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Clostridium difficile – what should be considered for an effective disinfection?

Summary

The vegetative *Clostridium (C.) difficile* cell can hardly survive in the inanimate environment and does normally not survive gastric acid. That is why it is only relevant for patients with atrophic gastritis or those who are treated with proton pump inhibitors or H_2-antagonists for transmission of infection. The *C. difficile* spore, however, can persist for 5 months on inanimate surfaces and can survive gastric acid even at a pH value of 1. In the small bowel it germinates within 1 hour. Hands are rarely contaminated with *C. difficile*. If contamination is suspected on hands they should be disinfected first of all to kill the vegetative *C. difficile* cells, and washed briefly and thoroughly afterwards in order to reduce the number of spores as much as possible. Up to 30 % of samples taken in the inanimate hospital environment is *C. difficile* positive and may be a reservoir for new infections. The highest rates of contamination were found around patients with diarrhea, especially around toilets and on bedpans. A sporicidal surface disinfection has resulted in a significant reduction of new *C. difficile* infections in most studies. In an outbreak situation it seems to have the largest potential for prevention apart from wearing protective gloves.
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Background

The supraregional increase in *Clostridium (C.) difficile* infections with new epidemic strains made it necessary to examine this nosocomial pathogen more closely [1]. A vital difference from previously known strains is the up to 23 times higher production of the toxins A and B, which can be explained by a mutation [2]. In some strains, another binary toxin has been found that contributes to the virulence [3]. In addition, the strains are resistant to fluoroquinolone [4]. Hence, it has to be assumed that also in Germany there will be an increase in infections caused by *C. difficile* [5].

Contamination of the environment

The vegetative *C. difficile* cell is able to survive on inanimate surfaces for up to 15 minutes only [6]. In comparison to other nosocomial pathogens this persistence is rather short [7] and can presumably be explained by the fact that the vegetative form of the *C. difficile* cell is obligate anaerobic and quickly killed by the oxygen in the air (Table 1). The *C. difficile* spore, however, is able to survive on surfaces for up to 5 months [8]. Its length of survival on copper is yet significantly shorter with 2 days [9]. Hence, it has to be assumed that in principle the spore form was found in environmental examinations to clarify outbreaks.

In several outbreaks of infections, the contamination of inanimate environment by *C. difficile* was examined. Fawley et al. reported that, during an outbreak on two geriatric wards, 29.2 % and 32.8 % of a total of 2550 environmental examinations yielded *C. difficile*-positive results

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[10]. *C. difficile* was most frequently found on floors (46 %), bedframes (19 %), radiators (19 %) and chests of drawers (8 %) [10]. A surgical intensive care unit reported that 11.1 % of a total of 432 environmental swab examinations were *C. difficile* positive [11]. The highest contamination rate was found on toilet seats (33.3 %), followed by bedpans (25.9 %) and the floor (14.5 %) [11]. By contrast, the contamination rate on a control ward was significantly lower (2.8 %) [11]. McFarland et al. could prove that the contamination rate correlates with the clinical symptoms [12]. The highest contamination rate was found in the environment of *C. difficile*-positive patients with diarrhoea (49 %), followed by *C. difficile*-positive patients without diarrhoea (29 %). The environment of culture-negative patients was *C. difficile* positive in 8 % of the cases [12]. When the inanimate environment is simply cleaned, the environmental contamination is only slightly reduced over 4 weeks. This points to the spore's long-lasting persistence on inanimate surfaces and the very limited effect of cleaning [13]. A possible reservoir in the inanimate environment are contaminated carpets [14], which, however, are not standard in German hospitals, because surfaces in patient care should be even and wipeable as recommended by the Commission for Hospital Hygiene and Infectious Disease Prevention at the RKI [15]. In case that own environmental examinations for isolating *C. difficile* are carried out, only suitable selective culture media should be used, e.g. *Clostridium difficile* agar [16].

Effective surface disinfection

Several studies prove the role that sporicidal surface disinfection plays for interrupting the chain of infection. As these studies were performed in the U.S. and UK respectively, mainly chlorine-releasing agents were examined. However, essential conclusions can still be derived. In 1988, a total of 1085 environmental examinations were carried out during an outbreak [17]. Both surfaces treated with 550 ppm hypochlorite (outbreak ward) and surfaces not treated with hypochlorite (control ward) were investigated. The use of the surface disinfectant could significantly reduce the rate of *C. difficile*-positive samples and the density of colonisation (Table 2), but not entirely to the level of the control ward.

