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10.21203/rs.3.rs-2174275/v1

RESEARCH

Characterisation of Clostridioides difficile spore response to sodium hypochlorite using EM-imaging and Raman Spectroscopy

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Abstract

Background: *Clostridioides difficile* is a spore forming bacterial species that is the major causative agent of serious infection in hospitals. *C. difficile* spores are highly resilient to disinfection methods and to prevent infection, common cleaning protocols use sodium hypochlorite solutions to decontaminate hospital surfaces and equipment. However, there is a balance between minimising the use of harmful chemicals to the environment and patients as well as the need to eliminate spores, which can have varying resistance properties between strains. In this work, we compare the effect of sodium hypochlorite on different *C. difficile* clinical isolates and the chemical's impact on spores' biochemical composition. Biochemical changes can, in turn, change spores' vibrational spectroscopic fingerprints, which can impact the possibility of detecting spores in a hospital using Raman based methods.

Results: First, we found that the isolates show different susceptibility to hypochlorite, with the R20291 strain, in particular, showing less than 1 log reduction in viability for a 0.5 % hypochlorite treatment, far below typically reported values for *C. difficile*. Second, TEM and Raman spectra analysis of hypochlorite-treated spores revealed that some hypochlorite-exposed spores remained intact and not distinguishable to controls. Finally, we saw differences in the ultra-structure of *C. difficile* and *B. thuringiensis* spores in response to hypochlorite.

Conclusion: This study highlights the ability of certain *C. difficile* spores to survive practical disinfection exposure as well as the changes in spore spectra that can be seen using Raman spectroscopy. These findings are important to consider to avoid a false-positive response when screening decontaminated areas.

Keywords: bacterial spores; laser tweezers Raman spectroscopy; Raman spectroscopy; LTRS; C. difficile

1 Background

Clostridioides difficile (also known as *Clostridium difficile*) is an anaerobic spore-forming bacterium and the most common cause of antibiotic-associated diarrhoea globally as well as the most common cause of healthcare-acquired infections (HCAI's) in the USA [1]. *C. difficile* is normally harmless to healthy adults, and ingestion of this common bacterium does not cause disease. Approximately 1-3 % of the population are reported to be asymptomatic carriers, while in the remaining population, existing gut microflora prevents colonization by *C. difficile* [2]. However,

as a side effect of oral broad spectrum antibiotics, this microflora can be depleted and trigger a *C. difficile* infection. Hospitals are a hotspot for infections as they contain a high concentration of susceptible patients. Indeed, the health impact of *C. difficile* infection is huge. The economic costs for management of *C. difficile*-associated disease in US hospitals alone was estimated at $ 4.8 billion in 2008 [3]. In addition, *C. difficile* infection also has a 5.6-6.9 % reported fatality rate leading to a significant loss of life [4].

The infectiveness and high outbreak management costs of *C. difficile* infection is due to its ability to form resilient endospores (spores). Spores can survive for months in the environment and cause infection when ingested. Spores are also capable of surviving many harsh conditions, such as 95 °C wash cycles for hospital bedding and gowns [5]. Common hospital disinfection approaches such as alcohol-based hand wash, low-concentration sodium hypochlorite (bleach), and quaternary ammonium are ineffective at decontaminating spores on surfaces [6, 7]. Thus, hospitals need strict hygiene and cleanup protocols to prevent *C. difficile* outbreaks.

Therefore, to avoid *C. difficile* outbreaks, it is important to have rapid and specific detection techniques that can detect spores both in hospital facilities such as patient-specific rooms and also at laundry rooms. Since Raman spectroscopy is a non-invasive, label-free and highly specific technique that can provide a spectral fingerprint of a sample, both on surfaces or in a liquid, it has been proposed as one possible method. In addition, Raman spectroscopy has an advantage compared to other spectroscopic methods such as infrared (IR), since Raman signals are only moderately affected by the presence of water, so testing aqueous suspensions is easier. Also, Raman bands are significantly narrower and therefore easier to identify than fluorescence bands [8, 9]. As proof of concept, Raman spectroscopy has been used successfully to detect spores and also to track chemical changes in the spore body in time series. For example, Raman spectroscopy has been successfully used to identify and distinguish different pathogens [10], identify spore strains [11], track the germination process [12, 13], and characterize the impact of disinfection chemicals on the spore body with time [14, 15].

