Decontamination Strategies for Control of Environmental Clostridium difficile (Decontamination and C. difficile)

Meenakshi Singh and Chetana Vaishnavi*

Department of Gastroenterology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Abstract: Clostridium difficile is a global nosocomial pathogen associated with increased morbidity and mortality particularly with the emergence of the hypervirulent strains. C. difficile is ubiquitously present in the environment due to contamination by the excreta of humans and animals. In the hospital environment C. difficile can be isolated from about 30% patients receiving antibiotics or those who are hospitalized. C. difficile spores are not only resistant to antibiotics, but they can also resist the harsh environmental conditions for longer times and thereby facilitate the spread of C. difficile infection (CDI). The primary mode of transmission of the disease is via the feco-oral route as symptomatic patients shed a large number of the pathogen resulting in contamination of the environmental surfaces. CDI occurs in patients with certain risk factors and who acquire the pathogen by ingestion or via contaminated equipments. In a hospital setting, outbreaks can occur in hospitals, nursing homes, and other extended-care facilities due to C. difficile. Therefore strategies to reduce the contamination of C. difficile in the environment will be of prime importance to every health care facility. In this review, the environmental reservoirs of C. difficile, the transmission and the risk factors of CDI are briefly described and the decontamination strategies for containing the pathogen are elaborated.

Keywords: C. difficile, Decontamination strategies, Disinfectants, Spores, Vegetative cells.

1. INTRODUCTION

Clostridium difficile is a Gram positive, obligately anaerobic, endospore-bearing bacilli which is an opportunist nosocomial pathogen causing self-limiting diarrhea to life-threatening colitis. C. difficile infection (CDI) has been reported from several healthcare facilities from all parts of the world, including India [1,2]. During recent years, there has been an increase in the incidence of CDI due to the emergence of hypervirulent strains of the organism, the most common among which is the BI/NAP1/027 strain. This epidemic strain has been reported from several parts of United Kingdom [3] America [4,5] and Europe [6] and has been found to be associated with increased morbidity and mortality in CDI patients.

C. difficile exists in the environment as vegetative and spore forms [7]. The vegetative form is fragile whereas the spore form is a resistant survival form [8]. C. difficile spores easily contaminates the healthcare environment and thereby facilitates the spread of CDI [9-11] leading to a substantial burden on the healthcare system. From 1999–2003 in Massachusetts, CDI management resulted in a total of 55,380 inpatient days and an expenditure of $55.2 million. The annual excess hospital costs in the United States was $3.2 billion per year for the years 2000–2002 [12].

Persistence of C. difficile spores in the hospital environment strengthened the need to decontaminate the environment in order to prevent CDI as well as potential CDI outbreaks. Therefore it is of extreme importance to control contamination of the environment by employing decontamination strategies against the organism. In this review, the environmental reservoirs of C. difficile, the transmission and the risk factors of CDI are briefly described and the decontamination strategies for containing the pathogen are elaborated.

2. ENVIRONMENTAL RESERVOIRS OF C. DIFFICILE

C. difficile can be isolated from about 30% patients receiving antibiotics or those who are hospitalized [13]. Spores can survive at low pH of the gastric contents of patients who are not on acid suppressive therapy, while vegetative cells can survive when the pH increases above 5 in patients who are on acid suppressive drugs [14]. These reservoirs continuously or intermittently contaminate the environment [15]. C. difficile is shed into the environment at the time of resolution of diarrhea of a patient and can continue even after therapy [16-18]. The number of vegetative cells is significantly higher in the stool of patients before antibiotic treatment for CDI than after initiation of treatment. After treatment, vegetative cells get eliminated and mainly spores are recovered from the stool of patients, suggesting that spores are resistant to antibiotics. When healthy individuals are needlessly given antibiotic treatment they also begin to shed large
amounts of *C. difficile* in the environment [19]. Thus, the internal environment of the host may influence the number of vegetative cells and spores released into the environment. Vegetative cells of *C. difficile* can survive on moist surfaces at room temperature under aerobic conditions for up to several hours [14]. Spores being more robust can persist on hospital floors for several months [20].

