levofloxacin	> 30*	> 32*	> 32*	> 32
Moxifloxacin	1	1	1	> 32
Clindamycin	4	4	4	2
Erythromycin	2	2	1.5	> 256

* Actual reading in the range 4–8 $\mu\text{g/mL}$. However, small colonies were seen within zone clearing.

severity or outbreak could result in late detection, thus potentially permitting the epidemic 027 strain to become established or even hyper-endemic.

C. difficile 027 outbreak strains demonstrate high resistance to erythromycin and newer fluoroquinolones, whereas historical 027 strains are usually susceptible to newer fluoroquinolones.^(1,8) The 027 isolates in the present study were susceptible to erythromycin and had susceptibility patterns that were more consistent with historical 027 strains than with the outbreak strains. They responded to routine treatment, did not trigger any outbreaks and appeared to have arisen as sporadic cases; this is again more consistent with the historical 027 strains.

The differences between *C. difficile* strains in Asia and Europe were explored in a recent study comparing isolates from Shanghai and Stockholm.⁽¹⁰⁾ Of the 75 isolates from Shanghai, 33% were toxin A-negative and toxin B-positive compared to 0% of the 80 isolates from Stockholm. The minimal inhibitory concentrations for fluoroquinolones, erythromycin and clindamycin were significantly higher for the Shanghai isolates than for the Stockholm isolates, but no 027 clones were detected.⁽¹⁰⁾

C. difficile is potentially an important emerging pathogen in Asia due to its impact on healthcare systems and costs. CDI can prolong admissions by five days,⁽⁷⁾ straining hospital systems struggling with high occupancy rates. The ageing demographic trend in developed Asian countries may worsen CDI rates, given its increased risk in elderly patients. The estimated annual cost of CDI to the United States healthcare system is US\$3.2 billion.⁽¹¹⁾ Such similar costs would constitute a disproportionately heavy burden to Asian economies.

The detection of *C. difficile* 027 in multiple Asian countries calls for continued vigilance in clinical and laboratory surveillance, enhanced infection control and active antimicrobial stewardship so as to address this emerging threat. Given its epidemic potential and virulence, health authorities need to actively monitor the spread of *C. difficile* 027 in their own countries and across Asia. Financial support for such surveillance and

prevention measures must be sought to strengthen this important component of public health preparedness, whether through public health funding or mixed cost-sharing mechanisms between healthcare facilities, patients and payors in the private sector, given its potential for nosocomial transmission.

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