Identification of *C. difficile* reservoirs in the patient environment and efficacy of aerial hydrogen peroxide decontamination

S. Yui MSc[a]

S. Ali PhD[a]

M. Muzslay MSc[a]

A. Jeanes MSc[b]

A P R Wilson MD[c*]

[a] Environmental Microbiology Research Laboratory, University College London Hospitals, London, UK

[b] Department of Infection Control, University College London Hospitals, London, UK

[c] Department of Microbiology, University College London Hospitals, London, UK

*Corresponding author

Address: Department of Clinical Microbiology & Virology, University College London Hospitals, 60 Whitfield Street, London W1T 4EU, UK. Tel. +44 2034479516; fax: +44 02034479211; peter.wilson@uclh.nhs.uk

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Abstract

Objective: To identify, using a novel enhanced method of recovery, environmental sites where spores of *C. difficile* persist despite cleaning and hydrogen peroxide aerial decontamination.

Design: Cohort study

Setting: Tertiary referral centre teaching hospital

Methods: 16 sites representing high-frequency contact or difficult-to-clean surfaces in a single-isolation room or bed area in patient bed bays were sampled before and after terminal or hydrogen peroxide disinfection using a sponge-swab. In some rooms individual sites were not present e.g. no en-suite room in ICU. Swab contents were homogenised, concentrated by membrane-filtration and plated onto selective media. Results of *C. difficile* sampling were used to focus cleaning.

Results: Over one year, 2529 sites from 146 rooms and 44 bays were sampled. *C. difficile* was found on 131/572 (22.9%) of surfaces before and 105/959 (10.6%) after terminal cleaning and 43/967 (4.4%) surfaces after hydrogen peroxide disinfection. *C. difficile* persisted most frequently on floor corners (97/334 29.0%) after disinfection. Between the first and third quarters there was a significant decrease in the number of positive sites (25/390 vs 6/256). However, there was no similar change in number of isolates before terminal cleaning.

Conclusion: Persistence of *C. difficile* in the clinical environment was widespread. Although feedback of results did not improve the efficacy of manual disinfection, numbers of *C. difficile* persisting following hydrogen peroxide gradually declined.

Introduction

Clostridium difficile infection is the most common cause of healthcare-associated diarrhoea; potentially leading to costly and life-threatening complications.[1] Spores shed in high numbers by *C. difficile* colonized or infected patients are resistant to some disinfectants and can be difficult to eradicate from the hospital environment by manual cleaning.[2] Spores remain viable for long periods on surfaces and may be a source of infection so reducing environmental contamination by *C. difficile* may decrease the risk of transmission.

Although disinfectants with sporicidal activity may be used during manual cleaning, it can be difficult to ensure staff members are consistent in applying the correct concentrations and ensuring full coverage of surfaces in a clinical setting. Sub-lethal concentrations of disinfectants allow bacteria to persist on surfaces and continued exposure may promote the development of tolerance. If insufficient volume of disinfectant is used on cloths or mops during cleaning, wet contact time is reduced and drying promotes transfer and spread of spores.

At a large teaching hospital, whole room aerial decontamination with hydrogen peroxide vapour (HPV) after patient discharge has been introduced to supplement manual cleaning in an effort to eradicate environmental reservoirs of *C. difficile* spores.[2,3] A sensitive method to detect *C. difficile* in the environment was developed in earlier work.[2] Sampling of surfaces in the patient rooms after manual cleaning (routine/terminal cleaning) and enhanced cleaning (hydrogen peroxide decontamination) was implemented to identify reservoirs of *C. difficile* contamination and direct cleaning towards areas most frequently demonstrating residual spores. Using sponge swabs, it was possible to isolate and quantify the amount of *C. difficile* bioburden on each surface. Where contact plates are limited to 25cm^2 sample areas, sponge swabs allow quantitative sampling of larger areas with greater sensitivity.[4] The purpose of this study was to demonstrate the most common surfaces in the ward environment for *C. difficile* to persist after routine cleaning or

terminal disinfection with and without hydrogen peroxide decontamination with a view to refining cleaning protocols.

