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Antimicrobial Activity of Selected Disinfectants Used in a Low Temperature Laundering Procedure for Textiles

Abstract

The aim of this study was to compare the disinfection effect of three disinfection agents (sodium chlorate (I), a combination of peroxyacetic acid and hydrogen peroxide, and hydrogen peroxide alone) in washing procedures at a low temperature (30 °C). The disinfection effect was determined using two indicator bacteria: *Enterococcus faecium* and *Enterobacter aerogenes* which were inoculated on pieces of cotton previously contaminated with artificial sweat to simulate real laundering conditions, and then they underwent the laundering procedures chosen. It was determined that the disinfection effect was appropriate for one of the three disinfection agents at 30 °C for a duration of the main washing of 43 min, after which both bacteria were inactivated. *E. faecium* proved to be more resistant as it survived after 43 min of main washing using two of the three disinfectants chosen. No bacteria were found on the cotton pieces after rinsing neither due to a disinfection effect nor their transfer into the washing bath. Low temperature washing procedures can prove to be effective with appropriate disinfectants, bath ratio, duration of laundering procedure and mechanical action. All disinfectants must be carefully chosen for particular laundering procedures in order to achieve efficient disinfection. This is important for sensitive users of textiles such as children, elderly people, and people with impaired immune systems, especially in hospitals.

Key words: laundering, textiles, indicators, disinfection, chemo-thermal process.

Introduction

The main aim of washing laundry is to remove soils and microorganisms from infected as well as dirty textiles and attain clean, fresh and disinfected textiles ready for use (Fijan et al., 2005; Fijan et al., 2006). Dirty textiles may contain many types of microorganisms that may be pathogenic depending on the origin of textiles. The origins of microorganisms in textiles are various, such as faecal microorganisms on bed linen from old peoples' homes, infected textiles from hospital wards with different pathogenic bacteria, fungi or viruses etc. Besides microorganisms these textiles contain obstinate soils (blood, urine, vomit, disinfection agents). They are laundered in industrial laundries. It is very important that the laundering procedure is efficient enough to destroy all pathogenic and potentially pathogenic microorganisms and at the same time not cause excessive damage to the textiles due to large amounts of effective bleaching and disinfecting agents. Textiles are disinfected using appropriate disinfection procedures such as thermal or chemo-thermal laundering procedures. According to the regulations of the Robert-Koch Institute valid in Germany (Anonymous, 1995), a laundering procedure has a thermal disinfection effect if the following criteria are met: bath ratio of 1:5, duration of at least 10 min at 90 °C or duration of at least 15 min at 85 °C. A disinfection effect is achieved if the number of bacteria is reduced for 5 log steps of 100.000 cfu.

In these and similar regulations there are lists of exactly defined chemo-thermal laundering procedures for individual washing and disinfecting agents (dosage of washing and disinfecting agents, bath ratio, duration of washing and disinfecting, washing temperature etc.). Chemo-thermal laundering procedures are becoming more common due to various reasons (Horvatic, 2002; Lange, 2001; Schmidt et al., 2005):

1. There is an increasing tendency to use clothes made of cotton blends that do not withstand high washing temperatures.
2. Minimising water consumption by using water-soluble detergents that do not need diluted washing baths for good performance and by lowering the washing temperature, thus not needing so many rinsing baths to achieve a cool down effect,
3. At least a 5% minimisation of energy consumption due to the decreased need for heating,
4. Use of non-toxic, biodegradable washing and disinfection agents etc.
5. And finally, decreasing costs in the ever competitive market of industrial laundries.

It is well known that the clothes of health workers, curtains, furniture covers, bed linen etc. (Kniehl et al., 2005; Malik et al., 2006) can become easily contaminated with microorganisms and thus become potential carriers. Therefore, the cleaning and disinfection of textiles and

surfaces in households is also important, especially for workers in healthcare facilities (Malik et al., 2006). Microorganisms can cause the degradation of natural fibres such as cotton or wool by excreting enzymes such as cellulases or proteases. The build-up of these microorganisms is exacerbated if the wash temperatures and/or base detergent formulations used allow dirt residues to build up in the fibres, which encourages microbial growth (Zoller, 1999).

In this research the disinfection effect of different low temperature washing procedures were determined using two indicator bacteria: *Enterococcus faecium*, ATCC 6057, and *Enterobacter aerogenes*, ATCC 13048. 100% cotton pieces were used as carriers for the selected microorganisms in the washing procedures. The cotton pieces were inoculated with artificial sweat as a substrate for simulating human excrements according to the research previously published (Fijan et al., 2007).