Sodium hypochlorite is a common disinfection chemical used in hospitals and in homes [16]. It is therefore of interest to know if this chemical affects the Raman signature of *C. difficile* spores to avoid any false-positive response. The aim of this work is to compare the effect of sodium hypochlorite on three different *C. difficile* clinical isolates and characterize if the sodium hypochlorite concentrations impact their Raman spectra. Raman spectra were acquired of single spores using laser tweezers Raman spectroscopy (LTRS). These findings were then linked to structural changes in the spores assessed using transmission electron microscopy (TEM).

2 Results and discussion

2.1 The *C. difficile* R20291 spores are highly resistant to disinfection with 0.5 % sodium hypochlorite

Sodium hypochlorite is a common decontamination agent used widely around the world [7], being the main active ingredient in household and industrial bleach. Sodium hypochlorite is a chlorine releasing agent (CRA) and works by degrading

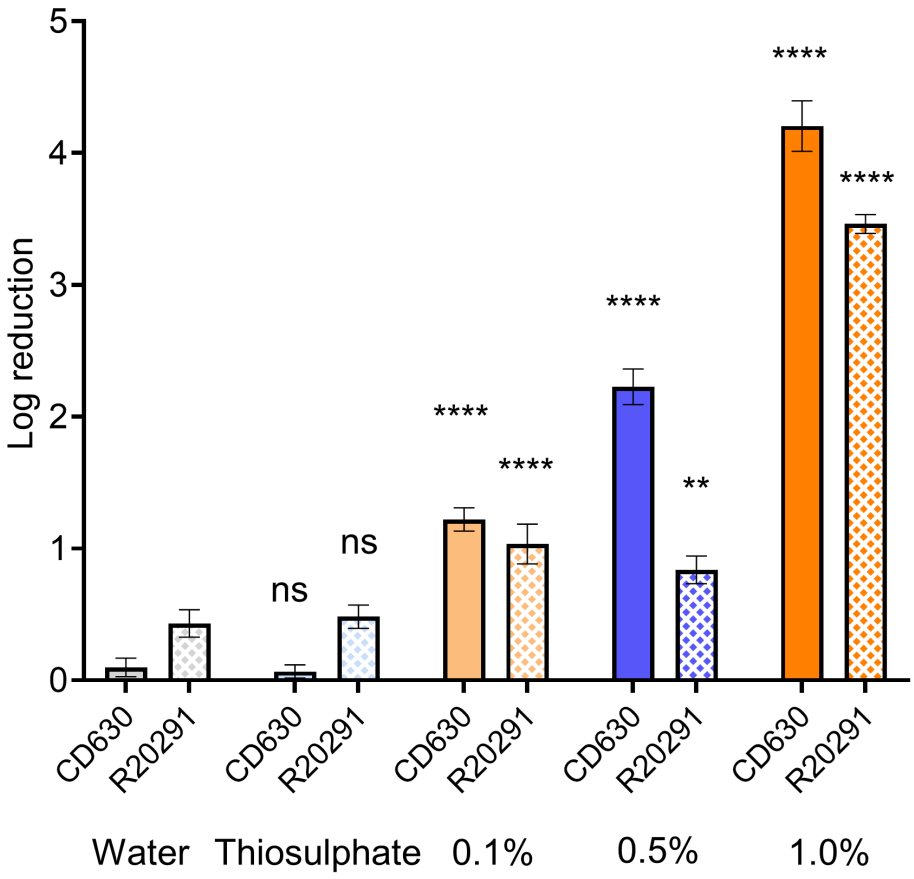

Figure 1 Disinfection of the CD630 and R20291 strains of *C. difficile*. Spores were incubated with 0.1 %, 0.5 % and 1.0 % hypochlorite for 10 min. Controls with water and thiosulphate alone are also shown. The R20291 strain in particular is very resilient to low concentrations below 1.0 % hypochlorite. Stars indicate statistical significance of the reduction compared to the respective water control; "ns" indicates no significance.