Table 1: Essential properties of Clostridium difficile for effective disinfection.

Characteristic	Vegetative cell	Spore
Oxygen tolerance	Obligate anaerobic	Aerotolerant
Survival on surfaces	15 minutes at most	Up to 5 months
Survival in the stomach (pH value of the gastric acid ⩽ 3)	No	Yes
Survival in the stomach (pH value of the gastric acid ⩾ 4)	Yes	Yes
Duration of germination in the small intestine	–	Approx. 1 h

Table 2: Relative frequency of C. difficile-positive cultures from inanimate surfaces, depending on the treatment with 500 ppm hypochlorite; adapted from [17].

Location of sampling	Positive cultures		Mean density (CFU per culture)
	n	%	
Control ward	25 of 584	4.3	1.6
Outbreak ward (prior to surface disinfection)	81 of 258	31.4	5.1
Outbreak ward (after surface disinfection)	40 of 243	16.5	2.0

On three different wards (bone marrow transplantation [BMT], neurosurgical intensive care unit, internal medicine), Mayfield et al. examined the effect of using hypochlorite solution, which was used for treating surfaces as soon as *C. difficile* was detected [18]. Routine treatment of surfaces was performed with a quaternary ammonium compound (QAC) solution. Only on the BMT ward, the hypochlorite solution resulted in a significant reduction from 8.6 to 3.3 *C. difficile* cases per 1000 patient-days. After the intervention (reverting to the QAC solution), the incidence of *C. difficile* in fact increased again to 8.1 cases per 1000 patient-days. On the other two wards, the rates remained unchanged (neurosurgical intensive care unit: from 3.0 to 2.7 per 1000 patient-days; internal medicine: from 1.3 to 1.5 per 1000 patient-days).

On two wards for geriatric medicine, Wilcox et al. carried out a cross-over study to determine the correlation between the incidence of *C. difficile* infection and the type of surface treatment [19]. Surfaces were either treated with a neutral cleaner or hypochlorite. Overall, the study did not provide a clear picture. On one ward, the incidence rate was reduced significantly (from 8.9 to 5.3 cases per 100 admissions), on the other ward there was no significant difference [19].

There also is another study that investigated the incidence rate of *C. difficile* infection depending on the type of surface treatment, which was carried out on a medical intensive care unit and a BMT ward [20]. After using hypochlorite solution, the *C. difficile* incidence significantly decreased by 50 % on both wards – compared to the control period without sporicidal surface disinfection.

There is another aspect. Different cleaners without antimicrobial agent apparently are able to promote the sporulation of *C. difficile* [21]. But whether this has a clinical relevance, especially in view of the short persistence of the vegetative *C. difficile* cell on surfaces [6], is very doubtful.

In summary, one can say that – in certain areas with particularly susceptible patients and with above-average incidence of *C. difficile* infection respectively – sporicidal surface disinfection can have a vital share in significantly reducing incidence rate. Therefore, disinfection of potentially contaminated surfaces is regarded as an essential element of infection prevention in case of *C. difficile* [22], in particular because of the possible transmission of the *C. difficile* spore from the surface via the air [23]. In this connection, surface disinfectants with sporicidal activity have to be used. In Germany, surface disinfectants based on peroxide compounds such as magnesium monoperphthalate or based on aldehydes are most frequently used for this.

Effective reprocessing of bedpans

Bedpans are classified as Class 1 medical devices. According to the guideline of the Commission for Hospital Hygiene and Infectious Disease Prevention at the RKI, reprocessing of medical devices should ensure that reprocessed medical devices do not pose a risk in terms of infection in the subsequent use [24]. Hence, bedpans should preferably be reprocessed automatedly. However, different aspects have to be kept in mind here. High temperatures of 80 °C or more will kill vegetative bacteria comprehensively [25], but not bacterial spores. Using active substances with sporicidal activity will normally not yield a practice-relevant killing of the bacterial spores as the short exposure time in the washer disinfector (partly only 1 minute) is by far not sufficient. In addition, active substances with fixing properties, e.g. aldehydes or peracetic acid, should absolutely not be used [26, 27]. On metal test pieces it could be shown that using a sporicidal active substance with fixing properties produces a worse reduction in spores than using a non-sporicidal active substance without fixing properties [28]. The main effect is achieved by mechanical removal of the bacterial spores [25]. Hence, it is important to be able to scientifically evaluate the cleaning power of the utilised washer disinfector on bedpans. Unfortunately there are hardly any scientific findings on this that could serve as basis for deriving how far the contamination of bedpans can be reduced by the cleaning process. However, a recently published Canadian study could determine that a commercially available washer disinfector for reprocessing bedpans (Steris Reliance 444 single-chamber WD, Steris Corp., USA) in different reprocessing settings (up to 85 °C for up to 5 minutes) only insufficiently reduces artificially applied *C. difficile* spores: Independent of the reprocessing procedure, each Rodac plate carried more than 100 CFU of *C. difficile* spores [29].

