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Assessment of Hygienic Effects of Laundering Using Spectrophotometry

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Authors' contributions

This work was carried out in collaboration between all authors. All authors managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Microbial cleanliness of textiles is an important issue in numerous industry sectors where microorganisms may contaminate manufactured goods and constitute a threat for consumers' health. The most common method of removing contaminants like microbes is laundering. Changes in the manner of laundering, which have taken place in the recent years due to ecological reasons, lead to decreased hygienic effects. The aim of the study presented in this paper was to determine the viability of bacteria in textiles following laundering with the use of the spectrophotometric technique. The tests were conducted on cotton textiles contaminated with bacteria and laundered. The concentration of bacteria was determined by spectrophotometry with the use of AlamarBlue™ dye. The quantitative analyses confirmed the possibility of using spectrophotometric measurements in determining the levels of the tested bacterial species in the extracts from the laundered textiles. It was demonstrated that laundering textiles contaminated with bacteria result in a decrease in their count irrespective of the species.

Keywords: Textile hygiene; microorganisms; laundering; spectrophotometry; AlamarBlue™.

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1. INTRODUCTION

Textile products become contaminated to varying degrees depending the fibre, type of product and conditions in which it is used. As a consequence, such products need cleaning or laundering due to hygienic, aesthetic and maintenance reasons. The primary aim of laundering is to remove various contaminants from the fibre/product. The most common type is laundering in water, i.e. washing dirty textile products in water colloidal solutions with adequately selected active agents, so-called washing agents, in strictly specified washing conditions [1-3]. All types of physical and chemical contamination may be accompanied by microbial contamination which is diversified with respect to the number and type of contaminating species. The main aim of the washing process (in the hygienic aspect) is to eliminate microbes present in textiles [4-8]. Microorganisms belong to contaminants that create a suspension in the washing bath, but the effectiveness of their elimination is based not only on removing them from the surface of the fibre, but also on limiting their viability by adequate selection of washing conditions.

Microbes in textiles may originate from the human skin, food or air, and when they find appropriately humid conditions and nutrients in the form of epidermal cells or other contaminants, they may remain in textiles for a longer period of time. Bacteria, including pathogens, settle on clothing products more often, and mould fungi or dust mites are more frequently identified on industrial textiles [8,9]. Textiles contaminated with microbes may then become the source of reciprocal human-textile infections and thus, contribute to the spread of infections. Therefore, it seems important to focus on selecting the best washing conditions that ensure a high level of microbial elimination from textiles and consequently, a proper level of textile microbial cleanliness [9-11]. A separate issue associated with the washing process is maintaining a proper hygienic level of laundered textiles. Both issues are significant in various industrial sectors, particularly in food, pharmaceutical and cosmetic industries as well as in hospitals and social care facilities since they translate into a proper level of health safety and, in the case of industry, into microbial quality of manufactured products [2,5,7,12,13].

In order to assess hygienic effects of laundering, it is necessary to determine the number of microorganisms viable on textile products following the washing process. This is a difficult task to accomplish since, so far, explicit research methods have not been created. In microbial counts, the following methods are used: turbidimetry, colorimetry, fluorescent method and commonly used culture-based methods [2,12,14,-21]. In the previous analyses, it was demonstrated that spectrophotometric measurements with the use of AlamarBlue™ dye may also be used to determine bacterial concentrations in extracts obtained from contaminated textiles [22]. The aim of this study was to verify whether this method is reliable in determining the number of bacteria that survive on textiles following individual stages of washing.

2. METHODOLOGY

The tests were conducted on 100% cotton textiles with the plain weave that have not undergone finishing processes. Samples with the size of 5 x 5 cm were cut from the textiles and sterilized in an autoclave with the use saturated steam under pressure in the temperature of 121-123ºC for 20 minutes. Subsequently, the previously prepared bacterial suspensions were placed on the sterile textile squares.