The rate of asymptomatic carriage of *C. difficile* is 3-5% in healthy adults [21]. Identifying and treating asymptomatic patients who are potential reservoirs with vancomycin (but not metronidazole) has been advocated for reduction in the number of new CDI cases to reduce horizontal spread of *C. difficile* to other patients [22]. Interventions to reduce environmental contamination by *C. difficile* have decreased the incidence of CDI [23-24]. The environments of asymptomatic patients or patients having mild diarrhea were found to be less contaminated (0-2.6%) compared to patients with more severe disease and frequent bowel evacuations (9.3-75%) [16, 20]. Vaishnavi and Singh [25] investigated for *C. difficile* on 79 fecal specimens from hospitalized patients, 176 swab samples from beds and 48 hand swab samples from hospital personnel in a tertiary care hospital. They reported *C. difficile* culture positive in 12.6% patients. Eight (10%) of them developed diarrhea and 5 (62.5%) were both culture and toxin positive. Of the bedding samples, 90 (51%) were positive by culture and 15 (8.5%) for both toxins A and B. Of the 48 hand swab samples 30 (62.5%) were positive by culture and 2 (4.2%) for both the toxins, suggesting that environmental cross-infection between patients and carriage by hospital personnel are sources of CDI spread.

Hafiz [26] was the first to screen and isolate *C. difficile* from the inanimate environment such as soil, dung and hay. Later on, Al Saif and Brazier [27] did an extensive environmental survey in Wales and reported moderate isolation of *C. difficile* from soil (21%), pets (7%) and hospitals (20%) but significant isolation from rivers (87.5%), lakes (46.7%) and swimming pools (57%) suggesting that the spores were resistant to salinity and common water treatment processes.

*C. difficile* has been isolated from a variety of animals [28] and are thus potential reservoirs for the organism. Carriage and infection of *C. difficile* have been reported in pigs [29] horses [30] calves [29] chickens [31] and household pets [32] and can account for CDI in humans.

Not surprisingly, *C. difficile* has also been found in raw food and packed food products. Recent studies showed 4.5% of vegetables purchased at grocers to be contaminated with *C. difficile* [33]. In another study, Bakri et al. [34] isolated *C. difficile* from 7.5% of packaged salads suggesting that contamination with *C. difficile* may be from soil and water as these strains were found to be earlier also associated with human disease. Various meat products also harbor *C. difficile* as heavy contamination has been reported from North America. Songer et al. [35] isolated *C. difficile* from approximately 40% of all uncooked beef, pork and turkey products as also from 47% of ready-to-eat products. Similarly, Weese et al. [36] observed *C. difficile* contamination in 12% of ground beef, 71% of ground pork products and 12.8% of all retail chicken samples. Interestingly, the strains of *C. difficile* identified in animal foods have also been isolated from human disease [37].

### 3. TRANSMISSION OF *C. DIFFICILE*

The primary mode of transmission of *C. difficile* from person-to-person is *via* the feco-oral route [38]. Adults caring for infants can acquire the pathogen as 70% of healthy neonates have toxigenic *C. difficile* in their gut as commensal [39]. Symptomatic patients shed large amounts of both vegetative cells and spores resulting in contamination of their skin, clothing, bedding, and nearby environmental surfaces and creating what has been termed a *fecal veneer* [40-41]. Patients may acquire spores or vegetative cells either by ingestion or direct inoculation *via* contaminated equipments [42] and fomites, which play a crucial role in spreading CDI. The airborne spread of *C. difficile* has also been reported [43].

The transmission of CDI in the hospital can also be affected by antibiotic usage. Rotimi *et al.* [44] documented the spread of *C. difficile* in 9.5% of previously *C. difficile*-negative patients during hospitalization, influenced by the use of antibiotics. Similarly, in another study 5% of hip fracture patients in a ward acquired the pathogen within six months [45]. However, the duration of hospitalization also aid in the dissemination of disease [46]. Transmission may also occur from the hands of health care workers in contact with contaminated patients or their feces. Transmission outside the hospital environment is best represented by community-acquired CDI [47-50].

Contaminated inanimate object play a crucial role in spreading nosocomial disease [51]. In hospitals, *C.
difficile has been isolated from a variety of items, mainly those in direct contact with a patient, including portable commodes, bathing tubes and electronic thermometers [52]. Other items that were found to be contaminated by feces were toilet seats, bedpans, floors, dust, mops and bed-linen [20, 53]. Verity et al. [54] found the bed-frame to be the most common site from which C. difficile was recovered. Most of the C. difficile strains isolated from the environment commonly matched those isolated from patients [55-56].

Additionally though C. difficile has been detected on nursing uniforms, no evidence of the uniforms being a source of transmission to patients has been found [57]. Higher rates of C. difficile acquisition was found in double rooms than in single rooms (17% vs 7%); and a significantly higher risk of acquisition after exposure to a roommate with a positive culture result [17]. Nosocomial acquisition of C. difficile by healthcare workers have been reported [58-59] though rare, and they can serve as primary transmitters of C. difficile by way of transient hand contamination.

