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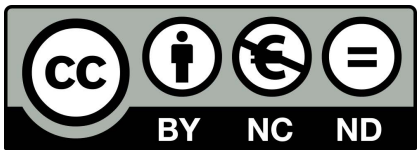

Microbiological evaluation of the ability of the DEKO-190 Washer/Disinfector to remove *Clostridium difficile* spores from bedpan surfaces

Running title: DEKO-190 Washer/Disinfector and *C. difficile*

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Abstract

Background and aims

Clostridium difficile is a major nosocomial pathogen causing mild diarrhoea to life-threatening pseudomembranous colitis, and its spores frequently contaminate hospital environments and equipment. Washer/Disinfectors (WDs) are commonly used to clean and decontaminate soiled equipment in health care facilities. This study aimed to evaluate the effectiveness of the DEKO-190 WD in removing *C. difficile* spores from bedpans.

Methods

Plastic carriers were inoculated with suspensions of *C. difficile* spores in autoclaved (sterile) human faeces. The carriers were then taped to a sterile plastic bedpan which was subjected to short, long or intensive wash cycles in the WD using one of two test detergents : Formula A (generic) and Formula B (highly alkaline). Mean $\log_{10}$ reductions in spores were calculated for each wash cycle.

Results

Mean $\log_{10}$ reductions were 3.21(SEM ±0.20) and 2.82 (±0.13) for Formula A and B, respectively, for the short cycle. The mean $\log_{10}$ reductions using the long wash cycle were 3.65 (±0.44) using Formula A and 5.30 (±0.43) using Formula B, while $\log_{10}$ reductions were 3.37 (±0.58) (Formula A) and 4.64 (±0.47) (Formula B) for the intensive cycle.

Conclusions

Washing with the DEKO-190 significantly reduced spore concentrations on carrier surfaces on a bedpan. Spore counts were most effectively reduced when carriers were washed on a long or intensive wash cycle using an alkaline detergent.

Introduction

Clostridium difficile, an anaerobic spore-forming bacillus, is one of the most significant hospital pathogens in the world. *C. difficile* causes toxin-mediated diarrhoeal disease that can progress to life-threatening pseudomembranous colitis and toxic megacolon. However, it can also be carried asymptomatically in hospitalised patients (1, 2). *C. difficile* infection (CDI) and carriage can result in shedding of spores which can survive for long periods of time measured in months or years and are particularly resistant to disinfectants, making them a frequent contaminant in hospital environments. Since the early 2000s, CDI has increased in both incidence and severity due to the emergence of new strain types, and advances in detection and surveillance (3-5), leading to investigation of ways to improve infection control and prevention, including enhanced cleaning and disinfection practices.

Bedpans are frequently used for hospital inpatients including those with diarrhoeal diseases such as CDI. Washer disinfectors (WDs) are now commonly used to clean and decontaminate soiled bedpans and urinals in health care facilities. If spores survive the cleaning process in WDs, contaminated bedpans could contribute to transmission of *C. difficile* to patients. Evaluating the ability of WDs to remove *C. difficile* spores from bedpans and other surfaces would indicate whether their use could reduce transmission of spores within the hospital environment. However, few studies have evaluated the effectiveness of WDs in removing *C. difficile* spores from contaminated bedpans (6-8). Some have shown effective spore reductions (8), with a recommendation to use alkaline rather than generic detergents (7). *C. difficile* spores have been reported to withstand temperatures within WDs of up to 91°C (8). The aim of this study was to evaluate the DEKO-190 WD (Franke Ltd, Finland) for its ability to remove *C. difficile* spores from strategically placed carriers on bedpans, and the potential for cross infection of other items washed with contaminated bedpans.

Methods

The DEKO-190 WD automatically flushes, washes and disinfects bedpans and lids, urine bottles, hand wash basins, suction bottles, buckets, kidney bowls, theatre overshoes and vases. It complies with all relevant Australian standards, EN ISO 15883-1 and EN ISO 15883-3 and has a Watermark licence, and is capable of maximum thermal disinfection A_0 value of 3000. There are five programmes available which include flush, short, medium, long and intensive washes, consisting of preliminary 5 s rinses, a variable length wash with detergent, a 15 s flush and finally thermal disinfection for 1 min at 91°C (Table 1). The WD was installed and commissioned to requirements as specified by the manufacturer according to reference standards EN ISO 15883-1, -3 (9), and IEC 61010-2-45 (10).

