

Contamination of hospital linen by *Bacillus cereus*

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SUMMARY

An investigation into two cases of post-operative *Bacillus cereus* meningitis revealed that hospital linen laundered by a batch continuous washing machine was heavily contaminated by *B. cereus* spores. The washing machine, detergents, other chemical additives and the water supply were eliminated as the source of contamination.

It was found that the linen introduced into the washing machine had a high *B. cereus* spore content and that this was still present after the wash process. The spores were not killed by either the heat disinfection stage of the wash or the addition of chemical disinfectants and were not removed by the dilution in the process.

The multiplication of *B. cereus* was thought to have occurred on used, damp linen stored in plastic bags, particularly when ambient temperatures were high. An increase in the water flow through the washing machine was the only measure associated with a decrease in *B. cereus* on laundered linen.

INTRODUCTION

Bacillus cereus meningitis in two patients following neurosurgery led to an investigation into the source of the organism [1]. It was found on some environmental samples and on the hands of staff in the operating theatre but an unexpected finding was heavy contamination of the theatre linen. Freshly laundered linen processed by the same laundry was similarly affected. In contrast *B. cereus* was found in very small numbers on hospital linen washed at three other laundries. Although little is known about the microbiology of clean linen these findings suggested that heavy contamination with *B. cereus* is unusual. This paper describes the laundering operation, methods for sampling linen, possible causes of the contamination at the laundry implicated in this incident and measures taken to eliminate it.

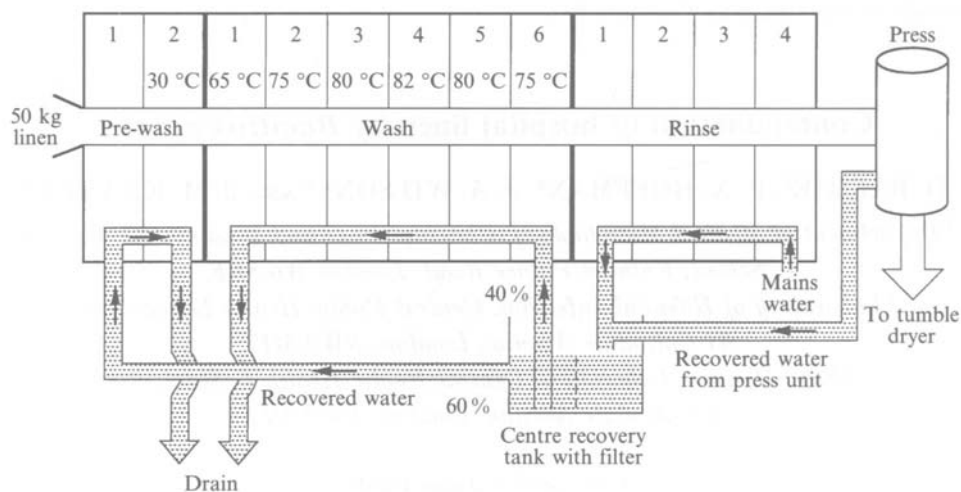


Fig. 1. Diagram of continuous batch washing machine.

THE LAUNDRY

Mains water was fed into a 40000 litre storage tank which supplied two independent plants on the same site, one used solely for hospital linen and the other entirely for non-hospital linen. Ninety percent of the hospital linen was processed in a batch continuous washing machine (BCWM), often known as a 'tunnel washer'. The remaining 10% was washed in washer extractors.

The BCWM (Fig. 1) was a single cylinder machine, of approximately 20 m length and 3 m diameter, divided internally by a spiral blade into 12 connecting compartments. Certain sections of the internal spiral were perforated to allow water to flow by gravity to an adjacent section whilst retaining linen. The machine operated on a counterflow principle, linen and water passing in opposite directions. Linen was agitated by repeated, reversed rotation of the cylinder through 270 degrees, with loads transferred to the next compartment when the cylinder turned a complete revolution. Every time the loads transferred, another 50 kg of linen was dropped into the pre-wash compartment so that, when fully operational, linen was present in each compartment. Each load remained in a compartment for 104 s and required 18 s to transfer to the next compartment. The cycle for each linen load lasted 25 m. Detergent, hydrogen peroxide and an alkali solution were added to the BCWM at various points in the process. After processing through the pre-wash, wash and rinse compartments each load was delivered to a piston press which removed most of the water and then moved by conveyer belt to tumble dryers (Fig. 1).