organic material in several reactions: saponification of fatty acids and neutralization and chloramination of amino acids [17, 7], which thereby will degrade the spore structure if exposed for long time scales or at high concentrations [15]. Spores of *C. difficile* were previously reported to be quite resilient to decontamination with lower concentrations of hypochlorite [18]. This is particularly important since, in a practical cleaning environment, spores might only be exposed for a shorter duration due to issues with cleaning protocols and time pressure for healthcare workers [19] or the concentration can be too low for effective decontamination [20]. Hospitals often use a 1:10 dilution of bleach (approximately 0.5 % or 5000 ppm) but reported spore disinfection varies [21, 22]. Decontamination of *C. difficile* with hypochlorite was previously reported at 4.3 log (0.5 %, 10 min) [18], 5.7 log (0.6 %, 10 min) [23], 6 log (0.5 %, <10 min) [24] and 4.64 - 5.39 log (0.5 %, 5 min) [25]. This is a substantial variance (4.3 - 6 log) across studies despite similar conditions. In addition, it has been noted that decontamination is also pH-dependent and at physiological

pH, the efficiency of hypochlorite is reduced to <1 log (0.5 %, 10 min) [26]. This is in contrast to *Bacillus* species, where sodium hypochlorite was reported to be more effective at decontaminating spores at physiological pH [27].

We found DS1813 *C. difficile* spores to be in line with previously reported results for spores with 5.1 ± 0.2 log reduction after 0.5 % hypochlorite treatment for 10 min. By contrast, the CD630 and the R20291 were more resilient than previously reported in the literature, with a decontamination in 0.5 % hypochlorite causing a 2.2 ± 0.1 log reduction in the CD630 strain, and 0.8 ± 0.1 reduction in the R20291 strain compared to control. This is significantly below the requirements for surface decontamination for an area in clinical environments, where usually at least 3 log is expected. We therefore made a viability assay in which the concentration was varied between 0.1 %, 0.5 %, and 1.0 %, with the results shown in Figure 1.

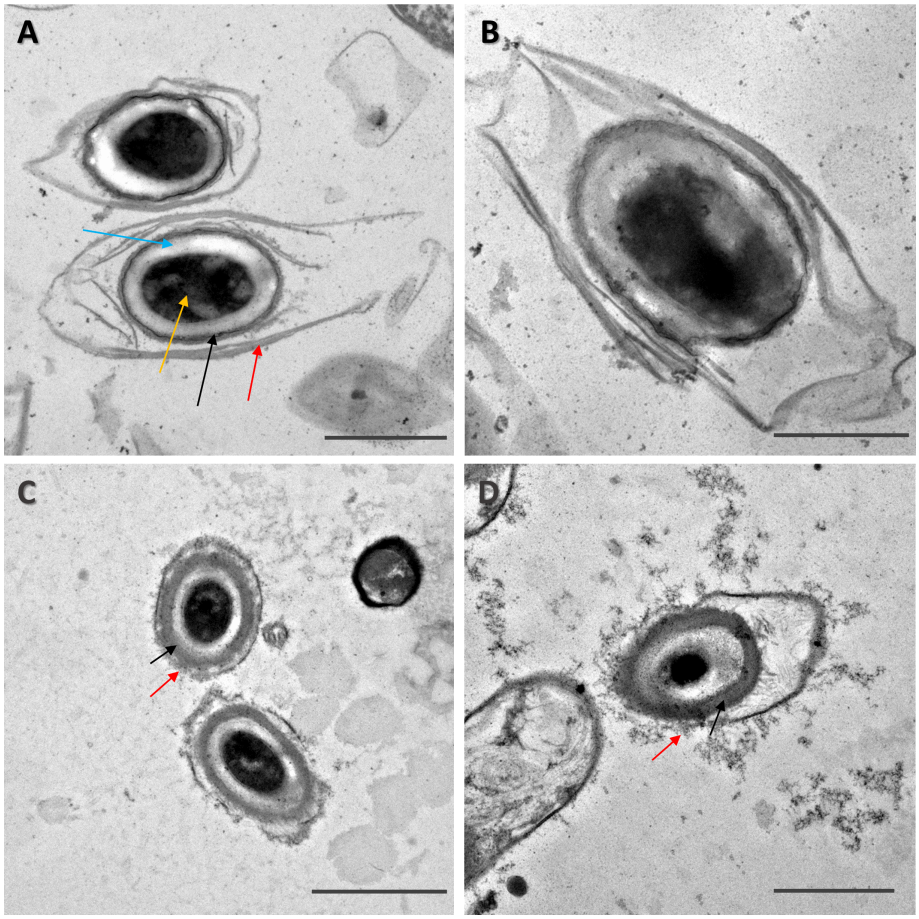

Figure 2 Representative TEM images of showing the effect of sodium hypochlorite treatment on *C. difficile* spores. Untreated spores are shown on A (R20291) and B (CD630). These spores are intact spores with spore layers clearly defined (arrows on A) and they have an intact exosporium. The spores appear disrupted after a 1 % sodium hypochlorite treatment, with the exosporium (orange arrow) and coat (black arrows) layers no longer intact in both C (R20291) and D (CD630). Some spores no longer have an electron-dense core. Scale bars are 1 μm.