Experimental

Microorganism carriers

Previously sterilised (121 °C, 15 min, 2 bar) cotton pieces measuring 1.5 × 1.5 cm were used as microorganism carriers. The cotton pieces were inoculated with 200 mL of artificial sweat prepared according to the standard method (ISO 105-E04, 1987). Characteristics of the cotton fabric are as follows:

Table 1. Description of disinfecting agents used; ^a The commercial names of the disinfecting agents are left out due to the impartiality of the research.

Mark	Composition	Active agent	Dosage ^a , mL	Description
DIS1	10% NaClO	ClO ⁻	10.0	A disinfection agent containing sodium chlorate (I). In addition, a bleaching agent.
DIS2	2,5% CH ₃ COOOH 10% H ₂ O ₂ 2,5% CH ₃ COOH	COOO ⁻ and HOO ⁻	12.5	A disinfection agent containing a solution of hydrogen peroxide, peroxyacetic acid and acetic acid. In addition, a bleaching agent.
DIS3	35% H ₂ O ₂	HOO ⁻	7.5	Basically a bleaching agent containing a solution of hydrogen peroxide with disinfection properties.

Table 2. Description of the investigated parts of the washing program for sensitive blends at low temperatures; ^a MW – main washing, ^b R – rinsing phase, NDIS – no disinfection agent used, only detergent.

Program at 30 °C	Dosage, mL of DISx	Duration of test, min.	Mark
Completed MW ^a (8 L) & R ^b (3 x 9 L)	10 DIS1	81	DIS1 81
	12.5 DIS2		DIS2 81
	7.5 DIS3		DIS3 81
Completed 43 min of MW (8 L)	10 DIS1	43	DIS1 43
	12.5 DIS2		DIS2 43
	7.5 DIS3		DIS3 43
	–		NDIS 43 ^c
First 20 min of MW (8 L)	10 DIS1	20	DIS1 20
	12.5 DIS2		DIS2 20
	7.5 DIS3		DIS3 20
First 10 min of MW (8 L)	10 DIS1	10	DIS1 10
	12.5 DIS2		DIS2 10
	7.5 DIS3		DIS3 10

100% cotton, tabby weave, weight per square meter 190 g/m², thread spacing warp/weft 30 threads/cm.

Bioindicators

The following indicator microorganisms were chosen: *E. faecium*, ATCC 6057 and *E. aerogenes*, ATCC 13048. *E. faecium* was chosen as a thermo-resistant representative of the Gram positive cocci, and *E. aerogenes* was chosen due to being a catalase positive thermo-resistant representative of the Gram negative family *Enterobacteriaceae* (Fijan et al., 2007). The method of preparing the indicator microorganisms for determining the disinfection effect of washing has been described previously (Fijan et al., 2007). A suspension of each microorganism chosen was prepared and inoculated onto the cotton carriers. After drying overnight the concentrations of *E. faecium* and *E. aerogenes* on the cotton pieces were 1,9×10⁷ cfu/per cotton piece and 4,6×10⁷ cfu/per cotton piece, respectively, assessed by serial dilutions and plating on appropriate agars for each microorganism, as noted in section 'Analysis of the disinfection effect of the washing'.

Disinfection agents

Three disinfection agents were used, designated as DIS1, DIS2 and DIS3. A description of the disinfectants is given in **Table 1**.

DIS1 is a disinfectant and bleaching agent based on sodium chlorate (I) (NaClO). NaClO is the most frequently used disinfection agent. The active compound is the chlorate anion (I) ClO⁻, occurring in dynamic acid–base equilibrium with a predomination of hypochlorous acid at pH 6–8. NaClO is active against all bacteria, fungi, viruses and even spores at relatively low concentrations (Zoller, 1999).

DIS2 is a disinfectant and bleaching agent consisting of peroxyacetic acid (CH₃COOOH) in hydrogen peroxide (H₂O₂). Peroxyacetic acid is always in dynamic chemical equilibrium with hydrogen peroxide, acetic acid and water. Bactericidal activity is due to the very strong oxidising power of the peracid, causing destructive changes to cellular enzymes and other cell constituents. It is very effective against gram-positive and gram-negative bacteria, spores and fungi (Zoller, 1999).

DIS3 consists of hydrogen peroxide and is basically a bleaching agent, though it is widely used for disinfection. H₂O₂ is less active than hypochlorite and has no effect on viruses and spores. Certain catalase excreting bacteria can deactivate hydrogen peroxide by using this enzyme to promote the conversion of hydrogen peroxide to water and molecular oxygen. It has a greater effect in combination with other active ingredients (Zoller, 1999).