Sterile plastic carriers (75mm×25mm, cut to size from a standard plastic bedpan) were inoculated with 0.2 mL of a suspension of autoclaved (sterile) human faeces spiked with ≥ 1×10^7 colony forming units (CFU)/mL non-toxigenic *C. difficile* spores (*C. difficile* clinical isolate CD062, Ribotype 010 (11)) and dried overnight in a vacuum chamber containing a calcium chloride dessicant. Carriers were then taped to a sterile plastic bedpan, positioned on the interior base surface (n=2), the interior under the lip (n=2) and the exterior side surface (n=1). Sterile negative control carriers (n=1 per cycle) were taped to the interior side walls of the WD. The bedpan and negative controls were subjected to short, long or intensive wash cycles (Table 1) in the WD using one of two test detergents : Formula A (1-10% potassium carbonate, 1-10% tetrapotassium (1-hydroxyethylidene) bisphospohonate, 1-10% disodium metasilicate; pH 12.4) and Formula B (highly alkaline; 10-30% potassium hydroxide; pH 14). The WD was thoroughly cleaned with bleach solution (6000 ppm free chlorine) after each experimental wash cycle.

In addition, 1 mL of spores (≥10^8 CFU/mL) in phosphate buffered saline (PBS) was placed in sealed 2.0 mL microtubes, taped inside the WD and subjected to short wash cycles, to determine the susceptibility of spores to heat within the WD.

Counts of residual spores on carriers were determined as follows. Test carriers and a positive control carrier (*n*=1; inoculated with test carriers, not put through wash cycle) were removed and placed into sterile 50 mL centrifuge tubes containing 20 mL PBS. The tubes were sonicated for 5 min, vortexed for 2 min and then the carriers were removed using sterile forceps. The tubes were centrifuged at 3000 *g* for 5 min and the pellets resuspended in 1.0 mL PBS. Post-wash spore suspensions were serial diluted and spread plated in duplicate onto pre-reduced blood agar plates for enumeration of colonies. The plates were incubated for 48 h at 35°C in an anaerobic chamber (Don Whitley Scientific Ltd, Shipley, West Yorkshire, United Kingdom), in an atmosphere containing 80% nitrogen, 10% hydrogen and 10% carbon dioxide at 75% relative humidity. The experiments were repeated three times and mean $\log_{10}$ reductions in spore counts were calculated for each wash cycle.

Results

Following the short wash cycle (Program 2) in the WD, viable spores were isolated from all carriers. Overall $\log_{10}$ reduction was greater for Formula A compared to Formula B (mean $\log_{10}$ reduction 3.21 vs 2.82, Table 2). Following runs on the long and intensive wash programs, $\log_{10}$ reductions of spores were greater than the short program. The mean $\log_{10}$ reductions using the long wash cycle (Program 4) were 3.65 (±0.44) using detergent Formula A and 5.30 (±0.43) using detergent Formula B. In the case of Program 5 (intensive wash cycle), $\log_{10}$ reductions were similar to Program 4 at 3.37 (±0.58) using detergent Formula A and 4.64 (±0.47) using detergent Formula B.

All sterile negative control carriers taped inside the WD during washing yielded post-wash residual spore counts averaging 4.2×10^3/mL for Formula A and 8.5×10^3/mL for Formula B on the short wash cycle, indicating some cross-contamination from inoculated test slides during the wash. Residual CFU titres were lower for the intensive wash program compared to the long program, averaging 1.1×10^5/mL for long wash with Formula A and 1.3×10^4 for intensive wash with Formula A, and 1.1×10^5/mL for long wash with Formula B and 2.1×10^4/mL for intensive wash with Formula B (Table 2).

For testing of temperature resistance of spores inside the WD, the average titre of spores subjected to wash cycles in sealed microtubes was 1.93×10^6/mL, which was reduced to 6.23×10^5/mL post-wash cycle, giving a mean $\log_{10}$ reduction of 0.51 (±0.15).

Discussion

CDI incidence rates are increasing, which is a major concern for infection prevention and control staff. WDs are being used increasingly on wards to wash and disinfect bedpans and other hospital equipment. It is important that their potential to reduce *C. difficile* contamination of such equipment is investigated, with a view to reducing transmission of *C. difficile* on hospital wards.