One clear observation is that the resistance to hypochlorite is much higher than previously reported for the R20291 specifically. A possible explanation is the inappropriate use of CRAs over time in clinical environments as the disinfectant of choice

[28, 20, 19] has resulted in evolution of biocide tolerance in *C. difficile*, specifically in hypervirulent R20291. This warrants further investigation to determine whether this matches trends in biocide tolerance of *C. difficile* persisting within clinical environments. This aligns with the final conclusions in Dawson *et al.,* 2011 that the type of disinfectant used should be carefully considered before deployment for decontamination of surfaces [22].

To assess the disruption of spores after exposure of sodium hypochlorite we used TEM imaging. A micrograph of spores at the higher 1 %, 10 min hypochlorite exposure is shown in Figure 2. Unexposed control spores (Figure 2A-B) are intact with the core (yellow), cortex (blue), coat (black) and exosporium (orange arrows) clearly visible. Spores treated with 1 % hypochlorite have a degraded or missing exosporium and a less electron-dense coat (Figure 2C-D). There are also some spores that are pale outlines with completely unstained internal content, similar to what we have previously observed in *B. thuringiensis* spores [15] treated with 0.5 % sodium hypochlorite for 30 min. This visual disruption was linked with the loss of the spore's CaDPA and in the more disrupted cases, the loss of most internal material. Nevertheless, there were no visual differences between the hypochlorite treated CD630 strain (Figure 2C) with 6 log killing and the R20291 strain (Figure 2D) with 1 log killing, despite such large differences in their rate of inactivation. To address this finding and quantify the changes in the spores with high accuracy, we turned to single spore analysis using LTRS.

2.2 Raman spectra of decontaminated spores fall into distinct groups

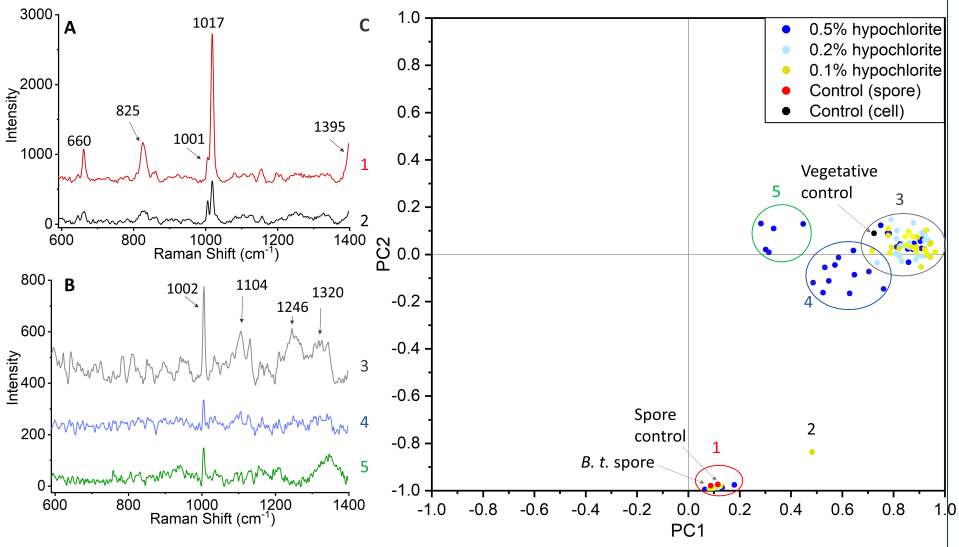

Figure 3 Principal component analysis (PCA) of sodium hypochlorite treated spores in the 600 - 1400 cm^{-1} spectral range (n=90). The representative observed spectra of spores with CaDPA (A) and without CaDPA (B) are shown. The numbering of the spectra corresponds to the numbering of the groups in the loadings plot (C). Untreated control spore, live vegetative cell, and a acquired the TEM data spore are also shown (marked with arrows).

