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Review

Approaches to the detection of *Clostridioides difficile* in the healthcare environment

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SUMMARY

Clostridioides difficile, a spore-forming bacillus, is a major cause of healthcare-associated infection, and can survive for prolonged periods in the inanimate environment. Environmental sampling to detect *C. difficile* is not routine but may be undertaken as part of outbreak management and during research projects. We conducted a literature search covering the period between 1980 and 2018 to review methods for the detection of this pathogen in the environment. There are many acceptable sampling methods used for environmental screening, including contact plates, cotton swabs, flocked swabs and sponges. Most recent studies suggest that sponges are the most effective method of sampling and have the added benefit of being capable of sampling larger and curved areas. Culture methods are the most common laboratory method of detecting *C. difficile* from environmental samples. However, the results are variable depending on the type of agar used and the turnaround times can be long. Molecular methods such as real-time polymerase chain reaction (RT-PCR), although more commonly used to detect *C. difficile* from faecal specimens, has been used with varying degrees of success in environmental sampling. Further studies are needed to determine whether molecular techniques could offer a more reliable, faster method of environmental sampling, giving infection prevention and control teams more reassurance that patients are being placed in adequately decontaminated hospital environments.

Introduction

Clostridioides difficile is a Gram-positive strict anaerobic bacillus with oval sub-terminal spores that was first described by Hall *et al.* (1935) as an anaerobe found in the intestinal flora of new-born infants [1]. *C. difficile* infection (CDI) is the most common cause of nosocomial diarrhoea in the industrialized world and is a significant burden on healthcare systems [2]. It is estimated that the cost of management of CDI for inpatients exceeds US$4.8 billion and €3 billion in the USA and Europe, respectively [2]. The spectrum of disease ranges from mild diarrhoea to severe, fulminant colitis, which may result in bowel perforation and shock, leading to colectomy and even death [3].

Patients become colonized with *C. difficile* by ingestion of the organism, either through direct contact with an infected patient or contact with the contaminated environment. *C. difficile* may also be transmitted on the hands of healthcare workers. The spores of *C. difficile* remain viable in the environment for prolonged periods of time and they can withstand

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extremes of heat and are resistant to the action of many cleaning agents [4,5].

The role of the healthcare environment in transmission

The significant roles played by the contamination of healthcare facilities and environmental cleaning in the transmission of nosocomial infections has been much discussed [6–9]. For many pathogens to be transmitted onwards, they must be capable of surviving in the environment and remaining viable for long periods of time. They should also be able to colonize patients, the hands of healthcare workers and have a small inoculating dose [6].

Transmission of *C. difficile* in the healthcare setting is highly effective. Direct contact with persistent spores on surfaces within the clinical environment increases the potential risk of transmission to patients either through the hands of healthcare workers or through direct contact with the contaminated items.

An outbreak report by Kaatz *et al.* implicated the contaminated environment in transmission of *C. difficile* [10]. Other studies have shown that the risk of acquiring CDI increases with increased levels of environmental contamination with *C. difficile* [11,12]. Placement of a patient in a room that was previously occupied by another patient known to be colonized with *C. difficile* has been shown to be a risk factor for the development of CDI [13]. This implies that terminal cleaning of rooms may be insufficient to prevent onward spread of CDI and emphasizes the need for adequate environmental decontamination.

Studies have shown frequent contamination of surfaces and equipment *with C. difficile* in the immediate environment of patients with CDI [10,14] and also from air samples of the environment of patients with CDI [15]. However, contamination of the environment is not limited to the patient's immediate surroundings, as contamination with *C. difficile* in staff areas has also been reported [16].

The degree of contamination of the patient's immediate environment may vary depending on the degree of severity of the patient's symptoms. Sethi *et al.* reported that the level of contamination of the environment of patients with CDI remained high at the time of resolution of diarrhoea and was less at the end of treatment [5]. Colonization pressure has also been shown to be a risk factor for the development of CDI, i.e. the more patients with *C. difficile* infections there are in a healthcare institution or specific clinical area, such as a ward, the more likely patients are to develop CDI [17].

The laboratory detection of *C. difficile* in the environment is not considered a routine investigation by most infection prevention and control teams, but is sometimes undertaken as part of outbreak management [16,18], and during research [5,19–33]. As a result, there is no standard approach and the laboratory methods vary. However, the availability of newer molecular methods has provided an exciting new option to increase detection. Consequently, we considered it timely to review methods and approaches to the detection of *C. difficile* in the environment, and specifically culture methods and molecular approaches, with a view to providing some conclusions and perspectives in this area.