The overall median faecal load of *C. difficile* in a study of 203 patients with CDI undertaken by Dionne *et al*. (2013)(12) was 6.67 $\log_{10}$ CFU/g (interquartile range, 5.57 to 7.54 $\log_{10}$ CFU/g). Thus the choice of approximately 7.0 $\log_{10}$ CFU/mL in the test suspension used was similar to *C. difficile* bacterial loads found in real life. As expected, the test spores used in our experiments showed resistance to high temperature, with a $\log_{10}$ reduction of only 0.51 when exposed to the heat conditions of a wash cycle, however spores contained within a suspension may react differently to heat compared to spores dried on a carrier, which is a limitation that

we did not explore further. The maximum temperature in the WD reaches 91°C and is held for 1 min for all wash cycles. Similar results have previously been reported for the DEKO-190 (8), while complete inactivation of *C. difficile* spores has been reported at higher temperatures 116°C for 7 min (6).

While the spores apparently withstood the temperatures within the WD, the action of rinsing and detergent reduced spore counts significantly. Overall, the mean $\log_{10}$ reduction of spores was 3.21 for Formula A and 2.82 for Formula B (Table 2) when washed on the short wash cycle. However, the $\log_{10}$ reduction of spores increased to 5.30 and 4.64, respectively, when the long and intensive wash programs were employed in combination with the highly alkaline detergent Formula B (Table 2), suggesting these are the optimal conditions for *C. difficile* spore reduction for the DEKO-190.

Spores were also isolated from sterile carriers taped inside the machine following wash cycles, suggesting cross-contamination of surfaces and survival of spores within the WD during the wash cycle. For this reason, it would be optimal to clean the WD with an appropriate strength bleach solution after all wash cycles to ensure minimal contamination of equipment with *C. difficile* spores, however this could be a cumbersome task and has the potential for exposure of personnel to *C. difficile* spores.

The $\log_{10}$ reductions estimated here for plastic carriers using the long and intensive wash cycles (5.30 and 4.64, respectively, Table 2) are comparable to those reported by MacDonald *et al.* (>5.76) where plastic bedpans were directly inoculated with spore suspensions and the intensive wash cycle (Program 5) was used (8). Since spore reductions were lower for the short wash cycle (Program 2) in these experiments, it appears that the long and intensive wash cycles, when run with alkaline detergent, are more effective in reducing spore counts than the short wash cycle.

Our results suggest that washing with the DEKO-190 significantly reduced but did not completely eliminate spores on carrier surfaces on a bedpan. Spore counts were most effectively reduced when carriers were washed on a long or intensive wash cycle using an alkaline detergent.

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Ethical approval was not required to perform this research because it did not involve human subjects or specimens.

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Table 1. Details of test wash cycle programs.

Wash program	Phase	Function	Duration
Short wash	1	Rinse, cold water	5 sec
	2	Rinse, mixed water	5 sec
	3	Circulation wash with detergent	1.5 min
	4	Flush, warm water	15 sec
	5	Disinfection at 91°C	1 min + heating
Long wash	1	Rinse, cold water	5 sec
	2	Rinse, mixed water	5 sec
	3	Circulation wash with detergent	3.5 min
	4	Flush, warm water	15 sec
	5	Disinfection at 91°C	1 min + heating
Intensive wash	1	Rinse, cold water	5 sec
	2	Rinse, mixed water	5 sec
	3	Circulation wash with detergent	5 min
	4	Flush, warm water	15 sec
	5	Disinfection at 91°C	1 min + heating

Table 2. Pre- and post-wash spore concentrations and mean $\log_{10}$ spore reductions.

Wash program	Average recovered spore concentration (CFU/mL)					
	Formula A			Formula B		
	Pre-wash	Post-wash	Mean $\log_{10}$ reduction (±SEM*)	Pre-wash	Post-wash	Mean $\log_{10}$ reduction (±SEM*)
Short	4.6×10^{7}	5.4×10^{4}	3.21 (±0.20)	3.3×10^{7}	7.3×10^{4}	2.82 (±0.13)
Long	3.6×10^{7}	3.4×10^{4}	3.65 (±0.44)	3.5×10^{7}	8.5×10^{3}	5.30 (±0.43)
Intensive	9.0×10^{6}	4.1×10^{4}	3.37 (±0.58)	6.4×10^{6}	4.5×10^{3}	4.64 (±0.47)

*SEM, standard error of the mean