Methods

Clinical Setting and selection criteria

At a London teaching hospital, single-isolation patient rooms and patient bed bays were selected randomly when terminal cleaning was requested regardless of the *C. difficile* infection status of the patient just discharged. All rooms and bed bays were selected between 9:00 and 17:00 on weekdays. No other selection criteria were applied. No room or bed bay was sampled more than once after it had been disinfected with aerial hydrogen peroxide. Rooms were cleaned daily at variable times. Sampling was immediately before and after terminal cleaning and hydrogen peroxide decontamination. Patients recovered from diarrhoea but still carrying *C difficile* were defined as colonized.

Decontamination and cleaning policy

All routine cleaning and terminal cleaning was performed manually using microfiber cloths (for surfaces) and microfiber mops (for floor areas) pre-treated with a peracetic acid-based disinfectant (DiffX, MTP Innovations, UK)[5] and prepared in-house by domestic cleaning staff. Both routine and terminal cleaning applied to all reusable equipment, furniture, non-porous surfaces and floors.

Routine cleaning was performed whilst a patient was admitted using microfiber and disinfectant at a concentration of 1000ppm for all surfaces. A higher concentration (3000 ppm) was only used when sporicidal activity was needed (e.g. patient had diarrhoea) to limit cost. Floors were mopped with solution at 750 ppm. A sachet of peracetic acid generating powder (20g) was dissolved in 1-4 litres of warm water as required with a 20 minute dwell time.[5] Equipment, curtains, bed handrail

and frame, furniture, doors, window sills and call bell were cleaned with cloths soaked in the solution. Surfaces were dusted and floors were dry and wet mopped. Spot cleaning was done by nurses with detergent or sporicidal wipes (Clinell, Gama Healthcare UK). On discharge of the patient, terminal cleaning was performed. Peracetic acid cloths were used to clean mattress, bed frame, clean equipment, surfaces, call bell, entertainment system, locker, bed table, furniture, switches, and ceiling vents. Bedding and crockery were removed. The walls were washed and curtains removed (if infected patient) and the floor mopped, dry then wet. If the patient had a known infection due to *C. difficile*, norovirus, vancomycin-resistant enterococci or multidrug resistant organisms, terminal cleaning was followed by decontamination using the Deprox system (Hygiene Solutions, UK), operated by dedicated personnel provided by the manufacturer. Aerial concentrations of hydrogen peroxide were 29-46 ppm at peak and a mean of 3.3 ppm at end of cycle. with mean peak delta relative humidity of 15.4% and a cartridge concentration of 4.9%.[6]

Sampling sites and processing of swabs

Up to 16 sites in patient single-isolation rooms and up to 10 sites in the bed-bay areas were selected representing high-frequency touch sites within and beyond the near-patient vicinity and difficult-to-clean surfaces.

As previously described, surfaces were sampled with sponge swabs (Technical Service Consultants Ltd., UK) pre-moistened with 10mL neutraliser solution (0.1% Sodium thiosulphate, 3.0% Tween 80, 0.3% Lecithin prepared in phosphate buffered saline [PBS]).[3] Sponge swabs were transferred into sterile blender bags (VWR, UK) containing 30mL neutraliser solution and manually homogenised (massaged between the fingertips) for 30 seconds. The solution was passed through a 0.45µm nitrocellulose filter membrane (Advantec, Ehime, Japan) by syringe filtration. Filter membranes were plated onto Braziers selective agar plates (90mm diameter; Oxoid, UK) and incubated anaerobically at 37°C for 48 hours. Presumptive *C. difficile* colonies were isolated using standard microbiology techniques (colony morphology, odour, Gram-stain)

and confirmed using a latex agglutination test (Oxoid, UK). Results of sampling were reported back to facilities staff on a fortnightly basis to highlight which surfaces were most likely to have residual contamination.

Statistics

Means (±standard deviation) were compared using a Chi-squared test . One tailed tests were used for all analyses and differences were considered statistically significant when $P<0.05$.

Results

Over a period of one year, 2,529 clinical sites in 146 single-isolation rooms and 44 bed bays were sampled for vegetative and spore *C. difficile* contamination. In bed areas occupied by known *C. difficile*-infected or colonised patients, *C. difficile* was recovered from 38.9% (83/213) of samples after routine cleaning, 20.6% (56/272) after terminal cleaning and 8.3% (23/276) after hydrogen peroxide decontamination. In bed areas where the *C. difficile* status of the occupying patient was unknown *C. difficile* was recovered from 13.4% (48/359) after routine cleaning, 7.1% (49/687) after terminal cleaning and 2.9% (20/691) after hydrogen peroxide decontamination (Table 1).