Methods

The scientific literature on the environmental detection of *Clostridioides difficile* published from 1980 to October 2018 was reviewed using searches conducted in Pubmed, CINAHL and EMBASE. Search terms, in addition to *Clostridium difficile*, included; environment, detection, screening, sampling, method and technique. The abstracts of references found in the searches were reviewed. Published research papers that focused primarily on methods of environmental screening were chosen. The references of chosen papers were also reviewed to determine whether they should be included in the review in case they were missed in the original literature search.

The environmental detection of C. difficile

There is currently no internationally accepted guidance on the use of environmental screening for *C. difficile* or when it should be undertaken. Given that the environmental reservoirs of *C. difficile* are known to be implicated in the transmission of CDI it would seem prudent to sometimes consider environmental screening particularly in the outbreak setting.

Sampling techniques

C. difficile has been frequently detected from various near-patient surfaces such as the bed rail, mattress, call button, bedside table and floor. It has also been isolated from patient equipment such as pulse oximeters, weighing chairs, commodes, and from surfaces in the patient bathroom such as toilet seat, toilet floor and toilet flush handle [18–22,25]. *C. difficile* has been recovered from other clinical areas such as alcohol hand gel dispensers, the desks of nurse stations and from the keyboards of computers used by clinical staff [16,18,25].

There have been many different techniques proposed for the sampling of environmental surfaces. These include adhesive paddles, rayon swabs, flocked swabs, contact (Rodac) plates and cellulose sponges. Buggy *et al.* compared Rodac contact plates, adhesive paddles, dry cotton swabs and moistened cotton swabs in the recovery of *C. difficile* from the environment. They found that Rodac plates achieved the highest recovery followed by moistened swabs. There was poor recovery of spores when using adhesive paddles or dry swabs, and they were deemed not to be useful [30].

Claro *et al.* demonstrated that recovery of *C. difficile* spores was greatest from contact plates followed by flocked swabs, with lowest recovery from rayon swabs [34]. There are some drawbacks when using contact plates. Firstly, the size of the area being sampled is limited to the area of the contact plate. Secondly, curved surfaces are difficult to sample when using contact plates.

Studies comparing the use of rayon and flocked swabs to cellulose sponges for the recovery of *C. difficile* have shown that the sponge method is more likely to be successful [20,32]. A study by Ali *et al.* compared the recovery of *C. difficile* spores using sponge swabs to recovery using contact agar plates. Sponge swabs recovered 76–98% of spores *in vitro* compared to contact agar plates which recovered 19–32% [19]. As part of this study, they were able to demonstrate environmental contamination close to a patient three days before the patient developed any symptoms of CDI. The environmental and

Table I
Different media used to culture *C. difficile* from the environment

Year	Country	Media	Findings	Reference
1991	USA	Cycloserine cefoxitin fructose agar (CCFA) with varying concentrations of cycloserine	Increased recovery of *C. difficile* from environmental sites when cycloserine concentration was reduced from 500 μg/mL to 250 μg/mL	[24]
2000	UK	Cycloserine cefoxitin agar with lysed blood with and without 5 mg/L of lysozyme	The addition of lysozyme resulted in significantly higher recovery of *C. difficile* from environmental samples	[33]
2006	Poland	0.1% Sodium taurocholate-enriched cycloserine-cefoxitin-amphotericin B cycloserine cefoxtin (TCCA) Columbia blood agar and TCCA broth	The majority of non-toxigenic strains only grew in the TCCA broth and not when plated directly on to TCCA agar	[26]
2012	UK	Cycloserine cefoxitin amphotericin B (CCAB) agar, cycloserine cefoxitin agar with egg yolk (CCEY), *Clostridium difficile* moxalactam norfloxacin agar (CDMN), fastidious anaerobic (FA) agar, glucose supplemented brain-heart infusion (GS-BHI) agar	Greatest recovery from non-selective media - GS-BHI and FA agar	[23]
2013	UK	Cefoxitin cycloserine egg yolk agar plus lysozyme (CCEY-L), chromID *C. difficile* (bioMerieux)	ChromID *C. difficile* was more sensitive (87.6%) than CCEY-L (26.6%) and had lower levels of non-*C. difficile* growth	[25]

clinical isolates were subsequently found to be the same ribotype [35].