What happens in the gastrointestinal tract?

On the basis of the current infection development through *C. difficile*, one has to act on the assumption that both the vegetative cell and the spore are excreted and that both contaminate surfaces and hands. Thus, both cell forms can reach the gastrointestinal tract of a patient or employee. Tests in the Syrian hamster show that the stomach normally is an invincible barrier for the vegetative cell.

When approx. 4 million vegetative *C. difficile* cells are inoculated intragastrically and examined 1 hour later, only 13 % of the cells can still be detected [30], i.e. the majority of the vegetative cells was killed by the acid. This finding is supported by a study that examined the survival of the vegetative *C. difficile* cell in the gastric juice at different pH values (Table 3). Vegetative *C. difficile* cells can only survive the gastric juice when the pH value is 4 or higher [31]. Here, particularly patients with atrophic gastritis or patients treated with proton pump inhibitors or H_2 antagonists are at risk [31]. In patients without limited gastric acid production, the vegetative *C. difficile* cell should hardly have a chance to pass the stomach.

But when approx. 4 million *C. difficile* spores are inoculated intragastrically, one will find an average of 78 % of the cells as heat-sensitive in the small intestine already after 1 hour, i.e. germination took place [30]. The share of heat-sensitive cells in the stomach was only 13 %, i.e. germination hardly took place [30]. In addition, the *C. difficile* spore is able to survive the gastric juice even with a pH value of 1 (Table 3). So, the *C. difficile* spore can well pass the stomach even in case of low pH values, germinate in the small intestine within rather short periods and can thus be transformed into a form being pathogenic for humans.

Table 3: Survival of C. difficile (vegetative cell or spore) in gastric juice, depending on the pH value, adapted from [31].

pH value	Survival of *C. difficile* in gastric juice	
	Vegetative cell form	Spore form
1	Killed	Survived
2	Killed	Survived
3	Killed	Survived
4	Survived	Survived
5	Survived	Survived
6	Survived	Survived
7	Survived	Survived

Contamination of flexible endoscopes

Particularly colonoscopes are expected to be contaminated with *C. difficile* after use. Hughes et al. could show that 10 of 15 (67 %) colonoscopes were *C. difficile* positive immediately after their use in CDAD patients [32]. So far, however, no transmissions of *C. difficile* after colonoscopies have been described, which is astonishing at first [33, 34]. The most likely reason for this is that the *C. difficile* spore normally germinates in the small intestine. But when the *C. difficile* spores are brought into the colon with the colonoscope retrogradely, they have to take different hurdles. On the one hand, germination in the colon is apparently more difficult than in the small intestine. In addition, compared to the entire gastrointestinal passage, the time they remain in the colon is rather short, perhaps too short for posing a risk to patients. These two factors seem to suffice for colonoscopes contaminated with *C. difficile* not to pose an evident risk to patients. For gastroscopes and duodenoscopes contaminated with *C. difficile*, the risk for the patient has to be classified as more serious.

Effective reprocessing of flexible endoscopes

When selecting suitable sporicidal disinfectants for reprocessing flexible endoscopes, three aspects have to be considered:

1. Fixation of organic material: Both aldehydes and peracetic acid are able to fix organic material like blood and biofilms

on surfaces to different extents [26, 27]. It could even be shown that a non-sporicidal instrument disinfectant with cleaning activity can achieve a higher reduction in bacterial spores in blood than a sporicidal instrument disinfectant with fixing properties [28]. Prior to the use of preparations based on these active substances, a thorough cleaning is therefore essential [39].