The floor corner and bathroom floor were the sites most frequently positive for *C. difficile* contamination after routine cleaning and terminal cleaning. Hydrogen peroxide decontamination reduced the numbers of spores and frequency of *C. difficile* isolation but was less effective on the bathroom floor, where 15.8% (9/57) of surfaces remained contaminated.

Ceiling vents were identified as reservoirs of *C. difficile* with 31.6% (6/19) of sites positive after terminal cleaning. Decontamination of these surfaces was not stipulated within the criteria for routine cleaning with only the exposed surfaces of the vent cleaned as part of the terminal cleaning protocols. All vents were covered and sealed during hydrogen peroxide decontamination to avoid circulation and spread to adjacent areas via the ducting. Despite enhanced-cleaning, *C. difficile* persisted on 33.3% (6/18) of vents. Of note, where single isolation rooms were decontaminated,

C. difficile was isolated on the outer door handles after both terminal cleaning (3.3% [2/61]) and hydrogen peroxide decontamination (4.9% [4/61]).

Efficacy of removal of *C. difficile* was assessed over 4 periods of 3 months in 2013/4 (Table 2). In the first quarter, *C. difficile* was recovered from 30.7% (31/101), 12.4% (43/347) and 6.4% (25/390) of sites after routine cleaning, terminal cleaning and terminal cleaning with hydrogen peroxide decontamination respectively. Following feedback of the commonly contaminated sites to the cleaners, this reduced to 20.9% (28/134), 8.2% (25/304) and 2.3% (6/256) respectively by the third quarter of the year. The reduction in recovery after hydrogen peroxide was significant between the first and third quarters ($P<0.05$). *C. difficile* isolated after terminal cleaning decreased from 12.8% to 8.2% between Apr-Jun and Jul-Sep quarters suggesting an improvement in cleaning technique as there was no change in *C. difficile* after routine cleaning for the same period (Table 2). This coincided with increased training of the cleaning staff on use of warm water to activate the disinfectant, coverage and the surface area to be cleaned by each mop or cloth and the quality of the microfibers, as observation by supervisors showed some deficiencies. The concentration of the solution could not be checked at the time.

The method used for sampling allows quantitative measurement of the amount of *C. difficile* on each surface (Table 3). The most contaminated surfaces by number of colonies were the nurse call button, bathroom floor and floor corner. Terminal cleaning reduced the amount of *C. difficile* on all surfaces with the exception of the ceiling vent. Hydrogen peroxide decontamination further reduced contamination in rooms with recent known colonised patients (Table 4).

During the study, rates of *C. difficile* infections were reported to Public Health England. Stool samples were tested without selection, both formed and unformed. The hospital–apportioned *C difficile* rate per 100,000 patient-bed-days was 37.1 in 2013/4, 40.2 in 2014/5 and 36.2 in 2015/2016. Cancer and medical specialties accounted for 60% of the patients with *C difficile* in the hospital, and this was increasing over the study period.

Discussion

In the healthcare setting, acquisition of *C. difficile* infection is associated with environmental contamination as much as with person to person spread.[7] Previous occupants of the same bed area who were infected with pathogens that survive well in the environment are a risk factor for acquisition of the pathogen.[8] *C. difficile* spores persist in the environment up to five months. [8] A retrospective cohort study demonstrated that even the administration of antibiotics to patients previously occupying a bed increased the risk of the next patient in that bed acquiring *C. difficile.* [9] Improved hand hygiene and source isolation can reduce transmission between patients but *C. difficile* persists in the environment with a wide range of ribotypes present, most likely disseminated by asymptomatic patients.[10] Previous studies in this hospital have shown that hydrogen peroxide systems are effective in reducing *C. difficile* in the environment.[3] Furthermore, the use of hydrogen peroxide or ultraviolet irradiation to decontaminate single-isolation rooms after discharge of the patient is associated with a gradual reduction in incidence of *C. difficile* infections in patients.[11,12] A large cluster randomised study of various types of terminal disinfection across 9 hospitals showed addition of UV-C to standard cleaning reduced the overall chance of a patient acquiring one of the four target organisms from a previous occupant. [13] However, the incidence of *C difficile* infection was not significantly different with or without UV-C devices nor were spore counts affected significantly in 92 rooms that were sampled. The tests were made at 10 sites using 10 Rodac plates each (5 aerobic 5 anaerobic media 125cm^2). For C. *difficile* using general anaerobic media there were only 2.9 to 4.5 mean cfu per room. In contrast the current study used hydrogen peroxide and a more sensitive environmental detection method. The rate of isolation of *C. difficile* from the environment following routine daily cleaning or terminal disinfection did not change significantly between the start and finish of the intervention period, suggesting that feedback of sites requiring additional cleaning to the cleaners had limited