The size of the area sampled was not clear in many of the studies reviewed. This may be because many of the common areas sampled in the near-patient areas such as the call bell, door handle, bedrail, etc., are curved and relatively small in area. This would contrast with flat surfaces, e.g., floor, mattress and bedside locker where it would be easier to quantify the area sampled. When Ali *et al.* compared the use of a 25-cm^2 contact plate to a 25-cm^2 sponge swab, it was possible to sample the entire test area with the sponge swab but not with the contact agar plate [19].

Engelhardt *et al.* used a 10 × 10 cm template when sampling flat surfaces and an approximated area of 10 × 10 cm for curved surfaces [20]. Otter *et al.* used cotton swabs to sample 25cm^2 areas and compared this to a sponge that was used to sample multiple surfaces totalling approximately 1 m^2 [32]. Brown *et al.* used cellulose sponges to sample small (32 cm^2) and large (1 m^2) areas of the floor and bedrails of several patient rooms. The samples were processed using enrichment culture or polymerase chain reaction (PCR) analysis. The authors found that large-surface-area samples were more likely to be positive for *C. difficile* than small-surface-area samples [21]. The added benefits of using sponges are that larger surface areas can be sampled and curved surfaces are easier to sample, which as mentioned previously, is often an issue when using contact plates.

Culture methods

Table I outlines details of some studies describing the different culture media used to isolate *C. difficile* from the environment. One of the first selective media recommended for the isolation of *C. difficile* from faecal specimens was cycloserine-cefoxitin-fructose agar (CCFA), which also contains egg yolk [36]. The use of cycloserine and cefoxitin inhibits the growth of other faecal flora such as the *Enterobacteriales* and the enterococci. Levett *et al.* demonstrated that when the concentration of cycloserine and cefoxitin in CCFA was halved, recovery of *C. difficile* from faeces increased. As a result the amount of cycloserine and cefoxitin was reduced to that now present in the agar [37]. This finding was echoed by Clabots *et al.* who demonstrated that the rate of *C. difficile* positive cultures from environmental sites increased from 4% to 17% by reducing the concentration of cycloserine by 50% [24].

These selective media are designed to isolate *C. difficile* from faecal specimens with the antibiotics present in the media preventing other faecal flora from growing. Malik *et al.* showed that more spores could be recovered when environmental sampling with non-selective media, such as fastidious anaerobic agar or glucose-supplemented brain-heart infusion agar, was used instead of a *C. difficile* selective medium. They proposed that the use of selective media in environmental monitoring could result in an underestimation of environmental contamination from samples [23].

A study by Wilcox *et al.* recommended the use of a modified Brazier's media for the environmental detection of *C. difficile* (CCEY agar without egg yolk and with the addition of lysozyme) as it was found to increase spore germination when compared to CCEY agar without the addition of lysozyme [33]. Martirosian recommended the addition of 0.1% sodium taurocholate to media used for the environmental detection of *C. difficile* to

increase spore germination as there are far fewer spores in the environment than there are in faeces [26].

A commercially available chromogenic *C. difficile* selective agar (CHROMID® *C. difficile*, bioMerieux) was originally developed and evaluated for the culture of *C. difficile* from faecal specimens. Hill *et al.* compared the use of this chromogenic agar to CCEY agar containing lysozyme (CCEY/L) in recovering *C. difficile* from environmental samples. The chromogenic media performed significantly better in this study with a sensitivity of 87.6% compared to that of the CCEY/L agar, which had a sensitivity of 26.6%. The authors calculated this by assuming that all *C. difficile* positive samples were detected by one of the culture methods. They also noted that only four non-*C. difficile* species grew on the chromogenic agar compared to 16 on the CCEY/L agar [25].

Several studies compared the use of an enrichment broth prior to sub-culturing on to agar plates and compared recovery of *C. difficile* to samples that were inoculated directly on to agar. Wilcox *et al.* sampled environmental sites and compared direct inoculation on to CCEYL agar to samples that were inoculated into Robertson's pre-reduced cooked media for 48 h, then sub-cultured on to CCEY and CCEYL agar and incubated for a further 48 h. The authors found that the use of the enrichment broth significantly improved recovery of *C. difficile* with 45% of samples being positive compared with 35% using direct inoculation without enrichment [33]. This contrasts with a study by Verity *et al.* who compared direct inoculation on to cycloserine cefoxitin agar supplemented with 5 mg/L of lysozyme to inoculation on to the same agar following enrichment with Robertson's cooked media. They found that *C. difficile* recovery was significantly greater using the direct inoculation method compared to enrichment (137 positive samples vs 61 samples) [22].