4. RISK FACTORS FOR C. DIFFICILE INFECTION

Epidemiological studies on decontamination have shown that patients admitted to rooms previously occupied by individuals infected with C. difficile [9] or sharing a room with such environment are more susceptible for the acquisition of nosocomial CDI [60]. In the same study, it was also found that being a ‘neighbor’ in a room adjacent to a patient with diarrhea was a greater risk for CDI and might reflect hand-to-hand transmission of the organism by healthcare workers [60].

Immunocompromised patients are at high risk of developing CDI due to an inability to mount an antibody response to C. difficile toxins [61-62]. Apart from host immune system, there are several factors that determine whether or not a patient develops a CDI. The major risk factors for CDI are advanced age, underlying diseases, prolonged hospital stay, non-surgical gastrointestinal procedures, presence of a nasogastric tube, anti-ulcer medications, stay on intensive care wards, duration of antibiotic course, administration of multiple antibiotics, etc [63]. An in-depth review of established and potential risk factors for CDI has recently been published [64]. The combination of the environmental presence of C. difficile in health care settings, host factors and medication in these settings can result in frequent outbreaks of CDI. Healthcare workers are increasingly being exposed to excretions and secretions contaminated with C. difficile and are therefore at a high risk of occupationally acquired infection [65]. Hospital healthcare workers may carry the pathogen from room to room and promote infection, but fecal carriage by staff is rare [17]. Outbreaks can occur in hospitals, nursing homes, and other extended-care facilities. The risk of acquiring C. difficile from the laboratory has been recognized, with two laboratory personnel acquiring CDI caused by the hypervirulent ribotype 027 [66]. The risk of both acquisition and potential severity of CDI among laboratory personnel is due to exposure to relatively high inocula of C. difficile which may be highly virulent.

C. difficile acquisition can be prevented by minimizing exposure to the organism and by decreasing the risk factors for patients to develop CDI, if exposure has occurred [67]. Multifaceted approach is generally required in minimizing exposure to C. difficile depending on the local epidemiology and the available resources. General precautions to reduce transmission of C. difficile must be taken. Recommendations for prevention of CDI in hospitals and other healthcare settings are given in Table 1.

5. OVERVIEW AND INTRODUCTION TO THE TYPES OF DECONTAMINATION STRATEGIES

Now, with the increase in the global burden of CDI, there is an urgent need for environmental decontamination strategies for C. difficile. Regular cleaning and disinfection must be performed as a precautionary measure in addition to use of disposable equipments and stringent hand-washing [68]. Hospital personnel should thoroughly disinfect objects contaminated with C. difficile in order to contain the pathogen. However the routine bacteriological surveillance for C. difficile is not mandatory because threshold levels associated with increased risk of clinical infection has not been established.

There are three types of disinfectants based on the level of their activities (i) High-level disinfectants (ii) Intermediate-level disinfectants and (iii) Low-level disinfectants. An overview on the types of disinfectants for C. difficile is given in Table 2. The advantages and disadvantages of decontaminating agents are provided in Table 3.

Among measures to control the dissemination of C. difficile various agents effective against the pathogen and especially the spores, should be used. Decontamination of C. difficile can be done by using chemical agents or by employing physical processes. Some of
the disinfectants and physical systems used as decontamination strategies against clostridial spores are as follows:

5.1. Glutaraldehyde

At a concentration of 2% glutaraldehyde is able to kill spores of *C. difficile* in 10 min, while it requires 30 min at 0.2% concentration [69]. Further dilutions of glutaraldehyde are unable to inactivate spores [70].

5.2. Oxygen Based Agents

Some oxygen based agents like sodium hypochlorite, ozone, hydrogen peroxide etc at a concentration of 2% are able to bring about a $10^4$ reduction in spores in one hour. When the concentration is decreased, the time required for the same level of reduction gets increased. For disinfecting bathrooms, toilets and furniture in a healthcare center, active oxygen-based agents have been found to be significantly more effective than quaternary ammonium compounds [71].

5.3. Chlorine Based Agents

Chlorine based agents have a broad spectrum of activity. To address environmental contamination in areas associated with endemic or epidemic CDI the use of chlorine-based decontaminants having a
minimum of 1000 parts per million (ppm) available chlorine is advocated. In UK, chlorine is used either with a detergent pre-clean or in combination with a detergent.