effect. However, there was a significant reduction over time in residual *C. difficile* numbers following hydrogen peroxide decontamination. The number of patients entering the hospital with *C. difficile* was not known as there was no universal screening. Any effect on the incidence of *C difficile* infections was confounded by a rising number of cancer patients admitted.

Many more bed areas contained sites where *C. difficile* was detected than had housed patients known to be carrying the organism. Contamination was found in 38.9% of sites after routine cleaning in patient bed areas occupied by patients known to be colonised with *C. difficile.* In bed areas with no known *C. difficile* patients, contamination was found in 13.4% of sites. After discharge of the patient, rooms were terminally cleaned in preparation for subsequent patients but 10.6% of sites sampled remained positive. Manual cleaning alone, even to the terminal disinfection standard, was inadequate to eradicate environmental contamination by *C. difficile*. The need for monitoring of the environment and the methods used have been extensively reviewed.[14] The main weakness of monitoring methods lies in not knowing the safe level of contamination with respect to preventing transmission.

A weak association has been reported previously between audit and feedback of cleaning performance using fluorescent markers and reduced rates of *C. difficile* infection.[15] Such markers were not used during this study but audit and immediate feedback of cleaning standards by domestic supervisors by direct observation and by using ATP (Adenosine-Tri-Phosphate) bioluminesence (Clean-Trace Clinical Hygiene Monitoring System, 3M Health Care Ltd. Loughborough, UK) had been in use at this hospital for several years.[16] Although not specific to bacteria, this method provides real-time results and indicates the adequacy of removal of organic debris from a surface to below a pre-determined threshold that may be relayed back immediately to the cleaner.

Aerial hydrogen peroxide was effective in reducing the level of contamination after terminal cleaning but had limited effect in areas where shielding occurred or when debris remained. In

some areas such as the bathroom floor, *C. difficile* persisted despite terminal disinfection and use of hydrogen peroxide. Previous studies here have shown uneven distribution of hydrogen peroxide, which may result in lower efficacy in en-suite bathrooms.[2] The hydrogen peroxide decontamination system used in this study consisted of a single unit. Systems are available which combine an hydrogen peroxide vapour generation unit with one or more aeration units to aid distribution. In some cases failures of eradication of pathogens have been reported but direct comparative trials between systems are needed.[17]

Hydrogen peroxide vapour systems, as used in this study, deliver 3-7% hydrogen peroxide with or without silver ions and reduce spores by at least 4 $\log_{10}$ cfu.[18] A micro-condensation hydrogen peroxide vapour systems that uses 35% hydrogen peroxide has been reported to reduce counts by 6 $\log_{10}$ cfu.[19] A trial at this hospital suggested similar efficacy against various pathogens, including *C. difficile* spores between the two types.[3] Another study compared the same vaporizator system and an aerosolizer using hydrogen peroxide and peracetic acid and found similar reductions in environmental load but did not test for *C difficile* spores.[20]

Failures are often associated with physical obstruction. In this study, the bed control and nurse call button were initially left in holders during aerial decontamination. However this may have shielded the posterior surface of the device from hydrogen peroxide. A change in protocol to suspend these items prior to the decontamination process was implemented to improve exposure. Ceiling vents must be sealed during decontamination to prevent leakage of hydrogen peroxide to other areas. Consequently, *C. difficile* spores remained in the vents. Ceiling vents and vent covers must therefore be included in all manual cleaning protocols. If local infrastructure allows, isolating air flow instead of using covers may be beneficial to allow hydrogen peroxide decontamination of vents.

