Clostridium difficile in Ready-to-Eat Salads, Scotland

**Marwah M. Bakri, Derek J. Brown,
John P. Butcher, and Alistair D. Sutherland**

Of 40 ready-to-eat salads, 3 (7.5%) were positive for
Clostridium difficile by PCR. Two isolates were PCR ribo-
type 017 (toxin A–, B+), and 1 was PCR ribotype 001. Iso-
lates were susceptible to vancomycin and metronidazole but
variably resistant to other antimicrobial drugs. Ready-to-eat
salads may be potential sources for virulent *C. difficile*.

Over the past decade, *Clostridium difficile* infection has
become a prominent cause of healthcare-associated in-
fection. Although *C. difficile* has been thought of tradition-
ally as a predominantly nosocomial infection, the incidence
of community-acquired cases has increased recently, as has
the incidence of cases from other healthcare settings such
as nursing homes (*1*). Notably, some evidence has shown
that *C. difficile* may be brought into the healthcare environ-
ment by asymptomatic carriers (*2*). The reported carriage
rates of *C. difficile* in healthy adults have varied from 0%
to 3% in Europe to up to 15% in Japan (*3*). Little is known,
however, about the prevalence of *C. difficile* in the environ-
ment and how it may be transmitted to humans.

C. difficile has been found in a variety of environments,
including water, soil, animal feces, and foods (*4,5*); these
findings suggest that *C. difficile* may be transmitted to hu-
mans through food, although no foodborne cases have been
reported. Because ready-to-eat foods have been implicated
in foodborne disease outbreaks associated with *Salmonella*
species (*6*) and *Escherichia coli* O157 (*7*), we examined
ready-to-eat salads for the presence of *C. difficile*.

The Study

We tested 50-g samples from each of 40 packaged
ready-to-eat salads purchased from 7 Glasgow supermar-
kets from May 1 through June 30, 2008, for the presence of
C. difficile spores. We essentially used the CDMN (*C. dif-
ficile*, moxalactam, norfloxacin) agar method of Rodriguez-
Palacios et al. (*4*) but also used direct plating and enrich-
ment broth culture. The contents of the 40 salads generally
differed, and any salads with the same contents carried

Author affiliations: Glasgow Caledonian University, Glasgow, Scot-
land, UK (M.M. Bakri, J.P. Butcher, A.D. Sutherland); and Scottish
Salmonella, *Shigella* and *Clostridium difficile* Reference Labora-
tory, Glasgow (D.J. Brown)

different supermarket brands, which eliminated replicate
sampling.

Isolates were identified as toxigenic (having genes for
toxins A and B) by PCR as previously described (*8,9*), and
ribotypes were identified by PCR (*10*). The MICs of 6 anti-
microbial drugs for these isolates were determined by using
E-test strips (AB Biodisk, Solna, Sweden). The following
MIC breakpoints were used to define resistance to these
drugs: metronidazole, ≥32 µg/mL; vancomycin, ≥16 µg/
mL; cefotaxime, 64 µg/mL; erythromycin, ≥8 µg/mL; mox-
ifloxacin, ≥8 µg/mL; and clindamycin, ≥8 µg/mL (Clinical
and Laboratory Standards Institute, Wayne, PA, USA).

C. difficile spores were detected in 3 (7.5%) of the 40
salad samples after culturing in enrichment broth (Table 1).
Thirty-five (87.5%) of the salads were marked as import-
ed from European Union countries; the remaining 5 were
from UK suppliers. The 3 contaminated salads were not of
UK origin. The 3 isolates were found to be toxinogenic by
PCR; 2 were PCR ribotype 017, and 1 was PCR ribotype
001 (Table 1).

The MICs of 6 antimicrobial drugs for each isolate and
the resistance profile of each isolate are shown in Table 2.
None of the isolates was resistant to vancomycin or metron-
idazole, and only the 001 isolate was resistant to moxifloxacin
and erythromycin. All 3 isolates were resistant or intermedi-
ately resistant to clindamycin and cefotaxime; breakpoints
for these drugs were highest for the 001 isolate.

Isolates were obtained after being cultured in enrich-
ment broth and not by direct plating, which suggests that
spore counts were low (<3.0 CFU/g). The infectious dose
required to colonize the healthy human gut is, however,
unknown. Isolates were of PCR ribotypes 001 (a common
clinical isolate in Scotland [*11*]) and 017 (a common Eu-
ropean PCR ribotype containing isolates that are negative
for toxin A and positive for toxin B [*12*]). No isolate was
resistant to vancomycin or metronidazole, which is in ac-
cord with findings for other *C. difficile* isolates found in
Scotland (*11*), but recent studies have highlighted the emer-
gence of increased resistance to metronidazole among *C.
difficile* isolates in England (*13*).

In general, the PCR ribotype 001 isolate was more
drug resistant than the 017 isolates; it was the only iso-
late resistant to moxifloxacin and erythromycin and had
the highest breakpoints to clindamycin and cefotaxime.
In a 2005 study in which 271 *C. difficile* isolates from
the UK were examined, all were found to be resistant to
cefotaxime (*14*).

