**Clostridium difficile** in retail baskets, trolleys, conveyor belts, and plastic bags in Saudi Arabia

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**ABSTRACT**

**Objectives:** To determine *Clostridium difficile (C. difficile)* prevalence on retail surfaces and shoppers plastic bags.

**Methods:** From 20 June to 10 August 2011, in a cross-sectional epidemiological study, 17 supermarkets from 2 cities, Albaha and Altaif, Saudi Arabia were sampled. A total of 800 samples, which comprised 200 samples per surveyed surface, were studied. These included baskets, trolleys, conveyer belts, and outgoing shoppers’ plastic bags. *Clostridium difficile* strains were isolated. The isolates were characterized using ribotyping and polymerase chain reaction for the detection of toxin A (*tcdA*), toxin B (*tcdB*), binary toxin (*cdtB*), and toxin C (*tcdC*) genes. Susceptibility to antibiotics was determined on a Muller-Hinton agar with 5% sheep blood agar using E-tests.

**Results:** Overall, the *C. difficile* prevalence on sampled surfaces was 0.75%. The highest prevalence was found on retail baskets and trolleys, followed by plastic bags. A total of 5 different ribotypes were identified. Alterations in *tcdC* were detected in ribotype 027 and BT1. All the identified isolates were susceptible to vancomycin, but resistant to levofloxacin.

**Conclusion:** In this study, *C. difficile* was present at a rate of 0.75% on supermarket surfaces. Spore disinfection of implicated surfaces may be necessary to control any community-acquired infections caused by this pathogen.

The spore-forming anaerobic rod, *Clostridium difficile* (*C. difficile*) is capable of causing gastrointestinal diseases, such as antibiotic-associated colitis, in humans and many animals, including dogs, horses, chickens, and pigs.1 Nosocomial colitis and diarrhea are commonly caused by this pathogen following the use of antibiotics. In addition, hyper-virulent strains, such as ribotype 027, are emerging and causing an increasing incidence, severity, and mortality.2 Moreover, 2 of the major sources of proteins in the Saudi diet, chickens and cows, are known to carry varying amounts of this offensive pathogen. This includes hyper-virulent strains, such as ribotypes 027 and ribotype 078, which were found in meats used for human consumption.1–3 Several studies were conducted to enumerate the amount of *C. difficile* in retail meat and chicken.4,5 Contamination of ready-to-eat foods was also noted.5 Nonetheless, studies showing the level of contamination of food retail fomites are lacking. Cross-contamination of foods during purchasing may cause the contamination of more risky foods, such as ready-to-eat foods, which unlike meats and poultry, are consumed without cleaning and cooking. To this date, no study was known to discuss the epidemiology of *C. difficile* cross-contamination in Saudi Arabia or abroad. A better understanding of *C. difficile* contamination within food retail fomites can help evaluate the risks of infection with community associated *C. difficile* and develop appropriate prevention and control methods. We aim to determine the prevalence and characteristics of *C. difficile* on 4 common food retail fomites: handheld shopping baskets, trolleys, conveyer belts, and outgoing shoppers’ plastic bags. The isolated *C. difficile* were further characterized into different ribotypes and their antimicrobial susceptibilities were determined.

**Methods.** This study was approved by the Faculty of Applied Medical Sciences, Al Baha University, Al Baha, Saudi Arabia. Prior related articles were retrieved and evaluated from PubMed (Medline) and EMBASE using the key words: “retail surfaces”, “*C. difficile*,” and “ribotype.”