Using the LTRS instrument we optically trapped individual spores and analysed their Raman spectra to quantify the chemical content. This approach provides a

spectral fingerprint of the trapped object and can thereby measure chemical differences between hypochlorite treated and untreated spores, as well as difference with vegetative cells. A typical Raman spectrum of a *C. difficile* spore in the 600-1400 cm^{-1} spectral range is shown on Figure 3A, with major peaks at 660, 825, 1001, 1017 and 1395 cm^{-1}. This is in line with the Raman spectrum of *C. difficile* spores described in literature [29, 30, 10]. The spectrum is dominated by the Raman peaks of calcium dipicolinic acid (CaDPA), a key chemical in the spore's wet heat resistance that makes up to 25 % of the dry weight of the spore core. The reported peaks of CaDPA are 660, 825, 1017, 1395 and 1575 cm^{-1}, while the 1001 cm^{-1} is due to phenylalanine [31, 29, 32, 10]. During germination, or if the spore body is damaged, the intensity of the CaDPA Raman peaks are significantly reduced or disappears completely as the spore loses its CaDPA store [15]. The Raman spectrum of spores in the 1400-1700 cm^{-1} range, which is outside the range of Figure 2, is shown in Figure S1A. In total, we acquired n = 6 spore spectra of untreated spores, all show consistent spectra.

While some hypochlorite treated spores appeared similar to the control spores, some spores produced different spectra, see Figure 3B and Figure S1B. These spores are missing CaDPA related peaks. This loss of CaDPA is expected as it was previously reported that hypochlorite-treated spores will release CaDPA [27, 33]. This is also consistent with TEM images in Figure 2, with some spores missing their electron-dense core. In addition to this lack of CaDPA, we see that there is variation in the other peaks among spectra in these spores, and with different prominence of different peaks. The main conserved peak among all spores is the 1001 cm^{-1} peak associated with phenylalanine, a structural amino acid in both spores and vegetative cells. Peaks at 1101 cm^{-1} and a double peak at 1246-1320 cm^{-1} are also present.

To compare spectra of different groups of spores/cells we quantified the differences between all of the measured spores with principal component analysis (PCA). PCA is a suitable method to compare complex data such as spectra to each other and quantify differences between them [34]. PCA has previously been used to compare Raman spectra of explosive chemicals [35], and to compare blood plasma spectra for when searching for viral biomarkers [36]. As such, this method was suitable for comparing the differences in the Raman spectra in the spores. The results from this analysis is shown on Figure 3C for the 600 - 1400 cm^{-1} range spectra (n=90), with representative spectra from each group in Figure 3A-B corresponding to the circled groups. The results for the 1000 - 1700 cm^{-1} range (n=90) are included in Figure S1C. Spores that retain the CaDPA peak make a compact cluster (Group 1) with 10 of 92 spores (11 %) in this cluster. Untreated control spores, both *C. difficile* and *B. thuringiensis*, also fall into Group 1, in line with earlier research that disinfection of bacterial spores does not necessarily lead to changes in the Raman spectrum [37]. A single outlier, marked as Group 2 also has the CaDPA peaks. The outlier is a spore with reduced CaDPA peaks prominence, but otherwise similar to the CaDPA containing spores.

The remaining spores lack CaDPA peaks and fall into three groups. Many spores retain their Raman spectrum peaks, except CaDPA. This is the most common group of spores among those observed (65 of 92 spores, 71 %) and has been labelled as Group 3. Spores in this cluster their retain their Raman spectrum, such as the

peaks at 1104 and 1246 cm^{-1}, and their similarity to Group 1 spectra can be seen seen on Figure S2, where the two spectra are shown on the same scale. *Bacillus subtilis* spores have been previously shown to release their CaDPA after hypochlorite treatment, so Group 3 appears made from such spores. Spectra in this group are also similar to vegetative cells, as shown by the marked vegetative control spectrum. These spectra are also similar to other vegetative cell spectra, with resonant peaks at 1101, 1245 and 1319 cm^{-1} [10].