Table 1. PCR profile for toxins A and B and PCR ribotype of
Clostridium difficile isolates, Scotland, 2008

Sample no./description	Toxin A	Toxin B	PCR ribotype
13/baby leaf spinach	–	+	017
24/organic mixed leaf salad	–	+	017
35/organic lettuce	+	+	001

Table 2. Susceptibility of 3 *Clostridium difficile* isolates to 6 antimicrobial drugs, by source of isolate, Scotland, UK, 2008*

Antimicrobial drug	Source of isolate		
	Baby leaf spinach (MIC, µg/mL)	Organic mixed leaf salad (MIC, µg/mL)	Organic lettuce (MIC, µg/mL)
Metronidazole	S (0.125)	S (0.094)	S (0.75)
Vancomycin	S (0.50)	S (0.38)	S (1.0)
Moxifloxacin	S (0.50)	S (0.75)	R (256)
Clindamycin	I (4.0)	I (6.0)	R (8.0)
Erythromycin	S (1.5)	S (0.75)	R (192)
Cefotaxime	I (48)	R (64)	R (256)
*S, sensitive; R, resistant; I, intermediate.			

Conclusions

The isolation of these PCR ribotypes from ready-to-eat salads is of concern and highlights the potential risk associated with consuming these salads, particularly since they are not cooked before being consumed. The consumption of these foods by vulnerable groups could possibly lead to *C. difficile* colonization and an increase in the asymptomatic *C. difficile* carriage rate among humans, thus increasing the risk for *C. difficile* transference within the healthcare environment (*2*). The presence of *C. difficile* in ready-to-eat salads could result from environmental contamination or transmission by food handlers. Further work is needed to investigate foods as a source of this pathogen and also to assess the role of soil and animals as its reservoirs.

Mrs Makri is a lecturer at Jazan University in Jazan, Saudi Arabia, and a candidate for a PhD in microbiology at Glasgow Caledonian University, Glasgow, Scotland, under the supervision of Dr Sutherland. Her research interest involves the molecular epidemiology of *C. difficile*.

References

1. McFarland LV, Beneda HW, Clarridge JE, Raugi GJ. Implications of the changing face of *Clostridium difficile* disease for health care practitioners. Am J Infect Control. 2007;35:237–53. DOI: 10.1016/j.ajic.2006.06.004
2. Clabots CR, Johnson S, Olson MM, Peterson LR, Gerding DN. Acquisition of *Clostridium difficile* by hospitalized patients: evidence for colonized new admissions as a source of infection. J Infect Dis. 1992;166:561–7.
3. Mulligan ME. *Clostridium difficile*—its role in intestinal disease. London: Academic Press; 2008. p. 229–56.
4. Rodriguez-Palacios A, Staempfli HR, Duffield T, Weese JS. *Clostridium difficile* in retail ground meat, Canada. Emerg Infect Dis. 2007;13:485–7.
5. Al Saif N, Brazier JS. The distribution of *Clostridium difficile* in the environment of South Wales. J Med Microbiol. 1996;45:133–7.
6. Sagoo SK, Little CL, Ward L, Gillespie IA, Mitchell RT. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. J Food Prot. 2003;66:403–9.
7. Delaquis P, Bach S, Dinu LD. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. J Food Prot. 2007;70:1966–74.
8. Ward SJ, Douce G, Dougan G, Wren BW. Local and systemic neutralizing antibody responses induced by intranasal immunization with the nontoxic binding domain of toxin A from *Clostridium difficile*. Infect Immun. 1999;67:5124–32.
9. Butcher J. Development of inhibitory analogs of *Clostridium difficile* toxins as novel therapeutics [dissertation]. Glasgow (UK): Glasgow Caledonian University; 2009.
10. Stubbs SL, Brazier JS, O'Neill GL, Duerden BI. PCR targeted to the 16S–23S rRNA gene intergenic spacer region of *Clostridium difficile* and construction of a library consisting of 116 different PCR ribotypes. J Clin Microbiol. 1999;37:461–3.
11. Mutlu E, Wroe AJ, Sanchez-Hurtado K, Brazier JS, Poxton IR. Molecular characterization and antimicrobial susceptibility patterns of *Clostridium difficile* strains isolated from hospitals in southeast Scotland. J Med Microbiol. 2007;56:921–9. DOI: 10.1099/jmm.0.47176-0
12. van den Berg RJ, Claas EC, Oyib DH, Klaassen CH, Dijkshoorn L, Brazier JS, 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;42:1035–41. DOI: 10.1128/JCM.42.3.1035-1041.2004
13. Emergence of reduced susceptibility to metranidazole in *Clostridium difficile*. Weekly Report, News Archives. Health Protection Agency. 2008;2 [cited 2009 Mar 31]. Available from http://www.hpa.org.uk/hpr/archives/2008/news0308.htm#cdiff1
14. John R, Brazier JS. Antimicrobial susceptibility of polymerase chain reaction ribotypes of *Clostridium difficile* commonly isolated from symptomatic hospital patients in the UK. J Hosp Infect. 2005;61:11–4. DOI: 10.1016/j.jhin.2005.01.020

Address for correspondence: Alistair D. Sutherland, Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Rm C131, Cowcaddens Rd, Glasgow G4 0BA, Scotland, UK; email: a.sutherland@gcal.ac.uk

Search past issues of EID at www.cdc.gov/eid