A cross-sectional epidemiological study was conducted between June and August 2011, wherein 17 supermarkets from 2 cities, Al Baha and Altaif, Saudi Arabia provided a total of 800 samples. These comprised 200 samples per surface type: baskets, trolleys, conveyer belts, and outgoing shoppers’ plastic bags. From each supermarket, 200 samples per surveyed fomite were obtained via simple random sampling through random number assignment to each available sampling surface. All the market-dominant supermarkets (4 from Al Baha and 5 from Altaif) are those listed by the Ministry of Commerce and Industry of Saudi Arabia5 were sampled. In addition, 8 randomly selected supermarkets from the Scenic Southwestern Tourist Road, between Al Baha and Altaif, Saudi Arabia connecting the many villages between the 2 cities, were sampled. To collect the samples, cotton buds were moistened with sterile saline containing 2% Tween 80 (Sigma-aldrich Chemie GmbH, Steinheim, Germany) and rubbed on the surfaces. The cotton buds were broken and placed in sterile test tubes (Fisher Scientific, Leicestershire, UK) containing 2 ml of *C. difficile* medium (40 g/L proteose...
peptone, 6 g/L fructose, 5 g/L disodium hydrogen phosphate, 2 g/L sodium chloride, 1 g/L potassium dihydrogen phosphate, 0.1 g/L sodium taurocholate and 0.1 g/L magnesium sulfate [Sigma-aldrich Chemie GmbH, Steinheim, Germany]). The tubes were incubated (37°C for 36 hours) anaerobically and then the resulting cultures were shocked with alcohol and incubated for 7 days to allow for sporulation; then, 3 mL of ethanol was vortexed with an equal volume of culture and the mixture was allowed to stand (at room temperature for one hour) before being centrifuged using a bench top centrifuge (Hettich Lab Technology GmbH, Tuttlingen, Germany) for 10 minutes at 1000 x g, and the resulting pellets were subcultured onto Columbia blood agar (CBA) (Oxoid, Nepean, Ontario, Canada) anaerobically for 2 days at 37°C. Seven probable C. difficile isolates were finally subcultured onto CBA plates for another 2 days. A L-proline aminopeptidase activity test (Prodisk, Remel, Lenexa, Kansas, USA) and triose phosphate gene polymerase chain reaction (PCR) were used for final identification of Clostridium isolates. A hybrid PCR express machine (Eppendorf AG, Hamburg, Germany) was used for triose phosphate gene PCR reactions.

Genes encoding production of toxin A (tcdA), toxin B (tcdB), and the binary toxin (cdtB) were PCR amplified and the gels were then inspected visually to determine the ribotype using the method previously described. Ribotype designations for the isolates were determined per HPA anaerobic reference laboratory (Cardiff, UK) standard nomenclature or given enteral designation if no standard nomenclature was applicable. The tcdC gene was detected by PCR and sequenced as previously described.8 Toxinotyping was carried out as described by Rupnik et al9 without modification. Susceptibility to levofloxacin, metronidazole, vancomycin, and clindamycin were tested on Mueller-Hinton agar with 5% sheep blood agar (Oxoid, Nepean, Ontario, Canada) using the E-tests and a 10⁹ cfu/ml exponential-phase inocula (AB Biodisk, Solna, Sweden). Breakpoints used are those described by the Clinical and Laboratory Standards Institute (CLSI),10 except for vancomycin, to which the breakpoints described by Martin et al11 were used.

**Results.** Prevalence of C. difficile. Overall, 0.75% of retail baskets, trolleys, conveyor belts, and plastic bags were contaminated with C. difficile. The fomites with the highest C. difficile prevalence were in retail baskets and trolleys (4 isolates/400 samples), albeit at an insignificant proportion from other surveyed fomites (p=0.25), followed by plastic bags (3 isolates/400), and conveyor belts (1 isolates/400 samples). The differences of prevalence rates of C. difficile in plastic bags (p=0.5) and conveyor belts (p=0.9) were also not statistically significant from that of other fomites. Twelve C. difficile isolates were obtained from 1,600 samples (0.75%) including 7 of 800 samples (0.875%) from Albaha and 5 of 800 samples (0.625%) from Altaif (Table 1). No statistical relationship or association between C. difficile occurrence rates and province was detected (p=0.562).