The basic principle of the BCWM is that water and linen flow in opposite directions, resulting in the commercially advantageous feature of low water consumption for weight of fabric washed. Fresh water, introduced to the BCWM at the final rinse compartment, left it at the first rinse compartment; 40% passed to the last wash compartment then, again flowing in opposite directions to the linen, to the first main wash compartment, finally draining to waste. The remaining 60% passed to the first pre-wash compartment, flowed in the same

direction as the linen to the second compartment from which it drained to waste. The water recovered when the linen was pressed after the final rinse was added to the water leaving the first rinse but comprised less than 10% of the total volume of water used.

MATERIALS AND METHODS

Environmental samples

Samples of water were collected from the mains supply as it left the storage tank (access to the tank was not possible) and from all compartments of the BCWM. The detergent, alkali solution and hydrogen peroxide were sampled from in-use and unopened containers.

Settle plates were exposed for 4 h during linen sorting.

Temperature-sensitive strips (RS Components, Corby) inside samples of linen were used to measure the maximum temperatures reached during the washing process.

Linen

Linen was sampled by impression plates. The fabric was pressed onto blood agar and *B. cereus* selective agar with a sterile-gloved hand. At the hospital, laundered scrub suits in theatre and various articles of linen delivered to the linen stores were sampled. At the laundry, linen was sampled after water extraction by the BCWM and after tumble drying. Test pieces, sterile cotton fabric, one metre square, were sampled after laundering in a BCWM either in a 50 kg load or as the sole item in a compartment.

Sampling was repeated to assess the effect of changes to the BCWM and to the water flow rate. An independent laboratory contracted to the laundry company also carried out routine weekly sampling. Linen laundered in another BCWM on the same site used for non-hospital linen and in BCWMs processing linen in two different laundries was sampled.

Microbiological methods

Blood agar, nutrient agar and *B. cereus* selective medium (Unipath, Basingstoke) were used for primary isolation. Liquid samples, 0.2 ml aliquots, were spread onto blood agar. Plates were examined after 48 h incubation at 37 °C. *B. cereus* was presumptively identified by the characteristic 'ground-glass', irregular edged appearance of colonies on blood agar and by the production of lecithinase and inability to ferment mannitol on the selective medium.

Efficiency of impression sampling

Three centimetre squares of sterile fabric, as used for sterile test pieces (above), were inoculated with 0.1 ml aliquots of serial dilutions of a suspension containing 1.3×10^8 ml⁻¹ *B. cereus* spores. Impressions of the fabric were made on nutrient agar either whilst damp or after drying overnight at 30 °C. To estimate the number of spores remaining after sampling, each square was immersed in quarter-strength Ringer's solution with 0.2% v/v Tween 80 and agitated for 15 min. Serial dilutions were spread onto nutrient agar and counted after 48 h incubation at 30 °C.

Comparison of the number of *B. cereus* colonies on the fabric impression plates with those recovered by immersion in Tween/Ringer's solution indicated the efficiency of the impression sampling method which had been used in the investigation.

Sampling of soiled linen before laundering

Soiled linen, from sealed bags awaiting transfer to the laundry, was sampled using an airborne particle sampler (Cassella London Ltd., Milton Keynes) with a fabric sampling attachment. Approximately 0.25 m² of 49 fabrics from 14 bags were sampled onto 15 cm diameter nutrient agar plates with bacitracin, 18 units ml⁻¹, as a selective agent. Using a single slit, the nominal sampling rate was 175 l/min⁻¹, although impedance by sampled fabric would, in practice, decrease this. The plates were incubated at 30 °C for 48 h.

Assessment of B. cereus on linen introduced into the BCWM

The spore burden of 50 kg loads of soiled fabric entering the first pre-wash compartment was assessed. This compartment received water mainly from the first rinse compartment, with a small amount from the press extractor, and flowed freely to the second pre-wash compartment. Volumes of 20 ml of water entering the first pre-wash and of that leaving the second pre-wash compartment were sampled. One hundred and sixty-three such samples were taken over 4 months. Vegetative bacteria were killed by the addition of phenolic disinfectant (Hycolin, William Pearson Ltd., Hull) to each sample to a final concentration of 1% v/v. After 20 min the disinfectant activity was neutralized by the addition of 2 ml 6% v/v Tween 80, held for 10 min, prior to serial dilution in quarter-strength Ringer's solution and spreading on nutrient agar. Colonies of *B. cereus* were counted after overnight incubation at 37 °C. The difference between the numbers of *B. cereus* spores in water entering and leaving the pre-wash compartments indicated the average number of spores introduced on 50 kg of soiled linen.