5.3.1. Sodium Hypochlorite

Use of hypochlorite, commonly called bleach has been found to be a widely tested disinfectant against C.
difficile, not only in the laboratory but also in the hospitals. However, less cleaning and disinfection might be achieved due to the quick drying of bleach and the difficulty in spreading it with even concentration over a surface [72]. Special Personnel Protective Equipment is required when bleach is used at concentrations of 5000 ppm [73]. Therefore, the Centers for Disease Control and Prevention has recommended the limited use of bleach for outbreak situations. A formulation of super-oxidised water releasing hypochlorous acid and free chlorine radicals can eliminate spores in five minutes. It is non-toxic and non-corrosive. However, its efficacy decreases in the presence of organic matter [74] thereby, requiring pre-cleaning.

5.3.2. Sodium Dichloroisocyanurate

Sodium dichloroisocyanurate (NaDCC) agents are considered to be superior disinfectants compared to bleach. They are also effective in the presence of organic matter [75]. NaDCC at a concentration of 1000 ppm along with detergent can decontaminate spores of ribotype 027 seeded onto stainless steel requiring three minutes for a $10^3$ reduction. However, a contact period of 9 mins is required to eliminate spores to levels below the detection limit of approximately $10^3$ [76]. Presence of organic matter further reduces the efficacy of the agent. Pre-cleaning with detergent and then wiping the surface with NaDCC increases the efficiency of the disinfectant as soil tends to quench its effect. Wilcox et al. [24] demonstrated a significant correlation between the use of a cleaning agent containing chlorine (dichloroisocyanurate; 1000 ppm available chlorine) and a reduction in the incidence of CDI. It is believed that higher concentrations of available chlorine (1000–5000 ppm) are more sporicidal than lower concentrations. Therefore the recommended concentration of available chlorine should be at least 1000 ppm and may ideally be 5000 ppm.

5.4. Ozone

Ozone is used frequently in the pharmaceuticals and food industry. It has greater effectiveness for disinfection of bacteria and viruses compared to chlorination. The ozonation process utilizes a short contact time (~10-30 mins). The toxicity of ozone is rated as 1.0 mg/l level by the US Environmental Protection Agency. However, ozonization requires efficient contacting system. The odor of ozone is noticeable at 0.01 mg/l level.

5.5. Hydrogen Peroxide

This system shows a reliable biocidal activity against a wide range of healthcare-associated pathogens. Two common modes of decontamination by hydrogen peroxide are (a) hydrogen peroxide dry mist system and (b) Accelerated hydrogen peroxide

5.5.1. Hydrogen Peroxide Dry Mist System

Hydrogen peroxide can easily decontaminate room surfaces and equipments without moving them. This procedure requires ~2.5 to 5 h. Hydrogen peroxide vapor (HPV) is found to be more effective for the contamination of entire rooms. Environmental contamination by C. difficile can be reduced by 94% with this system, and is also effective against the three main UK epidemic ribotypes 106, 001 and 027 [77]. A microscopic even layer film of HPV, invisible to the naked eye can be used for decontamination over all surfaces of a room or chamber which actually deactivates micro-organisms during the gassing process. A system of active distribution of HPV can be made by combination of a vapor generator and high velocity gas distribution nozzles and fans for the even spread of HPV which enables the uniform exposure of all surfaces. This system can be operated at ambient room temperature and relative humidity. The major advantage of this is that no further wiping down of surfaces is required on completion of decontamination as HPV decomposes to residue free oxygen and water vapor.

5.5.2. Accelerated Hydrogen Peroxide

It forms a uniform layer over the surface due to its viscosity and requires 10 mins to dry, thereby ensuring that the required contact time for sporicidal activity is achieved before the surface gets visibly dry [72].

5.6. Peroxygen Compounds

Peracetic acid is a distinct compound produced from acetic acid and hydrogen peroxide. Peracetic acid was found to be more efficacious than NaDCC even at a concentration of 10% [76]. Drawback of peracetic acid use is the requirement of 30 to 60 mins for inactivation of spores.

5.7. Acidified Nitrite

Acidified nitrite has been found to be effective for its sporicidal activity against C. difficile and the presence of organic matter does not hinder in the decontami-
nation process [78]. It is indeed a practical sporicide and harmless to users or surfaces.