Group 4 spores (13 of 92 spores, 14 %) partially overlap with Group 3 (2 overlapping spores), and consist of spores with reduced peaks, including the phenylalanine peak. These spores are likely the more degraded spores that observed under TEM, lacking not just the core content, but much of the other spore content, with the remaining content accounting for the smaller phenylalanine peak. Group 5 is the smallest group (5 of 92 spores, 5 %), and is an outlier to all the other groups. These spores have a small 1001 cm^{-1} phenylalanine peak, and a lack of other prominent peaks, similar to Group 4 spores. However, the spores in Group 5 also contain an additional broad peak centered at 1350 cm^{-1}. It is not a peak that is seen in spores, vegetative cells or in sodium hypochlorite. It is outside the Amide III band, which is usually placed at 1200-1300 cm^{-1} [38]. It is also not a peak seen in the subtracted background. A possible assignment of this peak is tryptophan and thymine from aggregates on the surface of the spore from lysed spore fragments [39].

We did not see any clinical isolate dependent distribution among the spectra (Figure S3), with all clinical isolates being present in all groups in the principal component analysis. Despite differing levels of resistance to sodium hypochlorite as well as differences in virulence and structure [40, 41], there were no changes visible under Raman spectroscopy between the strains.

We did, however, see spore spectra grouping based on the hypochlorite concentration used. All 5 spores in Group 5 and 10 of the 13 spores in Group 4 were spores treated with 0.5 % hypochlorite. This is consistent with the prediction that higher concentrations of sodium hypochlorite will lead to greater spore degradation, and more lysed components in solution. However, there were other 0.5 % hypochlorite-treated spores in Groups 1 and 3, with the same spectra as untreated controls. The observation that some spores remain visually and structurally intact despite extremely aggressive conditions like 0.5 % hypochlorite highlights the difficulty of decontaminating spores. This is compounded by the fact that spores can clump together [42], shielding the innermost parts of the clump from the chemicals.

2.3 *C. difficile* show higher resilience to hypochlorite than *B. thuringiensis*

The variations in the resistance properties of *B. thuringiensis* and *C. difficile* may lie in their structural differences or response to sodium hypochlorite. As noted in Figure 2, *C. difficile* spores show degradation of their outer layers, but many retain their electron dense core (with electron density correlated to the internal CaDPA) [43, 44, 45].

We compared this structural appearance to that of *B. thuringiensis*. We chose identical conditions of a 10 min treatment with 0.5 % sodium hypochlorite for each species. As shown in Figure 4, the appearance of spores differs markedly. Most spores of *C. difficile* R20291 appeared visually intact, with the core, cortex, coat and exosporium appearing whole. Spores of *C. difficile* CD630 strain also had an intact

core and cortex, but electron dense material appeared in the interspace between the coat and the exosporium, while the coat exhibited pronounced structural changes. By contrast, most spores of *B. thuringiensis* had a different appearance, with the core and cortex melding into no longer having a visible boundary (additional representative fields of view are shown on Figure S4-S6). This difference is of interest as it indicates that there are potential mechanistic differences in the way the spores respond to hypochlorite. Although in both species, spores lose their core content, as previously shown on 3, *B. thuringiensis* spores appear to go through a process of core content leaching into the cortex. It was previously reported that spore CaDPA release is a rapid process (of the order of less than minute) [33, 15] and does not happen simultaneously, so the appearance of the *B. thuringiensis* spores indicates a there is a stage before CaDPA is released, as it permeates into the cortex.

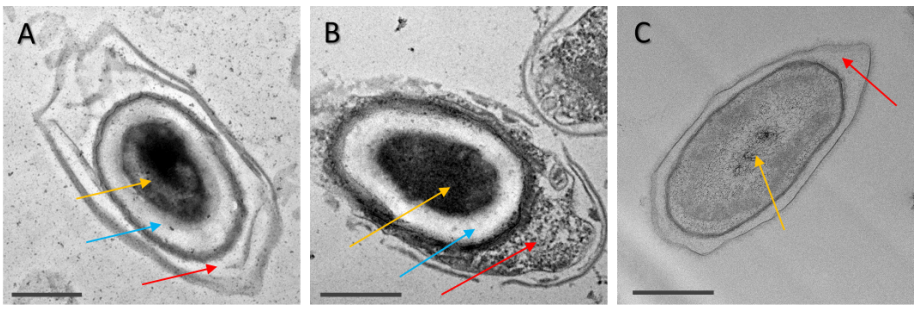

Figure 4 Representative TEM micro graphs of *C. difficile* R20291 (A) and CD630 (B) spores, and *B. thuringiensis* (C) spores treated with 0.5 % sodium hypochlorite for 10 minutes. In R20291, many spores remain visually intact, with the core (yellow arrows) remaining dense, the cortex (blue arrow), being clear and visible. In CD630, while the core and cortex are still intact, there is leakage of material from the coat into the interspace between the coat and exosporium (red arrow). In *B. thuringiensis*, the core loses electron density and cortex is no longer visible after treatment. Scale bars are 500 nm.