The duration of incubation of culture plates varies across the studies reviewed. Some authors examined plates for growth after 48 h [19,23,25,30,33] while others incubated plates for 48 h, reviewed the plates and re-incubated them for a further 72 h if culture-negative before deciding if there was no growth [26,29,32]. The duration of incubation of the enrichment broth when enrichment was used was 48 h [22,33].

Non-culture-based approaches

Both the European Society of Clinical Microbiology and Infectious Diseases' (2014) and the Infectious Diseases Society of America's guidelines (2018) on the management of CDI support the use of PCR (and other nucleic acid amplification techniques) or an enzyme immunoassay for the detection of glutamate dehydrogenase (GDH) as the first step of a multi-step algorithm for the laboratory diagnosis of CDI [38,39]. This is followed by the more specific enzyme immunoassay for Toxin A and Toxin B. Most PCR assays detect conserved regions of the Toxin B encoding gene *tcdB,* but there are others that can detect *tcdA* or binary toxin encoding genes such as *cdt* and *tcdC*. These tests are highly sensitive but there are concerns that if used as stand-alone tests, and especially in the absence of clinical correlation, they can over-diagnose CDI due to false-positive results. For this reason, a multi-step algorithm is preferred.

The use of real-time (RT)-PCR for the environmental detection of *C. difficile* was first described by Nonnenmacher *et al.* in 2007. In this study, a 16S rRNA target sequence was used. Moistened swabs were used to sample the hospital environment, inoculated into Schaedler bouillon and incubated for three days prior to DNA extraction. The authors found that 40.6% of environmental swabs were positive using this method [40]. In 2008, Mutters *et al.* examined the use of quantitative RT-PCR for the quantitative detection of *C. difficile* from hospital environmental samples. The target sequence of the probe used was a highly conserved region of the 16S RNA gene of *C. difficile*. Higher bacterial counts were observed in samples taken from the environment of *C. difficile*-positive patients, however, environmental contamination was also observed in areas with no known cases of CDI [41]. The study did not compare the PCR-method to culture-based methods and due to the target sequence used, this study was unable to determine whether the *C. difficile* detected was toxigenic. Therefore, it is possible that some of the strains of *C. difficile* detected may not have been responsible for clinical disease.

In a study by Deshpande *et al.*, a commercially available RT-PCR assay, which is routinely used for the detection of toxigenic *C. difficile* from faecal samples, was used to detect *C. difficile* in the environment. This approach was then compared to a culture-based method using swabs. In this study high-touch surfaces in the hospital environment were sampled using two swabs and a moistened piece of gauze. One swab was processed by RT-PCR for the toxin B gene *tcdb* and the other was used to directly inoculate a *C. difficile* Brucella agar plate. The gauze was placed directly into *C. difficile* Brucella broth and incubated. All isolates cultured were tested for toxin production using an enzyme immunoassay test. Twenty-three out of the 66 areas sampled were culture-positive for *C. difficile*. Four of the 66 of the environmental samples were positive using PCR. The PCR-method was compared to the anaerobic toxigenic culture method, which acted as a reference test to determine the sensitivity and specificity of the PCR method. The sensitivity of the PCR assay was 17.4%, but this increased to 52.2% when the PCR cycle threshold was increased from 37 to 45 cycles. The outcome of the study suggested that PCR was not as sensitive as culture-based methods, especially in areas of low-level contamination with *C. difficile* [27].

A study by Morales *et al.* used RT-PCR to detect environmental contamination in a hospital during and after an outbreak as the authors had previously sampled the environment using a culture-based method that yielded no positive results. They repeated the sampling using RT-PCR to determine whether this was a more sensitive method. They were able to detect *C. difficile* from 40% of their environmental sample using RT-PCR. A period of increased environmental cleaning using 1:10 hypochlorite solution was effective in controlling the outbreak. The sampling was then repeated two years after the outbreak. Results showed that the level of contamination of the environment was higher during the outbreak (72%) compared to after the outbreak (35%) [18]. One of the limitations of this study is that it did not compare the use of a PCR-assay to a culture-based assay.