Washing machine, the washing and disinfecting procedures

A small scale laboratory industrial drum washing machine was used (Gorenje Prological WA 132). The washing program used is shown in **Table 2**. A washing program for synthetic blends at different temperatures was chosen. The loading capacity for this program was 1.0 kg, total water consumption 35 L (main washing 8 L, 3× rinsing with 9 L), energy consumption 0.43 kWh, and the bath ratio in the main washing was 1:8. The total washing program at 30 °C lasted 81 min (main wash 43 min, rinsing 38 min). The water for the main washing (8 L) was heated to 30 °C with a boiler connected to the washing machine. The water was previously softened at a softening plant (WAK 10-KMN-1, Hidrotehnični biro, sp., Maribor, Slovenia).

The cotton pieces with dried microorganisms were washed according to the respective program and taken out at different intervals, given in **Table 2**. The bioindicators were taken out of the washing machine before rinsing to evaluate the disinfection effect, because rinsing would cause the bacteria to be flushed off the carriers and thus not be found on the carriers; however, this would not prove the disinfection effect.

For all the washing programs one commercial detergent was chosen with the following composition: 12% sodium alkylbenzene sulphonate, 2.5% sodium lauryl ether sulphate, 2.5% isopronaol, 3% fatty alcohol etoxylate, 1.5% sodium hydroxide. 10 g of washing agent/2.5 kg of dry linen was used. The washing machine was loaded with 2.5 kg of previously disinfected (10 min at 90 °C) ballast linen (Cotton/PES blend 50%/50%).

Analysis of the disinfection effect of the washing

After each procedure the cotton pieces were taken out and put into 20 mL of

sterile physiological saline solution (0.9%) with Tween 80 and vortexed to release the microorganisms from the cotton pieces. The CFU was assessed by serial dilutions and plating on selective agars as follows: (1) kanamycin-esculin-azide agar was used for determining the CFU of *E. faecium* after incubation at 37 °C for 48 h; (2) VRBD agar was used for determining the CFU of *E. aerogenes* after incubation at 37 °C for 24 h. The average CFU after the incubation periods was used to determine the percentage of inactivated microorganisms (PIM) after the washing procedures and the logarithmic reduction efficiency after washing (RED). These values are calculated according to the following equations (Fijan et al., 2007):

$$RED = \log \frac{\overline{CFU}_b}{\overline{CFU}_a}$$

$$PIM = \frac{\overline{CFU}_b - \overline{CFU}_a}{\overline{CFU}_b} \cdot 100$$

where:

RED reduction efficiency after washing

\overline{CFU}_b CFU on cotton pieces before washing

\overline{CFU}_a CFU on cotton pieces after washing

PIM percentage of inactivated microorganisms in %.

Each experiment was conducted 3 times to ascertain a general state.

Results and discussion

The results are shown in **Table 3** for the bacteria chosen: *Enterococcus faecium* and *Enterobacter aerogenes*. Three disinfecting agents were used: DIS1 sodium chlorate (I) (NaClO), DIS2 - consisting of an equilibrium of peroxyacetic acid (CH₃COOOH), hydrogen peroxide (H₂O₂), acetic acid and water, and DIS3 - only hydrogen peroxide (basically a bleaching agent with slight disinfecting properties). The dosages used are commonly used in industrial laundries (lower dosages would cause insufficient stain removal, whereas higher dosages would cause excessive damage to the textiles). From the results in **Table 3**, it is obvious that after completion of the chosen program at 30 °C for sensitive blends at low temperatures (81 min), no bacteria had survived; however, after completion of the main washing and before the rinsing phase (after 43 min), *E. faecium* had

Table 3. Results of the survival of selected bacteria after laundering procedures using different disinfecting agents; ^a PIM percentage of inactivated microorganisms, ^b RED logarithmic reduction efficiency after washing.

Mark		<i>E. faecium</i>		<i>E. aerogenes</i>	
		PIM ^a ,%	RED ^b	PIM,%	RED
30 °C MW & R 81 min	DIS1 81	100.000	> 7.28	100.000	> 7.66
	DIS2 81	100.000	> 7.28	100.000	> 7.66
	DIS3 81	100.000	> 7.28	100.000	> 7.66
30 °C MW 43 min	DIS1 43	99.9858	3.85	100.000	> 7.66
	DIS2 43	100.000	> 7.28	100.000	> 7.66
	DIS3 43	99.9995	5.28	100.000	> 7.66
	NDIS 43	99.9842	3.80	100.000	> 7.66
30 °C MW 20 min	DIS1 20	99.2632	2.13	99.9999	6.09
	DIS2 20	99.9996	5.36	100.000	> 7.66
	DIS3 20	99.7632	2.63	99.9999	6.30
30 °C MW 10 min	DIS1 10	97.7844	1.33	99.9953	4.33
	DIS2 10	99.9935	4.18	99.9989	4.98
	DIS3 10	98.8263	1.93	99.9326	3.17