3 Conclusions

Sodium hypochlorite is a common cleaning agent, both in homes and in hospitals. In this work, we looked at the chemical changes caused by decontamination of clinical isolates of *C. difficile* with sodium hypochlorite. We found that sodium hypochlorite inactivates *C. difficile* spores with rates of 0.8 log to 5.2 log (with 0.5 % (5000 ppm), 10 minute exposure) depending on clinical isolate, with the R20291 being the most resilient to disinfection, to the level where hypochlorite is largely ineffective against this strain.

We observed that hypochlorite treatment resulted in structurally intact R20291 *C. difficile* spores, whereas strain CD630 possessed a disrupted exosporium and outer coat layer, which is in contrast to *B. thuringiensis*, where disruption starts with leaching of material from the core into the cortex.

We compared the chemical content of spores using Raman spectroscopy and found that spore spectra fall into distinct groups, although spores that retain their internal CaDPA are indistinguishable from untreated spore controls.

Overall, this study highlights the changes in the structure and chemical content of *C. difficile* spores after hypochlorite exposure, both within the species and when compared to *B. thuringiensis*

4 Methods

4.1 Experimental setup and measurement procedure

We acquired Raman spectra from spores using our custom-built LTRS instrument. The instrument is built around an inverted microscope (IX71, Olympus) [46, 37]. We used a Gaussian laser beam operating at 785 nm (Cobolt 08-NLD) that is coupled into the microscope using a dichroic shortpass mirror with a cut-off wavelength of 650 nm (DMSP650, Thorlabs). Imaging and focusing of the beam were achieved by a 60× water immersion objective (UPlanSApo60xWIR, Olympus) with a numerical aperture of 1.2 and a working distance of 0.28 mm. The same laser was used for Raman light excitation. In general, we operated the laser at a fixed output power of 100 mW corresponding to a power of about 60 mW in the sample (total energy of 1.2 J when exposed for 20 seconds). This power was chosen as it is well below the energies that are able to damage spores [47, 48].

We collected the backscattered light by the microscope objective and passed it through a notch filter (NF785-33, Thorlabs) to reduce the Rayleigh scattered laser line. Further, to increase the signal-to-noise ratio, we have mounted a 150 μm diameter pinhole in the focal point of the telescope. The filtered light is coupled into our spectrometer (Model 207, McPherson) through a 150 μm wide entrance slit where a 600 grooves/mm holographic grating disperses the light [49]. The Raman spectrum was then captured using a Peltier cooled CCD detector (Newton 920N-BR-DDXW-RECR, Andor) operated at -95 °C. Our system has a Raman wavenumber spectral resolution of < 3 cm^{-1} and accuracy of ~ 3 cm^{-1}.

4.2 Strains, culture media, and conditions

C. difficile isolate DS1813, CD630 and R20291 spores were sourced from the Anaerobic Reference Unit, University Hospital Wales, Cardiff, UK [43]. All three isolates are clinical isolates, with the DS1813 and R20291 belonging to the hypervirulent 027 ribotype of *C. difficile*, while the CD630 belong to the 012 ribotype and is a commonly studied and fully gene sequenced [50]. Spores were grown on BHIS agar at 37 °C for 4 days. The colonies were collected and left overnight at 4 °C to sporulate and release from mother cells. The suspensions were then purified using non-damaging density gradient centrifugation in 50 % sucrose as described previously [51, 52, 20]. Spores were then washed in deionised water and stored at 4 °C. This method avoids spore purification steps such as lysozyme or proteinase, to ensure that spores and their resilience to chemicals are representative of the spores typically found in hospital environments [53].

We determined the concentration of viable spores in the stock by serially diluting in deionized water down to 10^{-7} concentration and 10 μl drops plated onto BHI agar plates and grown at 37 °C in anaerobic conditions [54].