5.8. Metal

Various metals and metal salts are commonly employed to prevent microbial growth. Copper-based biocides can achieve a log102-3 reduction in C. difficile spores in 30 mins [79]. Ultramicrofiber cloth dipped in these agents is also effective for the surfaces contaminated with spores of ribotype 027 [79]. Copper when used in alloys with 58% or more copper has been shown to reduce surface microorganisms in high-touch areas, though the results are not the same with C. difficile, especially spores [80-82]. Another metal, silver is also toxic to microorganisms at low levels. However no published report on its effectiveness on C. difficile is available. Moreover the cost involved with using silver for decontamination may be prohibitive [82].

5.9. Triclosan

Triclosan has a broad range of antimicrobial activity at concentrations of 0.2% to 2%. Under clinical conditions, a 63% reduction of aerobes and an 81% reduction in anaerobes have been reported [82]. The development of self-disinfecting surfaces has limited or no effectiveness against all microorganisms, especially C. difficile spores.

5.10. Ultraviolet-C Radiation

UV-C radiations in the range of 255-275 nm are effective decontaminating agents which function by destroying the reproductive capability of the microorganisms. Triggering germination by treating surface-dried spores with a solution containing sodium taurocholate also increased their susceptibility to UV-C radiation [83]. A UV-C device is also used to decontaminate rooms. A log102-3 reduction with spore contaminated surfaces is achieved in approximately 45 mins. For rooms, an 80% reduction has been observed [84]. This system has extensive usage as room decontamination is rapid (~15 to 25 mins) against vegetative bacteria but requires longer exposure (~50 mins) for C. difficile spores.

5.11. Formaldehyde

Formaldehyde is used mainly as a water-based solution in the form of formalin (37% formaldehyde by weight). A wide range of microorganisms is destroyed by varying concentrations of aqueous formaldehyde solutions.

6. LABORATORY MEASURES OF EFFECTIVENESS AGAINST ENVIRONMENTAL RESERVOIRS

Hand hygiene is one of the important factors for minimizing transmission of C. difficile in the hospital but proper compliance is required. Alcohol based hand antiseptics has improved compliance with hand hygiene, but they only help in reducing hand carriage of most vegetative bacteria and many viruses, but C. difficile, in its spore form, is highly resistant to killing by alcohol [78]. In fact the risk of transferring C. difficile to patients under their care is increased when healthcare workers decontaminate their hands with alcohol-based products which only displace spores over the skin surface, as opposed to mechanical removal by washing with soap and running water. McFarland et al. [17] found that C. difficile persisted on the hands of 88% (14/16) of personnel who had washed their hands with plain soap whereas washing with 4% chlorhexidine gluconate reduced the rate to 14% (1/7 personnel) thereby suggesting the superior efficacy of chlorhexidine containing antiseptic for removing C. difficile from the hands of healthcare workers. No difference between bland soap and chlorhexidine gluconate in removing C. difficile from experimentally seeded hands [85]. A prospective controlled trial of vinyl glove use showed a significant decline in CDI rates, from 7.7 cases per 1000 discharges before institution of glove use to 1.5 cases per 1000 discharges after institution of glove use [86].

Kaatz et al. [87] reported that unbuffered hypochlorite solution with 500 ppm available chlorine reduced environmental contamination by 79% and resulted in the end of an outbreak whereas buffered hypochlorite (1600 ppm available chlorine) could bring about a 98% reduction in environmental contamination. The use of a glutaraldehyde-based disinfectant was found effective in gradually controlling an outbreak without patient isolation [68]. Hypochlorite as 1:1000 solution for regular surface cleaning resulted in negative environmental samples [88]. The authors reported that deep cleaning of wards involving removal of all curtains and linen as well as washing the floors and the walls decreases the environmental burden of C. difficile to below detectable levels. Wullt et al. [78] showed that glutaraldehyde, peracetic acid and acidified nitrite only reduced spore counts by 99% after 15-min exposure, whereas isopropanol showed no effect on spore viability even after 30-min exposure. Oxygen, aldehyde and alcohol-based disinfectants are also useful for cleaning floors with reduction in the total bacterial count. However when only detergent is used...
there is introduction of a greater bacterial load into the environment [71]. Use of a mop with detergent for cleaning of floors is as ineffective as using water alone. The bacteria also spread from the area of contamination to clean areas.

The use of a 10% sodium hypochlorite solution mixed fresh daily has been associated with a reduction in CDI (Mayfield et al. 2000) and in controlling outbreaks [89] in some settings. In a bone marrow transplant unit with a relatively high infection rate cleaning with a hypochlorite-based solution (5000 ppm available chlorine) reduced the incidence of CDI (Mayfield et al. 2000). A $10^5$ reduction in C. difficile spores in 10 mins has been obtained by using 5000 ppm bleach. More time is required with lesser concentration viz. 15 mins with 3000 ppm and 15 to 25 mins with 1000 ppm for the same effect [90]. Bleach of 5000 ppm tested on C. difficile spores dried on stainless steel also had a similar effect [72]. When 5000 ppm bleach is sprayed onto the surface with contact of 3 mins and then wiped with a cloth dipped in the same bleach, its efficacy is enhanced [73].