Nurse call buttons were the most highly contaminated surfaces after both routine and terminal cleaning. This high-frequency touch surface, regularly used by patients, may be an important

reservoir of *C. difficile* and highlights the importance of hand hygiene policies for staff, patients and visitors. Whole room aerial decontamination was effective at reducing *C. difficile* on surfaces inaccessible or hard to reach by manual cleaning. However, effective removal of dirt and organic debris by manual cleaning was essential for highest efficacy and may have improved as a result of feedback.

Manual cleaning was often insufficient to remove all *C. difficile* from the environment. Identification of highly contaminated sites led to a temporary improvement in terminal cleaning of affected areas and reduction in *C. difficile* isolated. Removal of soil was important in improving the long term efficacy of hydrogen peroxide decontamination with the aim of reducing the risk of transmission.

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Potential conflict of interest: APRW is on advisory panels for 3M and Merck. SY, SA, MM and AJ have no potential conflicts of interest.

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Table 1. Percentage of sites positive for *C. difficile* contamination after cleaning (number of positives/total number of samples).

		Known *C difficile* patient			**No known *C difficile* patient**		
Sample Sites	**Area sampled/cm^2**	**Routine**	**Terminal**	**Terminal + HPV**	**Routine**	**Terminal**	**Terminal + HPV**
Patient							
Floor Corner	225 - [15 x 15cm]	**85.7%** (12/14)	**57.9%** (11/19)	**0.0%** (0/19)	**37.0%** (10/27)	**27.3%** (15/55)	**7.4%** (4/54)
Bed Rail	180 - [3 x 60cm]	**50.0%** (7/14)	**11.8%** (2/17)	**5.6%** (1/18)	**7.4%** (2/27)	**4.1%** (2/49)	**0.0%** (0/48)
Bed Control	225 - [15 x 15cm]	**42.9%** (6/14)	**17.6%** (3/17)	**11.1%** (2/18)	**3.7%** (1/27)	**4.1%** (2/49)	**0.0%** (0/48)
Nurse Call	50 - [25cm^2 front +back]	**28.6%** (4/14)	**21.1%** (4/19)	**15.8**% (3/19)	**7.4%** (2/27)	**0.0%** (0/55)	**0.0%** (0/54)
Bedside Table	300 - [15 x 20cm]	**57.1%** (8/14)	**22.2%** (4/18)	**0.0%** (0/19)	**7.4%** (2/27)	**5.9%** (3/51)	**0.0%** (0/51)
Chair Arm	150 - [5 x 30cm]	**46.2%** (6/13)	**22.2%** (4/18)	**22.2%** (4/22)	**14.8%** (4/27)	**3.8%** (2/53)	**0.0%** (0/52)
Bin Lid	120 - [10 x 12cm]	**28.6%** (4/14)	**26.3%** (5/19)	**0.0%** (0/19)	**5.0%** (1/20)	**2.0%** (1/49)	**2.1%** (1/48)
Door Handle (inner)	50 - [Whole handle]	**28.6%** (4/14)	**10.5%** (2/19)	**5.3%** (1/19)	**0.0%** (0/10)	**2.4%** (1/42)	**0.0%** (0/42)
Door Handle	50 - [Whole handle]	**0.0%** (0/14)	**10.5%** (2/19)	**15.8**% (3/19)	**0.0%** (0/10)	**0.0%** (0/42)	**0.0%** (0/42)

(outer) Ceiling vent	200 - [10 x 20cm]	**20.0%** (1/5)	**0.0%** (0/5)	**16.7%** (1/6)	**33.3%** (1/3)	**42.9%** (6/14)	**41.6%** (5/12)
Bathroom							
Bathroom Floor	225 - [15 x 15cm]	**64.3%** (9/14)	**52.9%** (9/17)	**17.6%** (3/17)	**45.0%** (9/20)	**23.7%** (9/38)	**15.0%** (6/40)
Toilet Assist Bar	150 - [3 x 50cm]	**42.9%** (6/14)	**5.9%** (1/17)	**11.8%** (2/17)	**10.0%** (2/20)	**5.3%** (2/38)	**2.5%** (1/40)
Toilet Flush	50 - [Entire handle]	**21.4%** (3/14)	**5.9%** (1/17)	**11.8%** (2/17)	**5.0%** (1/20)	**5.3%** (2/38)	**0.0%** (0/40)
Toilet Seat	800 - [10 x 80cm]	**42.9%** (6/14)	**17.6%** (3/17)	**5.9%** (1/17)	**25.0%** (5/20)	**5.3%** (2/38)	**5.0%** (2/40)
Tap Handle	50 - [Hot + Cold]	**28.6%** (4/14)	**11.8%** (2/17)	**0.0%** (0/17)	**5.3%** (1/19)	**0.0%** (0/38)	**2.5%** (1/40)
Door Handle	100 - [Both handles]	**42.9%** (6/14)	**11.8%** (2/17)	**0.0%** (0/17)	**20.0%** (4/20)	**0.0%** (0/38)	**0.0%** (0/40)
Total		**38.9%** (83/213)	**20.6%** (56/272)	**8.3%** (23/276)	**13.4%** (48/359)	**7.1%** (49/687)	**2.9%** (20/691)