survived when there was no disinfectant (NDIS), and when two of the three disinfecting agents chosen were used: hydrogen peroxide (DIS3) and sodium chlorate (I) (DIS1). Therefore, the main washing phase did not succeed in inactivating all bacteria and therefore has no disinfection effect for these two disinfecting agents. In the rising phase, after the main washing phase (81 min), there were no surviving bacteria, thus achieving 100% PIM after completion of the procedure. On the other hand, the disinfection effect for *E. faecium* had already been achieved after 43 min of main washing in a procedure using a combination of peroxyacetic acid and hydrogen peroxide (DIS2). *E. faecium* also showed slightly lower results for PIM for DIS1 (sodium chlorate (I)) after 10 and 20 min of main washing in comparison with DIS3 (hydrogen peroxide), perhaps due to the fact that it is a catalase negative bacteria that cannot produce an enzyme to inactivate hydrogen peroxide. On the other hand, it has been reported that *E. faecium* is resistant to sodium chlorate (I) (Kerns et al., 1995). *E. faecium* also proved to be the more resistant of the bacteria chosen as *E. aerogenes* did not survive any procedure after 43 min of main washing, despite the fact that *E. aerogenes* had survived after all the investigated procedures of main washing after 10 min with somewhat higher results for PIM using sodium chlorate (I) (DIS1) in comparison to those for hydrogen peroxide (DIS3), the possible reason for which is that it can produce an enzyme catalase that inactivates a limited concentration of hydrogen peroxide. It should also be noted that the PIM scales of all the results look very similar since they are all above 97.7%; however, comparing the results

according to the logarithmic RED scale shows that there are in fact differences for example if the initial CFU is 10⁷, then a PIM of 99.000% means a reduction RED of 2 or the survival of 100,000 cfu; a PIM of 99.900% means a reduction RED of 3 or the survival of 10,000 cfu; a PIM of 99.990% means a reduction RED of 4 or the survival of 1,000 cfu, and a PIM of 99.999% means a reduction RED of 5 or the survival of only 100 cfu. This comes to light especially in the case of the testing of MW for 43 min for *E. faecium*. From the results it can be seen that the reduction RED was 3.8 for the test without disinfectant, whereas all the other results have a much higher RED: >7.28 for DIS2 and 5.28 for DIS3, thus achieving a disinfection effect according to RKI regulations, where the disinfection effect is assured when the RED level is higher than 5. The only exception is DIS1, which is sodium chlorate (I), and it has been reported that *E. faecium* is resistant to sodium chlorate (I) (Kerns et al., 1995).

It has also been reported that *E. faecium* can survive laundering temperatures of up to 60 °C (Fijan et al., 2007) and thermal treatment of 1 min up to 85 °C (Kerns, 1995) and that *E. aerogenes* can survive laundering temperatures of up to 60 °C (Fijan et al., 2007). However, in our research, a higher bath ratio was used (1:8) in the main washing phase in comparison to the research conducted by Fijan et al., 2007, where the bath ratio was only 1:3. An additional influence on the results, besides the use of disinfecting agents, was the use of a drum washing machine with a higher mechanical action, achieving higher results of PIM on the cotton pieces due to the combination

of mechanical removal and transfer to the water medium as a factor at lower temperatures. It is, however, normal in laundering procedures that all soils (including bacteria and other microorganisms) are removed and inactivated from the linen by a combination of the following: (1) inactivation by disinfecting agents, (2) mechanical removal from the linen into the bath by the activity of washing agents and the mechanical action of the washing machine, and (3) longer duration of laundering, causing a higher rate of mechanical removal and inactivation by disinfecting agents. Therefore, a stronger mechanical action, higher bath ratios and longer duration of laundering procedures will succeed in removing bacteria from linen, thus achieving higher values of inactivation at lower temperatures along with the effect of disinfection.

Conclusions

From the results we can conclude that the most efficient disinfecting agent used at lower temperatures was the combination of peroxyacetic acid and hydrogen peroxide, achieving slightly higher results of microbial inactivation in comparison with the other two disinfectants used. However, when using lower laundering temperatures, extra caution must be taken to have enough duration of laundering

procedures, appropriate bath ratios and mechanical action, otherwise some microorganisms may survive the laundering procedures.

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