The first ozone disinfection experiment was conducted in France in 1886 by de Meritence who demonstrated that diluted ozonized air could sterilize polluted water [91]. An ozone dosage of 20-30 ppm, for 20-30 min, and a short burst of humidity in excess of 80% relative humidity were able to inactivate $>10^3$ colony forming units of most of the bacteria, or bring about in many cases complete eradication of bacteria [92]. As a gaseous sterilant in a concentration of 25 ppm ozone has also been tested and found to be effective in bringing about at least $10^4$ reductions in C. difficile spores [92].

Hydrogen peroxide in the form of vapor or dry mist, with prior cleaning of surfaces, can be an effective method of disinfection of the hospital environment when unoccupied. Boyce et al. [93] investigated whether HPV decontamination can reduce environmental contamination and nosocomial transmission of C. difficile in a hospital affected by an epidemic strain of C. difficile. Intensive HPV decontamination of 5 high-incidence wards followed by hospital-wide decontamination of rooms vacated by patients with CDI was carried out. They observed that 11 (25.6%) of 43 cultures of samples collected by sponge from surfaces before HPV decontamination yielded C. difficile, compared with 0 of 37 cultures of samples obtained after HPV decontamination ($P < .001$). On 5 high-incidence wards, the incidence of nosocomial CDI was significantly lower during the intervention period than during the pre-intervention period (1.28 vs 2.28 cases per 1000 patient-days; $P = .047$). The hospital-wide CDI incidence was lower during the intervention period than during the pre-intervention period (0.84 vs 1.36 cases per 1000 patient-days; $P = .26$). Analysis limited to time in which the epidemic strain was present during both the pre-intervention and the intervention periods, CDI incidence was significantly lower during the intervention period than during the pre-intervention period (0.88 versus 1.89 cases per 1000 patient-days; $P = .047$), showing the efficacy of HPV decontamination in eradicating C. difficile from contaminated surfaces. Barbut et al. [94] showed 91% reduction in contamination by using hydrogen peroxide dry mist system, as compared to the 50% reduction obtained by hypochlorite cleaning. Boyce et al. [93] observed that the prevalence of C. difficile was significantly reduced from 5% to 0% after the use of hydrogen peroxide, possibly because of previous hypochlorite based cleaning.

Accelerated hydrogen peroxide gel could kill $10^6$ dried spores within 10 mins [90] as effectively as bleach at 5000 ppm. These compounds even produce a $10^{2-3}$ reduction in surface-dried spores in one minute [73] and are found to perform better than stabilized hydrogen peroxide disinfectants [73].

Wilcox et al. [24] reported that the use of sodium dichloroisocyanurate was able to reduce the incidence of CDI cases on a hospital ward where environmental contamination corresponded to incidence rates. Conversely, in one study it was observed that both bleach and NaDCC were equally sporicidal [95]. Block [96] observed that peracetic acid can completely disinfect stainless steel contaminated with C. difficile spores in 10 mins, but was less effective on polyvinyl chloride. Rutala et al. [97] showed that nanostructured UV-C emitting device having reflective coating had the ability to reduce the time required to decontaminate a room for C. difficile spores, from 43 mins 42 secs to 9 mins 24 secs. Levin et al. [98] reported that the number of deaths and colectomies attributable to hospital-associated CDI also declined dramatically after using portable pulsed xenon UV light. This system has been found to be effective in 95% reduction of C. difficile [98].

7. SPORES AND DISINFECTANTS

Spores are resistant to several physical and chemical disinfectants especially in the presence of
organic matter [76, 95]. The stability of spores can be attributed to specific structures in the spore, changes in the lipid composition, mineralization processes, dehydration effects and synthesis of novel protein species. Vegetative cells have a lower ability to survive in air and are more susceptible to the effect of sporicides [14, 99]. A wide range of disinfectants suitable for decontamination of the environment has in vitro activity against C. difficile spores [78, 97]. According to US Environmental Protection Agency, List K provides 36 registered antimicrobial products effective against C. difficile spores [100]. Fawley et al. [95] found that the working strength concentrations of 5 different cleaning agents inhibited growth of C. difficile cultures in vitro, though only chlorine-based cleaning agents used at the recommended working concentrations were able to inactivate C. difficile spores. The in vitro exposure of epidemic C. difficile strains, including NAP1/BI/027, to sub-inhibitory concentrations of non-chlorine-based cleaning agents significantly increased sporulation capacity, not seen with chlorine-based decontaminants [101]. Thus it is likely that some cleaning agents in low concentrations could promote C. difficile sporulation and thereby the transmission.