ACTIONS TAKEN AT THE LAUNDRY TO REDUCE LINEN CONTAMINATION

Cleaning

As a result of finding *B. cereus* in several compartments of the BCWM and on linen, the machine was cleaned thoroughly; its interior was descaled and steam cleaned. Whilst empty of linen, water was introduced to all compartments, the temperature raised to 82 °C and maintained at that level while the cylinder rotated for 3 h. Hypochlorite was then added to the water and left overnight. Eighteen hours later the water was drained to waste and the machine flushed through with fresh mains water.

Overnight shutdown procedure

At the beginning of each working day, the rinse sections of the BCWM were heat disinfected by raising the temperature of the water to 80 °C and maintaining it for 11 min [2]. At the time of the outbreak water and linen were left overnight in the wash compartments of the BCWM. This practice appears to be widespread,

although not in accordance with current guidance [2]; all eight other laundries contacted had practices identical to the laundry studied. In an attempt to reduce the level of contamination and to comply with Department of Health procedural recommendations, all compartments were drained and left empty of linen and water overnight.

Additives

The amount of hydrogen peroxide added was increased by 25% for one month, new drums of detergent and alkali were brought into use, the practice of topping up the drums was discontinued and the PVC tubing from the drums to the machine was changed.

Water flow

When cleaning, changes in the overnight shutdown procedure and additives did not reduce the observed levels of *B. cereus* contamination on processed linen, the water flow to the BCWM was increased initially by 30% and then by a further 10%.

RESULTS

Environmental samples

B. cereus was not isolated from the mains supply leaving the storage tank or from the water softener. Samples of water taken from all compartments of the BCWM contained greater than 10^3 colony forming units (c.f.u.) ml^{-1} of *Bacillus* spp, predominantly *B. cereus*. Bacteria were not isolated from the alkali or hydrogen peroxide solutions. Detergent from the in-use container supplying the BCWM contained approximately 10^3 c.f.u. ml^{-1} mixed coliforms, but not *B. cereus*. This was attributed to the practice of topping up the same container for a month. No bacteria were isolated from unopened containers of detergent or from in-use solutions after the practice of topping up ceased.

All ten settle plates in the linen reception and sorting area gave semi-confluent growth of *B. cereus*. Due to the large colonial size of *B. cereus*, the maximum number of colonies that could be counted accurately was about 19; any greater numbers were recorded as ≥ 20 .

The temperature sensitive strips indicated that a maximum temperature of 83 °C was consistently attained in the BCWM.

Linen

Results are shown in Table 1. When the investigation began a large proportion of the scrub suits in theatre were heavily contaminated by *B. cereus* as were many articles in the linen store. At the laundry damp linen, including 10 of 12 sterile test pieces, sampled after water extraction and linen after tumble drying were similarly contaminated. Dry linen processed by the non-hospital linen BCWM on the same site and by the BCWM of two different hospital laundries had low levels of contamination.

Heavy contamination persisted after the BCWM had been extensively cleaned, additives increased and the overnight shutdown procedure changed. Samples

Table 1. *Recovery of B. cereus from linen laundered by BCWMs*

Source of linen	Number of items sampled c.f.u./impression plate*			
	0	1-5	6-19	≥ 20
Hospital				
Theatre scrub suits	0	4	7	20
Linen in store	0	3	11	12
Laundry				
At start of investigation	3 (3)	1	2	18 (11)
After changes in shutdown procedure and extensive cleaning	0	0	0	18 (6)
After water flow increase	14 (5)	37 (12)	21 (6)	8† (1)
Three other laundries	74	33	3	1

* Figures in brackets refer to the number of items out of the total which were added to the BCWM as sterile test pieces.

† Mostly *Bacillus* spp other than *B. cereus*.

Table 2. *Efficiency of impression sampling*

Recovery of spores from damp and dried fabric by agar impression and by detergent-buffer immersion

Method	c.f.u. cm ⁻² damp fabric		
	290	230	260
Immersion			
Impression	2.9	3.6	2.7
Recovery by impression method (%)	0.86	1.6	1.0
Method	c.f.u. cm ⁻² dried fabric		
	1400	730	1800
Immersion			
Impression	2.7	1.8	3.0
Recovery by impression method (%)	0.19	0.25	0.17

taken after the flow of water to the BCWM had been increased had lower levels of contamination. When the result of weekly sampling by the independent laboratory contracted to the laundry showed an increase in contamination of the linen, the water flow rate was increased again. This increase in contamination on linen coincided with an increase in *B. cereus* isolates from clinical specimens.