**Characterization of isolates.** The 12 isolates were classified into 5 ribotypes. Five isolates shared an identical ribotype profile with the epidemic strain ribotype 027. Three of these were obtained from the Altaif province, and 2 from Albaha. All 5 were different ribotypes and toxinotypes.

| Isolate | Province | Source     | Ribotype | Toxin gene profile | Toxinotype | tcdC alteration type |
|---------|----------|------------|----------|-------------------|------------|---------------------|
| B1      | Albaha   | Retail basket | V        | tcdA, tcdB         | III        | None                |
| B2      | Albaha   | Retail basket | 027      | tcdA, tcdB, cdtB   | III        | None                |
| B3      | Albaha   | Retail basket | 027      | tcdA, tcdB, cdtB   | III        | None                |
| B4      | Albaha   | Trolley     | A2       | tcdA, tcdB         | XXVI       | None                |
| B5      | Albaha   | Trolley     | BT1      | tcdA, tcdB, cdtB   | III        | ∆117A               |
| B6      | Albaha   | Plastic bag | A2       | tcdA, tcdB         | XXVI       | None                |
| B7      | Albaha   | Plastic bag | V        | tcdA, tcdB         | 0          | None                |
| T1      | Altaif   | Retail basket | 027      | tcdA, tcdB, cdtB   | III        | ∆117A               |
| T2      | Altaif   | Trolley     | T1       | Not applicable     | Not tested |                    |
| T3      | Altaif   | Trolley     | 027      | tcdA, tcdB, cdtB   | III        | ∆117A               |
| T4      | Altaif   | Conveyor belt | BT1     | tcdA, tcdB, cdtB   | III        | ∆117A               |
| T5      | Altaif   | Plastic bag | 027      | tcdA, tcdB, cdtB   | III        | ∆117A               |

| tcdA - toxin A, tcdB - toxin B, cdtB - binary toxin (cdtB), NA |
toxinotype III with genes for tcdA, tcdB, and cdtB toxins. The 3 isolates of the strain ribotype 027 from Altaif had tcdC genes with the ∆117A truncation mutation, whereas the other 2 isolates from Albaha showed no truncation. In addition, 2 other isolates (one from Albaha and one from Altaif) shared an identical ribotype profile (designated ribotype BT1), had genes for tcdA, tcdB, and cdtB toxins, were toxinotype III, and had tcdC genes with the ∆117A truncating mutation. Two isolates from Albaha (ribotype V) and another one from Altaif (designated ribotype T1) lacked the binary toxin genes. The 2 Albaha ribotype V C. difficile strains gave positive tcdA and tcdB PCR results and happened to be toxinotype 0 with no truncations in the toxic C gene. The other isolate from Altaif, classified as T1, had no tcdA and tcdB genes and was not typeable by toxinotyping. Two other ribotypes from Albaha were classified as ribotype A2 and had tcdA, tcdB, and cdtB toxin genes. It has no noticeable toxin gene deletions and was determined to be toxinotype XXVI.

Susceptibility of isolates to antimicrobials. Most of the identified isolates were vancomycin and metronidazole susceptible and levofloxacin resistant (Table 2). The metronidazole MIC<sub>50</sub> was 0.5 μg/ml and MIC<sub>90</sub> was 1 μg/ml. The metronidazole MIC range was 3-8 μg/ml. Vancomycin MIC<sub>50</sub> was 0.5 μg/ml and MIC<sub>90</sub> was 1 μg/ml, and the MIC range was 0.75 to 2 μg/ml. Inversely, all isolates were resistant to levofloxacin since the MIC range was 8-24 μg/ml. The MIC for clindamycin ranged from 2-16 μg/ml (Table 2).

Discussion. Supermarket fomites were sampled to determine the prevalence of C. difficile and demonstrate the risk of this organism during food purchasing. C. difficile are known to be present in animals, ready-to-eat foods, and environmental samples. But, the level and type of C. difficile contamination of food-purchasing fomites was not yet determined. In this study, which surveyed food-handling fomites inside supermarkets for virulent strains, we recovered 3 of the epidemic ribotype 027, which all showed a ∆117 mutation in the down regulator of tcdA and tcdB production, the tcdC gene, and were toxinotype III. In addition, 2 other isolates (both ribotype BT1) contained the same mutation in the tcdC gene. Mutations in the tcdC probably cause a higher production of toxin A and B, as seen in ribotype 027, which was detected in humans suffering from C. difficile infections. In addition, ribotypes BT1 and V were detected in human infections.

