

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.^{3,4} 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, Albaha University, Albaha, 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, Albaha 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 Albaha and 5 from Altaif) are those listed by the Ministry of Commerce and Industry of Saudi Arabia⁶ were sampled. In addition, 8 randomly selected supermarkets from the Scenic Southwestern Tourist Road, between Albaha 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.⁷ 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

Table 1 - Ribotype and toxinotype data for 12 *Clostridium difficile* isolated from 2 cities, Albaha and Altaif, Saudi Arabia.

Isolate	Province	Source	Ribotype	Toxin gene profile	Toxinotype	<i>tcdC</i> alteration type
B1	Albaha	Retail basket	V	<i>tcdA, tcdB</i>	0	None
B2	Albaha	Retail basket	027	<i>tcdA, tcdB, cdtB</i>	III	None
B3	Albaha	Retail basket	027	<i>tcdA, tcdB, cdtB</i>	III	None
B4	Albaha	Trolley	A2	<i>tcdA, tcdB</i>	XXVI	None
B5	Albaha	Trolley	BT1	<i>tcdA, tcdB, cdtB</i>	III	Δ117A
B6	Albaha	Plastic bag	A2	<i>tcdA, tcdB</i>	XXVI	None
B7	Albaha	Plastic bag	V	<i>tcdA, tcdB</i>	0	None
T1	Altaif	Retail basket	027	<i>tcdA, tcdB, cdtB</i>	III	Δ117A
T2	Altaif	Trolley	T1		Not applicable	Not tested
T3	Altaif	Trolley	027	<i>tcdA, tcdB, cdtB</i>	III	Δ117A
T4	Altaif	Conveyor belt	BT1	<i>tcdA, tcdB, cdtB</i>	III	Δ117A
T5	Altaif	Plastic bag	027	<i>tcdA, tcdB, cdtB</i>	III	Δ117A

tcdA - toxin A, *tcdB* - toxin B, *cdtB* - binary toxin (*cdtB*), NA

toxintype III with genes for *tcdA*, *tcdB*, and *cdtB* toxins. The 3 isolates of the strain ribotype 027 from Altaif had *tcdC* genes with the Δ117A 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 toxintype III, and had *tcdC* genes with the Δ117A 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 toxintype 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 toxintype XXVI.

Susceptibility of isolates to antimicrobials. Most of the identified isolates were vancomycin and metronidazole susceptible and levofloxacin resistant (Table 2). The metronidazole MIC₅₀ was 0.5 µg/ml and MIC₉₀ was 1 µg/ml. The metronidazole MIC range was 3-8 µg/ml. Vancomycin MIC₅₀ was 0.5 µg/ml and MIC₉₀ 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. *Clostridium difficile* are known to be present in animals,

ready-to-eat foods, and environmental samples.^{1,5,12} 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 Δ117 mutation in the down regulator of *tcdA* and *tcdB* production, the *tcdC* gene, and were toxintype 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.¹¹ In addition, ribotypes BT1 and V were detected in human infections.¹¹

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.¹³

The incidence of infections with *C. difficile* from community sources in Saudi Arabia is estimated at 38% of the total infected patients.¹⁴ 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%).¹² Secondly, the meat, poultry, and vegetables, unlike cheese; for example, are not ready-

Table 2 - Minimum inhibitory concentration (MICs) of antibiotics for *Clostridium difficile* isolates.

Isolate	Province	Source	Vancomycin	Metronidazole	Clindamycin	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	6 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.¹⁵ 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.^{12,15}

Study limitations. The study was designed for the cities of Albaha and Altaif 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 Albaha and Altaif 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.²

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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