In a study by Brown *et al.*, a quantitative PCR using target sequences of 16S RNA and the *tcdB* was compared to a culture-based assay that involved an enrichment step. Environmental sampling was undertaken in the rooms of patients who were known to have CDI, in the rooms of patients who had recently had antibiotics but did not have CDI, and in the rooms of patients without a history of either CDI or antibiotic use. Large and small areas of the floor and bedrail were sampled using

pre-moistened sponges. Sixty-four percent of samples were positive using the 16S PCR compared to 43.8% using enrichment culture and 39.6% using the PCR for *tcdB*. Large surface area samples were more likely to be positive than small surface area samples and floor samples were more likely to be positive than samples from bedrails [21].

The main advantage of PCR-based methods for the environmental detection of *C. difficile*, over culture, is the rapid turnaround time in the diagnostic setting. Accounting for the DNA extraction step, the overall turnaround time for PCR may be a matter of hours compared to culture-based methods, which may take days. This may be particularly important if environmental screening is being used to confirm adequate decontamination of a clinical area prior to reopening the area for use.

Other approaches

Bomers *et al.* proposed the use of dogs for the detection of *C. difficile* from patients and faeces [42]. More recently, Bryce *et al.* reported the successful use of scent detection by a dog for environmental reservoirs of *C. difficile*. Jars containing cotton gauze that had been soaked with a 1-mL solution of saline mixed with a colony of toxigenic *C. difficile* were hidden on simulation wards. The dog was evaluated on odour recognition and search capability, the overall sensitivity of which was 92.3% with a specificity of 95.4% [31].

Discussion

C. difficile is an important healthcare-associated pathogen and inadequate decontamination of the environment can result in transmission and outbreaks. The environmental detection of *C. difficile* remains challenging and there are currently no internationally agreed guidelines or consensus on when to sample and with which methods. While the literature reviewed does not lend itself to firm or conclusive recommendations, it is possible to make some observations.

Environmental surveillance for *C. difficile* should not be considered routine but there may be a role for it during outbreak investigation or as part of research into transmission patterns and when trying to determine reservoirs. Environmental surveillance of *C. difficile* could also be used as a tool for monitoring the effectiveness of environmental decontamination prior to reopening a clinical area for use.

Although there are a number of methods of sampling, more recent studies have shown greater recovery of *C. difficile* when using sponges. This method allows for larger and curved areas to be sampled, which likely explains the increased recovery of spores in the studies reviewed.

The literature also suggests that culture-based tests are currently the most commonly used methods and will likely continue to be for the time being. Moreover, there does not appear to be a consensus on which agar medium to use when culturing as this was variable. The use of selective media does appear necessary to limit the growth of other organisms commonly found in the environment. However, often the selective component of many of the media used in these approaches is insufficient to prevent the growth of contaminating environmental organisms. While there is evidence to potentially underestimate the level of environmental contamination when using selective media, we would still recommend some form of selective media to aid in the identification of *C. difficile* from environmental samples.

There were conflicts in the literature regarding the effectiveness of using an enrichment step as part of the culture process. However, when this additional step was used, there was a consistent increase in the time to reporting results, which may have had an impact on the practical use of the method in the clinical setting.

There is less evidence in the literature for using molecular methods to detect *C. difficile* in the environment as it remains a novel technique in this area, although this is likely to change as PCR becomes more routine and accessible. While the rapid turnaround times for molecular methods are impressive, this method is not without drawbacks. Firstly, if the target used for the PCR is not a toxin gene, it cannot determine whether the *C. difficile* being detected is toxigenic or not. If it is not toxigenic, then the significance of the isolate in an outbreak setting needs to be examined. Secondly, even if *C. difficile* DNA is detected in the environment, it could represent DNA from non-viable vegetative organisms rather than the DNA from a *C. difficile* spore or a viable vegetative cell. Detecting spores is important given the difficulties they present for current decontamination methods. Despite these drawbacks, PCR remains a possibility for more rapid and sensitive detection of *C. difficile* from the environment. However, further research in the area is required to evaluate the effectiveness and implementation of this approach in the clinical setting.

Infection prevention and control practitioners would benefit from greater clarity on when to sample, where to sample, how to sample and how to interpret results, irrespective of whether they were generated from a culture- or molecular-based method. This literature review highlights the need for further comparative studies both *in vitro* and in the clinical setting to develop more consistent approaches to environmental sampling, which in turn would guide infection prevention and control experts to develop guidelines for the detection of *C. difficile* in the environment. The increasing availability of molecular methods may result in more routine sampling as the rapid availability of results may be used to inform the effectiveness of hospital decontamination strategies as well as providing some reassurance in patient placing when the environment is deemed *C. difficile* 'free'.

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Conflicts of interest statement

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