Metronidazole, to which many isolated C. difficile strains were susceptible (Table 2), is one of the antimicrobials of choice for C. difficile-associated diarrhea management because it spares the use of vancomycin and thereby reduces the selective pressure capable of increasing vancomycin-resistant Enterococcus (VRE) and vancomycin-resistant Staphylococcus aureus (VRSA) pathogens. Our study is consistent with other studies showing vancomycin as a second choice antibiotic for the management of C. difficile-associated diarrhea.

The incidence of infections with C. difficile from community sources in Saudi Arabia is estimated at 38% of the total infected patients. Infection due to contamination is likely more important than infection from primary sources for several reasons. First, the level of contamination of primary sources, such as the feces of cattle, sheep, fish, and poultry, and vegetables, was low (0-2.3%). Secondly, the meat, poultry, and vegetables, unlike cheese; for example, are not ready-

| Isolate | Province | Source          | Vancomycin | Clindamycin | Metronidazole | Levofloxacin |
|---------|----------|-----------------|------------|-------------|---------------|--------------|
| B1      | Albaha   | Retail basket   | 2 μg/mL    | 8 μg/mL     | 4 μg/mL       | 12 μg/mL     |
| B2      | Albaha   | Retail basket   | 1.5 μg/mL  | 6 μg/mL     | 16 μg/mL      | 8 μg/mL      |
| B3      | Albaha   | Retail basket   | 2 μg/mL    | 6 μg/mL     | 6 μg/mL       | 12 μg/mL     |
| B4      | Albaha   | Trolley         | 0.75 μg/mL | 4 μg/mL     | 2 μg/mL       | 12 μg/mL     |
| B5      | Albaha   | Trolley         | 2 μg/mL    | 3 μg/mL     | 8 μg/mL       | 24 μg/mL     |
| B6      | Albaha   | Plastic bag     | 2 μg/mL    | 6 μg/mL     | 12 μg/mL      | 16 μg/mL     |
| B7      | Albaha   | Plastic bag     | 1.5 μg/mL  | 4 μg/mL     | 8 μg/mL       | 12 μg/mL     |
| T1      | Altaif   | Retail basket   | 2 μg/mL    | 8 μg/mL     | 12 μg/mL      | 12 μg/mL     |
| T2      | Altaif   | Trolley         | 2 μg/mL    | 3 μg/mL     | 4 μg/mL       | 24 μg/mL     |
| T3      | Altaif   | Trolley         | 1.5 μg/mL  | 6 μg/mL     | 8 μg/mL       | 8 μg/mL      |
| T4      | Altaif   | Conveyor belt   | 2 μg/mL    | 6 μg/mL     | 12 μg/mL      | 16 μg/mL     |
| T5      | Altaif   | Plastic bag     | 2 μg/mL    | 4 μg/mL     | 16 μg/mL      | 24 μg/mL     |

Breakpoints were: metronidazole: ≤8 μg/ml (susceptible), 16 μg/ml (intermediate), and ≥16 μg/ml (resistant); clindamycin and levofloxacin: ≤ 2μg/ml (susceptible), 4 μg/ml (intermediate), and ≥8 μg/ml (resistant); vancomycin: ≤2 μg/ml (susceptible), 4 μg/ml (intermediate), and ≥8 μg/ml (resistant).
to-eat foods; hence, they are cleaned or cooked before consumption. Yet, other studies estimated that the feces of chicken are contaminated with *C. difficile* at a rate of 17.5% and that other fecal samples of other animals are contaminated at a 4.3% rate. Similarly, in line with the low prevalence of *C. difficile* in the aforementioned primary sources, *C. difficile* was also uncommon in this study (0.75%). It is difficult to identify the source of the contamination. Nonetheless, soil, which was found contaminated at 22.4%, vegetables (2.3%), poultry (1.6-17.5%), or other animals (0-4.3%) could be possible sources. 

**Study limitations.** The study was designed for the cities of Alba and Altaf only and due to minimal representation of isolates due to the limited geographic region, it cannot be used to make national inferences and it has no direct clinical implication. Nonetheless, the study sought to identify the prevalence of *C. difficile* in Alba and Altaf supermarkets and their antibiotic-susceptibility and virulence profiles. A nation wide study may be needed to determine the general prevalence of this pathogen. The actual risk of exposure to *C. difficile* through retail equipment is difficult to determine. Nonetheless, spore formers, such as *C. difficile*, present a difficult challenge to the retail business and the food industry. 