It is not known if different strains of C. difficile have any differences in the susceptibilities to different kinds of decontaminating agents. The sporulation of ribotype 001 was found to be further enhanced when exposed to non-chlorine-based disinfectants [89], which could explain its extensive dissemination and persistence in the environment. The hypervirulent ribotype 027 is capable of robust spore production [102-103] while the epidemic ribotype 001 produces significantly high numbers of spores compared to non-prevalent strains. Wullt et al. [78] reported no differences in susceptibilities of different strains to glutaraldehyde, peracetic acid or acidified nitrite while using strains belonging to serogroups A and C along with a common nosocomial strain. Similarly, during the testing of a peroxy compound, it was found that the spores of ribotype 027 were inactivated as efficiently as those of the other strains tested [104]. It is not clear whether other agents can have similar effects on both vegetative cells and spores and further studies on this aspect will be required. Speight et al. [105] assessed the efficacy of 32 commonly available sporicidal agents against C. difficile spores after 1 min and 1 h of contact under clean and dirty conditions. The workers observed that the application of these so called sporicidal disinfectants was not a universal cure in the environmental control of C. difficile.

In a comparative investigation of disinfectants, Büttgen et al. [106] found that the hypervirulent ribotype 027 strain were more resistant to glutaraldehyde and peracetic acid than the other laboratory C. difficile strains, thereby requiring independent verification of sporicidal activity and quality control of so called sporicidal disinfectants. Ferreira et al. [107] reported that the disinfectants under the trade name ClorOrio (chlorine releasing agent) and Cidex Opa (aldehyde) are the most efficient agents for eliminating spores of C. difficile when compared with Virkon (peroxygen), which decreases only 50% of the spores [108]. Some studies showed a higher rate of reduction of C. difficile spores when a disinfectant under the trade name Peresal (0.5%) containing hydrogen peroxide and peracetic acid was used [107, 109]. Cationic bis-biguanide (50% concentrated) under the trade name Riohex has also shown sporicidal activity against C. difficile spores [110] but Ferreira et al. [107] found it to be less effective probably because it was sold at a lower concentration of 2%. A consensus conference highlighted the difference in approach to routine environmental decontamination in US (disinfectant-based) versus UK (detergent-based) hospitals [62]. Evidence is generally lacking to support one or the other approach. Thus the choice of cleaning agent may have a substantial effect on C. difficile spore persistence in the hospital environment.

8. CONCLUSION

Environmental surfaces contaminated by C. difficile and its spores play a crucial role in spreading CDI in the hospital. Interventions to reduce environmental contamination are therefore required, particularly because of the increasing incidence of hypervirulent C. difficile strains. Comprehensive approach to minimize exposure to C. difficile should be taken depending on the local epidemiology and the available resources apart from the general precautions to reduce transmission of C. difficile. In areas associated with endemic or epidemic CDI the use of chlorine-based decontaminants having a minimum of 1000 ppm available chlorine with a detergent pre-clean will be quite useful. Environmental contamination by C. difficile can also be reduced tremendously with hydrogen peroxide and UV-C radiations system. Carefully chosen environmental disinfectants effective against the pathogen and especially the spore, inclusive of a range of antimicrobial products as given in the US EPA List K could help to reduce the environmental contamination of C. difficile.
REFERENCES