2. Exposure time: Some disinfectants with sporicidal activity require longer exposure times than typically applied in WDs. Under these conditions of use, it is questionable whether sporicidal activity is actually achieved.
3. Process parameters: For different disinfectants there are different process parameters that have to be followed. Preparations based on glutaraldehyde are often used at temperatures around 55 °C, preparations based on peracetic acid, however, at lower temperatures. Process parameters are stipulated by the manufacturer of the disinfectant and cleaner respectively.

Contamination of hands

Hands are contaminated with *C. difficile* rather seldom. Fawley et al. reported that 2.4 % and 5.4 % of 527 samples of the employees' hands were *C. difficile* positive [10]. Feketey et al. detected *C. difficile* in 4 of 31 (13 %) samples taken from employees' hands [40]. Malamou-Ladas et al. described a contamination rate of 1.7 % [41]. In another study, 2 of 12 hands were *C. difficile* positive [11]. Normally, hands become contaminated through direct contact with infected patients. In case protective gloves are not worn hands become contaminated in 57 % of the cases [12]. Then, *C. difficile* is most frequently detected under the fingernail (43 %), followed by fingertips (37 %) and palm (37 %) [12]. When protective gloves are worn, it has to be expected that the gloves are contaminated after the contact with colonised skin of the patient [42]. Contamination of the glove is between 1 und > 100 CFUs [42]. The contamination is particularly high after contact with the inguinal region [42].

Hands or gloves can become contaminated through the contact with surfaces or objects as well as through the contact with patients' skin. The skin of patients with *C. difficile*-associated diarrhoea is often contaminated, particularly the inguinal region (approx. 60 %), the abdominal region (approx. 55 %), the chest (approx. 45 %), and the hands (approx. 35%) [42]. The skin of the abdominal and chest regions remain contaminated with *C. difficile* for one week after the diarrhoea [42]. Patients are normally not on isolation during this time, and the healthcare workers may not wear protective gloves at that time when having direct contact with the patient's intact skin. Also this way, the employees' hands can become contaminated through direct contact with the skin of the patient.

Wearing a simple protective glove provides the best protection against contamination [12]. A contamination of the hands does not automatically imply that *C. difficile* will be detected in the same person's stool afterwards [11].

Effective hand hygiene

In case hands become contaminated, it has to be assumed that the skin is temporarily colonised by vegetative cells and spores of *C. difficile*. The bacteria's survival on the skin is considerably worse than on inanimate surfaces [43]. As vegetative *C. difficile* cells are only able to survive on inanimate surfaces for up to 15 minutes [6], their survival on hands is presumably much shorter. Alcohol-based hand disinfectants reduce the vegetative *C. difficile* cell within 30 seconds by more than 5 $\log_{10}$ steps [44, 45]. Hence, it has to be assumed that only spores remain on the hands after hygienic hand disinfection. These can be reduced through a 10-second handwash with simple soap by approx. 2 $\log_{10}$ steps [46, 47]. Prolonged handwashing (30 to 60 seconds) does not improve the reduction in spores [46]. The use of antimicrobial soap does also not have an advantage over simple soap [46].

When caring for *C. difficile*-positive patients, protective gloves are normally worn. In this case it is assumed that the hands are not roughly soiled, as proven by the low number (up to 3) of detectable *C. difficile* spores per hand [11]. So, hygienic hand disinfection followed by a short thorough handwash with simple soap is the most effective hand hygiene in case the hands are suspected of being contaminated with *C. difficile* [48]. If these two measures were carried out in reverse order, the environment could additionally become contaminated with vegetative *C. difficile* cells. Furthermore, disinfectants can be slightly less effective immediately after handwashing [49], as handwashing – despite thorough drying – leads to an epidermal hyperhydration of about 10 minutes [50]. Rough soiling is certainly the exception, for example when protective gloves are not worn. In this case it is unclear which order of hygienic hand disinfection and handwashing achieves the better overall result. However, in case handwashing is performed at first it is imperative to thoroughly dry the hands before hygienic hand disinfection is carried out.

Does more hand disinfection promote the spread of *C. difficile* spores?