Efficiency of impression sampling

Results are shown in Table 2. The more efficient method of recovering these bacteria from the fabric was by immersion in Tween 80 quarter-strength Ringer's solution and these counts were taken as the total number recoverable from the fabric. The dilutions that gave counts on agar impression closest to those obtained from naturally contaminated linen were the 10⁻³ for damp fabric and 10⁻² for dry fabric. The counts obtained on impression of damp fabric represented around 1.2% of the bacteria recoverable by immersion. When dry fabric was pressed onto the agar, the equivalent recoverability fell to about 0.2%. This implies that 20 colonies on an impression plate represents around 83 c.f.u. cm⁻² from a damp fabric sample; 500 c.f.u. cm⁻² when the fabric sampled was dry.

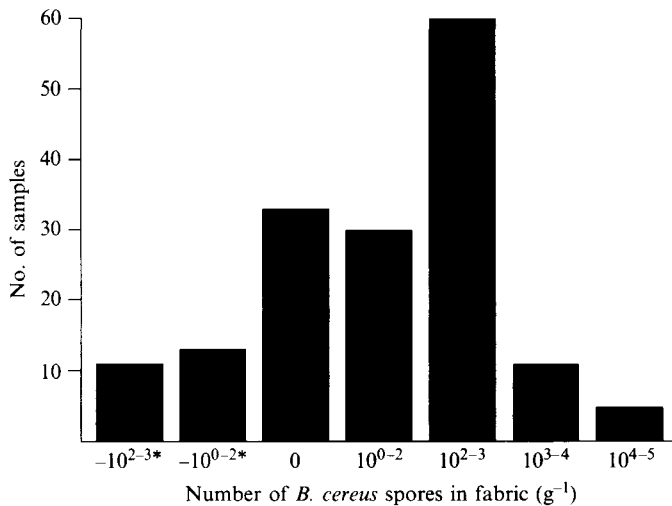


Fig. 2. Assessment of *B. cereus* on linen introduced into the BCWM. Derived from the difference between the number of *B. cereus* spores in water entering and leaving the pre-wash compartments. * Fewer *B. cereus* spores in water leaving the pre-wash than water entering pre-wash.

Sampling of soiled linen before laundering

Forty-nine fabrics from 14 sealed plastic bags yielded an average of 15 discrete colonies (range 0–62) per 0.25 m². In addition, and probably of greater importance, 14 lines of confluent growth were observed, presumably due to the sampling attachment passing over areas of heavy bacterial growth, resulting in very heavy inoculation of the agar under the slit at the time.

Assessment of B. cereus on linen introduced into the BCWM

Results, expressed in terms of spores of *B. cereus* per gram of fabric added to the first pre-wash compartment, are shown in Fig. 2. The overall balance of the results indicated large numbers of spores being added to the BCWM with the linen. In 14 samples there were more *B. cereus* spores in water entering the pre-wash than leaving it. This coincided with a high spore count in the water entering the pre-wash (derived from rinse water), probably eluted from a previous heavily contaminated load.

DISCUSSION

Microbiological examination of laundered hospital linen is not done routinely and there are no generally accepted levels of bacterial contamination. A method of sampling fabric which is reproducible, reliable and practical has not been agreed. Liquid extraction methods give accurate results, but use complicated techniques and fabrics must be cut to obtain samples. Impression plates, used in this investigation, are a low efficiency sampling method [3] but are quick and easy to perform and do not involve destruction of the item. Their accuracy is limited by the number of colonies countable on the agar and by the variation in recovery from damp and dry fabrics.

We demonstrated that the impression plate method recovers around 0.2% of the total spores present on dry fabric. It was not unusual to recover > 20 c.f.u. from an impression plate of a dry scrub suit. Twenty colonies on a standard Petri dish of agar (equivalent to about 20 cm² of fabric) thus represents about 500 spores cm⁻² fabric and around 10⁷ spores on each scrub suit in theatre. Some of these spores will be dispersed on lint during surgery and enter susceptible sites on patients, either directly by falling into the wound or by settling on exposed surgical instruments which are then used in the wound. It has been found [4] that, during hip and knee joint replacement surgery, only 30% of bacteria in the wound had fallen in directly from the air; the remainder were presumed to have fallen onto other surfaces before being transferred to the wound. It could be speculated that during more complex and lengthy surgery, laid up instruments spend more time exposed. The operations on the two patients who acquired *B. cereus* meningitis were of 10 and 6 h duration.