http://dx.doi.org/10.1111/j.1572-0241.2000.03227.x

http://dx.doi.org/10.1111/j.1572-0241.2000.03227.x

http://dx.doi.org/10.1128/CMR.00082-09

http://dx.doi.org/10.1056/NEJMoa051590

http://dx.doi.org/10.1056/NEJMoa051639

http://dx.doi.org/10.1111/j.1469-0691.2006.01580.x

http://dx.doi.org/10.1016/j.resmic.2010.09.007

http://dx.doi.org/10.1128/CMR.00089-01

http://dx.doi.org/10.1086/658669

http://dx.doi.org/10.1016/j.jhin.2013.05.016

http://dx.doi.org/10.1186/1471-2334-13-342

http://dx.doi.org/10.1086/522676

http://dx.doi.org/10.1086/502603

http://dx.doi.org/10.1128/AAC.01443-06

http://dx.doi.org/10.1016/j.ajic.2010.04.196

http://dx.doi.org/10.1093/ajcn/32.6.2453

http://dx.doi.org/10.1056/NEJM198901263200402

http://dx.doi.org/10.1086/649016

http://dx.doi.org/10.1128/AAC.37.7.1432

http://dx.doi.org/10.1093/infdis/i43.1.42

http://dx.doi.org/10.1067/mge.1997.93052

http://dx.doi.org/10.7326/0003-4819-117-4-297

http://dx.doi.org/10.1086/381819

http://dx.doi.org/10.1016/S0195-6701(02)00400-0

http://dx.doi.org/10.4103/0255-0857.93052

http://dx.doi.org/10.1099/00222615-45-2-133

http://dx.doi.org/10.1099/00222615-45-2-133

http://dx.doi.org/10.1128/AAC.37.7.1432

http://dx.doi.org/10.1128/JCM.00224-07

http://dx.doi.org/10.1016/j.vetmic.2006.10.013
http://dx.doi.org/10.1086/593109

http://dx.doi.org/10.1111/j.1469-0691.2008.01992.x

http://dx.doi.org/10.1099/00222615-26-2-125

http://dx.doi.org/10.1016/S0195-6701(85)80016-5

http://dx.doi.org/10.1093/infectiousdiseases/14.8.713-a

http://dx.doi.org/10.1053/jhin.1999.0567

http://dx.doi.org/10.1016/j.ajic.2010.02.009

http://dx.doi.org/10.1186/1471-2334-10-268

http://dx.doi.org/10.1016/S0195-6701(99)0046-4

http://dx.doi.org/10.1016/S0195-6701(85)80014-1

http://dx.doi.org/10.12968/bjon.2008.17.5.28827

http://dx.doi.org/10.1016/j.jhin.2008.06.008

http://dx.doi.org/10.1086/502129

http://dx.doi.org/10.1093/jac/dkm201

http://dx.doi.org/10.1086/383260

http://dx.doi.org/10.3201/aid1205.051064

http://dx.doi.org/10.1086/430315

[83] Nerandzic MM, Donskey CJ. Triggerng germination represents a novel strategy to enhance killing of Clostridium difficile spores PLOS One 2010; 5(8): e12285.
http://dx.doi.org/10.1371/journal.pone.0012285

http://dx.doi.org/10.1186/1471-2334-10-197

http://dx.doi.org/10.1038/30148335

http://dx.doi.org/10.1016/0002-9343(90)90462-M

http://dx.doi.org/10.1093/aje/137.7.1289

http://dx.doi.org/10.1016/0195-6701(94)90063-9

http://dx.doi.org/10.1053/jhin.1999.0253

http://dx.doi.org/10.1016/j.ajic.2005.04.240


http://dx.doi.org/10.1016/j.ajic.2007.10.021

http://dx.doi.org/10.1086/589906

http://dx.doi.org/10.1086/597232

[95] Fawley WN, Underwood S, Freeman J, Barnes SD, Saxton K, Stephenson K, et al. Efficacy of hospital cleaning agents...
http://dx.doi.org/10.1086/519201

[96] Block C. The effect of Perasafe and sodium dichloroisocyanurate (NaDCC) against spores of *Clostridium difficile* and Bacillus atrophaeus on stainless steel and polyvinyl chloride surfaces. J Hosp Infect 2004; 57; 144–48.  
http://dx.doi.org/10.1016/j.jhin.2004.01.019

http://dx.doi.org/10.1086/670211

http://dx.doi.org/10.1016/j.ajic.2013.02.010

http://dx.doi.org/10.1093/jac/dkn219


http://dx.doi.org/10.2307/30141374

http://dx.doi.org/10.1128/JCM.01964-07

http://dx.doi.org/10.1128/JB.00445-10

http://dx.doi.org/10.1016/j.ijheh.2010.10.004

http://dx.doi.org/10.1016/j.jhin.2011.05.016


http://dx.doi.org/10.1016/j.anaerobe.2013.04.008

http://dx.doi.org/10.1099/jmm.0.030288-0

http://dx.doi.org/10.1139/m93-008

http://dx.doi.org/10.1371/journal.pone.0025754

Received on 28-06-2014 Accepted on 07-08-2014 Published on 31-08-2014

DOI: http://dx.doi.org/10.14205/2310-8703.2014.02.01.4

© 2014 Singh and Vaishnavi; Licensee Pharma Publisher. This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.