This concern is expressed again and again and is based on the fact that alcohols are practically not active against bacterial spores [51, 52], which has been known for more than 100 years [53–56]. Two studies tried to address this concern. Over a period of 6 years, Gordin et al. examined the incidence of MRSA, VRE and *C. difficile* per 10,000 patient-days [57]. After 3 years, the antimicrobial soap used until then was replaced by an alcohol-based hand disinfectant. The MRSA and VRE incidence rate was significantly lower during the use of the alcohol-based hand disinfectant, the incidence rate of *C. difficile*, however, remained unchanged (Table 4). The vegetative *C. difficile* cell on the employees' hands obviously does not play a measurable role for nosocomial transmission.

Boyce et al. examined the incidence of *C. difficile* per 1,000 patient-days over a period of 4 years, in which alcohol-based hand disinfection was promoted [58]. In these 4 years, compliance rate with hand hygiene increased from 38 % to 63 %. Within the same time period, the share of alcohol-based hand disinfection rose from 10 % to 85 %; the share of handwashing fell from 90 % to 15 % accordingly. The number of *C. difficile*-positive patients even decreased from 1.74 to 1.18 per 1,000 patient-days [58]. These two studies show that less handwashing and increased hand disinfection does not result in a higher risk of *C. difficile* in the hospital.

Table 4: Mean prevalence rate of nosocomial pathogens per 10,000 patient-days; 1998–2000: Hand hygiene with antimicrobial liquid soap; 2001–2003: Hand hygiene with alcohol-based hand disinfectant; adapted from [57].

Species	Mean rate per 10,0000 patient-days		p-value
	1998–2000	2001–2003	
MRSA	8.44	6.32	0.005
VRE	4.33	2.46	0.001
C. difficile	3.24	3.38	0.78

Comparison of the chemoresistance of different spore-formers

Several studies examined the activity of disinfection procedures against *C. difficile* spores [59–61]. However, *C. difficile* cannot be found in standard tests for determining the activity of chemical disinfection procedures. Here, one finds either *C. sporogenes* [62, 63] or surrogates of aerobic spore-formers such as *Bacillus (B.) subtilis* [62–64] or *B. cereus* [64], which partly have also been examined in comparative studies on the chemoresistance to disinfectants [61].

Compared to *C. difficile*, the spore of *C. sporogenes* is less resistant, as tests with chlorine dioxide, hydrogen peroxide and bleach proved [61]. On the basis of the available study, the spore of *B. subtilis* possesses a chemoresistance which is comparable with the one of *C. difficile* [61, 65]. Hence, it can currently be assumed that adequate activity (e.g. reduction by at least 4 $\log_{10}$ steps in the suspension test) against the spore of *B. subtilis* likewise provides activity against the *C. difficile* spore. As *B. subtilis* is test microorganism in both national methods and European norms, it would be appropriate for determining sporicidal activity.

Importance of the spore enrichment for the disinfection result

Firstly, test spores are obtained through cultivation of the spore-former on standard culture media [59, 60]. At this time point, there is a mixture of vegetative cells and spores. Afterwards, the share of vegetative cells is minimised to obtain an almost pure spore suspension. Vegetative cells can be killed by exposure to heat (75 °C, 10 minutes) [66], to oxygen in the air [64] and by alcohol (e.g., 95 % ethanol or 65 % isopropanol) [59, 64, 67]. In either case, however, it is decisive to verify the share of spores in the overall cell count in the spore suspension, e.g. through Gram staining [59]. A study on the activity against *C. difficile* spores, for instance, could show that 70 % isopropanol can reduce the number of *C. difficile* spores by 0.2 – 0.4 $\log_{10}$ steps, which was exactly equivalent to the share of vegetative cells in the overall cell count (10–15 %, determined by Gram staining) [59]. Only experimental determination of the share of spores in the overall cell count can ensure that "sporicidal activity" is to a considerable extent not only a "bactericidal activity".

Résumé

Sporicidal surface disinfection and wearing protective gloves are essential measures to prevent transmission of *C. difficile* in healthcare. Potentially contaminated hands should firstly be disinfected to kill the vegetative form of *C. difficile* and then washed shortly and thoroughly with simple soap. For reprocessing flexible endoscope, thorough cleaning and not using active substances with fixing properties such as aldehydes or peracetic acid in the cleaning phase play a decisive role for the reprocessing result. Another source of infection are contaminated bedpans, which – according to the current state of knowledge – cannot be adequately decontaminated from *C. difficile* spores through automated reprocessing.

Conflict of interest

The author is an employee of Bode Chemie GmbH & Co. KG.

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