In conclusion, *C. difficile* is present in retail surfaces and can contaminate outgoing shoppers’ plastic bags. They harbor pathogenic toxins and can be a potential link to community-acquired infections. Further, studies are now needed to understand their medical impact and to enact any necessary preventative measures.

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**References**

1. Janezic S, Zidaric V, Pardon B, Indra A, Kokotovic B, Blanco JL, et al. International *Clostridium difficile* animal strain collection and large diversity of animal associated strains. *BMC Microbiol* 2014; 14: 173.

2. Valiente E, Cairns MD, Wren BW. The *Clostridium difficile* PCR ribotype 027 lineage: a pathogen on the move. *Clin Microbiol Infect* 2014; 20: 396-404.

3. Weese JS, Reid-Smith RJ, Avery BP, Rousseau J. Detection and characterization of *Clostridium difficile* in retail chicken. *Lett Appl Microbiol* 2010; 50: 362-365.

4. Visser M, Sephi S, Olson N, Du T, Mulvey MR, Alfa MJ. Detection of *Clostridium difficile* in retail ground meat products in Manitoba. *Can J Infect Dis Med Microbiol* 2012; 23: 28-30.

5. Eckert C, Burghoffer B, Barbur F. Contamination of ready-to-eat raw vegetables with *Clostridium difficile* in France. *J Med Microbiol* 2013; 62 (Pt 9): 1435-1438.

6. Ministry of Commerce and Industry of Saudi Arabia [Internet]. Riyadh (KSA): Ministry of Commerce and Industry of Saudi Arabia; [date unknown] [cited 2011 Feb 14]. Available from: http://www.mci.gov.sa/

7. Lee JH, Lee Y, Lee K, Riley TV, Kim H. The changes of PCR ribotype and antimicrobial resistance of *Clostridium difficile* in a tertiary care hospital over 10 years. *J Med Microbiol* 2014; 63 (Pt 6): 819-823.

8. Spigaglia P, Mastrantonio P. Molecular analysis of the pathogenicity locus and polymorphism in the putative negative regulator of toxin production (*TcdC*) among *Clostridium difficile* clinical isolates. *J Clin Microbiol* 2002; 40: 3470-3475.

9. Rupnik M, Avesani V, Janc M, von Eichel-Streiber C, Delmee M. A novel toxinotyping scheme and correlation of toxinotypes with serogroups of *Clostridium difficile* isolates. *J Clin Microbiol* 1998; 36: 2240-2247.

10. Hecht DW, Citron DM, Dzink-Fox J, Gregory WW, Jacobus NV. Clinical Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A7. 2007. *Clinical and Laboratory Standards Institute* 2012; 32: 1-39.

11. Martin H, Willey B, Low DE, Staempfli HR, McGeer A, Boerlin P, et al. Characterization of *Clostridium difficile* strains isolated from patients in Ontario, Canada, from 2004 to 2006. *J Clin Microbiol* 2008; 46: 2999-3004.

12. al Saif N, Brazier JS. The distribution of *Clostridium difficile* in the environment of South Wales. *J Med Microbiol* 1996; 45: 133-137.

13. Johnson S, Louie TJ, Gerding DN, Cornely OA, Chasan-Taber S, Fitts D, et al. Vancomycin, Metronidazole, or Tolvamer for *Clostridium difficile* Infection: Results From Two Multinational, Randomized, Controlled Trials. *Clin Infect Dis* 2014; 59: 345-354.

14. Al-Tawfiq JA, Abed MS. *Clostridium difficile*-associated disease among patients in Dhahran, Saudi Arabia. *Travel Med Infect Dis* 2010; 8: 373-376.

15. Simango C. Prevalence of *Clostridium difficile* in the environment in a rural community in Zimbabwe. *Trans R Soc Trop Med Hyg* 2006; 100: 1146-1150.