4.3 Spore biocide treatment

Each of the 3 isolates was decontaminated with 0.1 %, 0.5 % and 1.0 % sodium hypochlorite (1,000, 5,000 and 10,000 ppm active chlorine), for 9 samples in total. The decontamination procedure for each sample was as follows. 100 μl of spore suspension in water of known concentration was mixed with 100 μl of double concentrated NaOCl (0.2 %, 1.0 % and 2.0 %) and left for 10 minutes. The biocide was

then neutralised with 0.5 % sodium thiosulphate as described previously [20]. The spores were then washed with deionised water, by centrifuging and discarding the supernatant twice, to remove reacted chemicals.

The reduction in the viable spore counts was determined by spreading 100 μl culture onto BHI plates agar supplemented with 0.5 % yeast extract, 1% L-cysteine and 0.1 % sodium taurocholate (BHIS-ST) [55]. Spores were grown in anaerobic conditions at 37 °C for 48 hours and colonies were counted from a plate with appropriate dilution.

4.4 Sample preparation and reference spectrum acquisition

We prepared a sample by placing a 1 cm diameter ring of 1 mm thick vacuum grease on a 24 mm × 60 mm glass coverslip. We added 5 μl of the spore suspension into the ring, after which we sealed it by placing a 23 mm × 23 mm glass coverslip on top. After the sample was placed in the LTRS instrument, we measured the Raman spectra of the spores using 2 accumulations of 10 seconds. We measured 20 individual spores for each sample (10 measurements in the 600-1400 cm^{-1} and 10 in the 1000-1700 cm^{-1} range), for 180 measurements in total. There were also triplicate controls at each spectral range. The background spectrum of the spore suspension was also measured and subtracted.

4.5 Data processing and analysis for reference spectra

The statistical significance of spore decontamination results was calculated using two-way Anova with Dunnett's multiple comparisons test, done in Graphpad Prism 9 (Prism 9.3, GraphPad Software). We used an open-source Matlab script (using Matlab R2022, Mathworks) provided by the Vibrational Spectroscopy Core Facility at Umeå University to process Raman spectra [56]. To baseline correct the spectra we used an asymmetrical least-squares algorithm [57] with $\lambda = 10^5$ and $p = 10^{-3}$. We smoothed spectra using a Savitzky-Golay filter [58] of polynomial order 1 and a frame rate of 5. Principal component analysis of the spectra (PCA) was carried out in Graphpad Prism 9. Data was mean-centered and PCA was based on the correlation matrix. Graphs were plotted in Origin 2018 (OriginLab).

4.6 Electron microscopy

Samples for TEM were prepared as liquid suspensions of spores after treatment with sodium hypochlorite and neutralisation with thiosulphate, while untreated control samples were suspended in water. After the incubation, samples are centrifuged and resuspended in MQ water twice to wash off any aqueous chemicals. Spores are fixed with 2.5 % Glutaraldehyde (TAAB Laboratories, Aldermaston, England) in 0.1 M PHEM buffer and further postfixed in 1 % aqueous osmium tetroxide. They are further dehydrated in ethanol, acetone and finally embedded in Spurr's resin (TAAB Laboratories, Aldermaston, England). 70 nm ultrathin sections are then post contrasted in uranyl acetate and Reynolds lead citrate.

Acknowledgements

The authors acknowledge the facilities and technical assistance of the Plymouth Electron Microscopy Unit (PEMC); and of the Umeå Core Facility for Electron Microscopy (UCEM) at the Chemical Biological Centre (KBC), Umeå University, a part of the National Microscopy Infrastructure NMI (VR-RFI 2016-00968)

Funding
This work was supported by the Swedish Research Council (2019-04016); The University of Plymouth; Kempestiftelserna (JCK-1916.2).

Abbreviations
BHIS - Brain heart infusion supplemented CaDPA - Calcium dipicolinic acid CRA - Chlorine-releasing agent HCAI - Healthcare-acquired infection LTRS - Laser tweezers Raman spectroscopy PCA - Principal component analysis

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
Not applicable

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable

Authors' contributions
DM, LTJ and MA and conceived the experiments for this work. DM and MA were responsible for design and interpretation of the results of the Raman spectroscopy experiments. LTJ and DM were responsible for the design and interpretation of disinfection experiments. DM acquired and analysed Raman spectroscopy data and acquired the TEM data for B. thuringiensis. IAJ, MM and LTJ acquired and analysed disinfection data on C. difficile. GH acquired the TEM data for C. difficile. DM and MA wrote the draft manuscript. DM, MA, LTJ and MM revised the manuscript. All authors read and approved the final manuscript.

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