HPV = hydrogen peroxide vapour system

Table 2. Percentage of *C. difficile* positive sites over assessment periods after routine cleaning, terminal cleaning and terminal cleaning with hydrogen peroxide decontamination in 2013/4.

Cleaning Protocol	Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec
Routine	**30.7%** (31/101)	**20.8%** (57/274)	**20.9%** (28/134)	**25.0%** (22/88)
Terminal	**12.4%** (43/347)	**12.8%** (30/234)	**8.2%** (25/304)	**10.3%** (21/203)
Terminal + HPV	**6.4%** (25/390)*	**5.8%** (14/243)	**2.3%** (6/256)*	**2.8%** (3/108)

***P=0,03 χ^2 test**

HPV = hydrogen peroxide vapour system

Table 3. *C. difficile* contamination on surfaces after cleaning. Counts expressed as mean colony forming units (CFU) per 100 cm^2 ± standard deviation.

Sample Sites	Routine	Terminal	Terminal + HPV
Patient bed/room			
Floor Corner	3.78 ± 14.71	0.49 ± 3.10	0.74 ± 5.85
Bed Rail	2.62 ± 11.72	0.26 ± 1.43	0.00 ± 0.14
Bed Control	0.89 ± 2.52	0.06 ± 0.27	0.01 ± 0.09
Nurse Call Button	4.34 ± 17.62	1.70 ± 9.50	0.14 ± 0.95
Bedside Table	0.94 ± 3.00	0.04 ± 0.43	0.00 ± 0.00
Chair Arm	2.23 ± 5.37	0.15 ± 0.76	0.04 ± 5.85
Bin Lid	0.03 ± 0.09	0.02 ± 0.10	0.00 ± 0.01
Door Handle (Inner)	0.75 ± 2.15	0.20 ± 1.09	0.07 ± 0.51
Door Handle (Outer)	0.00 ± 0.00	0.10 ± 0.56	0.07 ± 0.43
Ceiling vent	0.11 ± 0.19	0.60 ± 0.27	0.95 ± 0.42
Bathroom			

Bathroom Floor	4.21 ± 10.41	1.16 ± 6.66	0.14 ± 0.55
Toilet Assist Bar	0.55 ± 1.11	0.08 ± 0.48	0.04 ± 0.21
Toilet Flush	0.53 ± 1.83	0.36 ± 2.72	0.18 ± 1.08
Toilet Seat	0.43 ± 1.14	0.09 ± 0.44	0.01 ± 0.07
Tap Handle	1.12 ± 3.44	0.04 ± 0.19	0.00 ± 0.13
Door Handle (Inner & Outer)	2.06 ± 5.29	0.22 ± 1.47	0.00 ± 0.00

HPV = hydrogen peroxide vapour system

Table 4. *C. difficile* contamination in bed areas after cleaning. Counts expressed as mean colony forming units (CFU) per single isolation room (SIR) or bed bay (± standard deviation).

	Routine		Terminal		Terminal + HPV	
	SIR	Bay	SIR	Bay	SIR	Bay
Known *C difficile* colonised patient						
Number	14		17		14	
Mean (SD)	86.9 (98.8)		21.2 (38.7)		7.1 (17.9)	
No known *C difficile* colonised patient						
Number	10	10	33	10	34	9
Mean (SD)	2.9 (4.7)	12.5 (30.0)	4.4 (15.4)	0.4 (1.0)	4.0 (19.0)	0.9 (2.51)

HPV = hydrogen peroxide vapour system