The source of *B. cereus* on clean linen formed the main part of this investigation. It was not found in laundry additives (water, detergent and alkali). Initially, the machine being shut down at the end of the day with fabrics left inside the wash compartments was thought to contribute to the problem. This could have led to overnight fermentation resulting in high numbers of spores by the following morning. Whereas the recovery of large numbers of *B. cereus* from an unemptied machine at morning startup could be expected, they were present 4–8 h into full capacity operation of the BCWM, by which time any present at startup should have been diluted out. However, high numbers continued to be isolated when the BCWM was operated in accordance with current policy [2] and in excess of those recommendations.

Our findings suggest that *B. cereus* spores, present on used hospital linen, multiplied during storage in plastic bags. They were then introduced with the linen to the BCWM and not adequately removed during the laundering process. Linen as the source of *B. cereus* is suggested by the recovery of greater numbers from water leaving, than from water entering, the pre-wash and the isolation of high numbers from bagged, soiled linen sampled before washing. That the contamination arises in areas of high density growth on stored soiled fabrics is supported by finding 'hot spots' of *B. cereus* during vacuum sampling of used pre-laundry fabrics and high numbers on settle plates exposed in the linen sorting area. The *B. cereus* were present as spores, as indicated by their survival in wash compartments at 80 °C and in phenolic disinfectant. High numbers of *B. cereus* were present in water sampled from other BCWM compartments suggesting that contamination passed along the machine. Subsequent redistribution within and between loads in the BCWM is indicated by the presence of the organism on previously sterile test fabrics after their passage through the BCWM whether included in a load or in a compartment by themselves. *B. cereus* in high numbers on damp processed linen at the laundry, and on unused linen stored at the hospital, completes the cycle of contamination. This situation would be more likely to occur when ambient temperatures favour multiplication of *B. cereus* in bagged, soiled linen. It is noteworthy that *B. cereus* meningitis in the two patients which led to the investigation occurred during a particularly hot summer. The number of isolates of *B. cereus* from clinical specimens increased significantly at

the same time [1]. A year later, in a period of high ambient temperatures, linen contamination recurred and clinical isolates of *B. cereus* increased coincidentally.

One of the advantages of BCWMs is low water consumption. This may be a disadvantage in some more water economic machines should microbes not susceptible to heat disinfection be present in numbers too high to be removed by dilution. It may be that increasing water-to-linen ratios is a method of reducing spore contamination on fabric.

There exists a widespread belief that the majority of water is 'recycled'. This is not the case. Fresh water is introduced at the final rinse compartment, passes back through the machine in the opposite direction to the linen and leaves the first rinse compartment; one third is fed to the main wash and two thirds to the pre-wash compartments from which it is discharged to waste. Thus whilst water is used in more than one compartment, it is not reused once it has completed its set passage through the BCWM. It is not therefore in the true sense recycled, with the consequent concentration of microbial contamination that may involve. The only reused water in the system is that from the post-rinse press which is then added to water leaving the first rinse. The volume of this recovered water is low compared to the volume to which it is added and it is dumped with the rest of the water. Only a small proportion will be extracted by the press, so that if it were contaminated, this small proportion of recycled water would be rapidly and greatly diluted.

Used hospital linen is placed in plastic bags which are then sealed. There are likely to be areas on this linen where water and nutrients will favour the growth of bacteria. *B. cereus* has a fast growth rate at a range of temperatures, is motile, produces factors inhibiting competing species and can grow well on minimal nutrients, and consequently is found in wide range of habitats [5, 6]. Under normal circumstances the laundry process can remove low numbers of *B. cereus* entering the BCWM. High ambient temperatures during storage before transfer to the laundry may ensure that *B. cereus* which is present reaches high spore numbers before the linen enters the BCWM. Their removal by the washing process will require dilution greater than that required to produce a visually clean product. This was shown by the reduction in contamination of linen when the water flow was increased by 30%. Subsequently a further increase in water flow rate coincided with a similar reduction.

There was no evidence of a buildup of spores on linen processed by the laundries studied as controls, indicating that the usual levels of dilution used in laundry processes are sufficient to prevent this occurring. The quality control of the laundry process is far greater than could ever, in practice, be achieved in the storage time and conditions of used linen when still in hospitals. We therefore consider that control of the laundry process should be focused on control of such contamination rather than on any factor within the hospital. The findings of this investigation apply only to those hospital pathogens that form heat resistant spores and are acquired from the environment; fortunately these are notable by their rarity.

Microbiological monitoring of linen is impractical on a routine basis but is helpful in investigating hospital cluster of *B. cereus* infection or an unaccountable increased isolation of *B. cereus* or *Bacillus* spp from clinical specimens.

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