Three bacterial species used in the tests includes *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922*, Enterococcus faecalis* ATCC 29212*.* After 24-hours of culturing in the temperature of 37ºC on a broth agar, the bacterial cell suspensions were prepared in a physiological saline with the concentration of 6 x 10^8 CFU/ml, (2 in McFarland's standards). Optical density of such an inoculum was measured with the use of a densitometer by DENSIMAT (bioMérieux, France) [23]. The suspensions of the bacterial strains were placed on the cotton textile samples in the amount of 1 ml per textile square and spread over the surface using a glass spreader. Subsequently, the samples inoculated with bacteria were incubated at room temperature for 24 hours [14,19,21]. The culture test was performed six times for each bacterial strain. Two samples were controls, two samples after incubation underwent the main wash and two samples underwent a complete washing cycle.

Following a 24-hour culture, a part of textile samples inoculated with bacteria were underwent a model washing process. The washing agent used in these tests was a solution of the certified, standard soap manufactured by Enterprises Limited in Bradford (UK) [24]. In accordance with

the recommendations, the preparation contained no optical brightening agents, phosphates or enzymes. The washing bath was a 0.5% standard washing agent solution in the distilled water, and the bath module was 1:50. The laundering process consisted of the main wash and three rinses. The main wash was conducted in the temperature of 40℃ for 12 minutes using "Elan" shaker, type 357 with 50±2 revolutions per minute [13]. Following the main wash, some samples were separately placed in beakers with sterile distilled water and rinsed thrice, each time for 3 minutes, at room temperature by means of manual shaking every 15 seconds [25]. The rinsings were poured out after each stage and replaced with another portion of sterile distilled water in the weight ratio corresponding to the module of the washing bath, i.e. 1:50. Following the washing process, the textile samples were placed in flasks with sterile saline, and the extraction process began. Extraction of bacteria that remained on the textiles after washing and those present on control textiles was conducted in accordance with the previously created procedure [22,23]. Each sample was placed in a separate flask with sterile saline and subsequently, shaken in a type 357 Elan shaker for 6 minutes at 250 rpm.

To determine the number of viable bacterial cells in the suspensions, AlamarBlue™ preparation (manufactured by AbD Serotec) was used which contained a dye - resazurin CAS: [62758-13-8] [22]. For each tested microorganism, the following were examined: extracts obtained from the textiles inoculated with a suspension of given bacteria - control; extracts from the contaminated textiles that underwent the main wash; extracts from the contaminated textiles that underwent a full washing cycle, i.e. the main wash and three stages of rinsing. Moreover, the spectrophotometric measurements were conducted for the samples of the washing bath that remained after laundering the textiles inoculated with bacteria and of water after the last rinsing. To perform the measurements, 3 ml of extracts were collected and placed into measuring cuvettes with the transmittance in the range of the visible spectrum. Subsequently, 0.3 ml of AlamarBlue™ dye was added and, having mixed the contents, the cuvettes were closed with a parafilm and tightly covered with aluminium foil in order to protect the contents from light. The cuvettes remained in the incubator for 24 hours in the temperature of 37℃. The conditions in which the bacteria and dye were incubated were selected on the basis of the literature data and results of the previous experiments [22,19].

The determination of bacterial concentrations in the suspensions obtained after the extraction from the laundered textiles required conducting spectrophotometric measurements of absorbance in the range of 450-650 nm with the use of a MARCEL spectrophotometer, model S330 PRO by MARCEL. The study involved testing the suspensions labelled quantitatively with AlamarBlue™ dye. Based on the absorbance measurements, the amount of dye reduced due to microbes was determined. The absorbance values for the maximum absorption at a light wave of 570 nm for a reduced form and at a light wave of 600 nm for an oxidized form were taken into account. The amount of the dye form reduced by bacteria was expressed in percentage according to the following formula (1) [19,20]:

$$\text{Reduced dye \%} = \frac{(\varepsilon_{ox})^{\lambda 2}A^{\lambda 1} - (\varepsilon_{ox})^{\lambda 1}A^{\lambda 2}}{(\varepsilon_{red})^{\lambda 1}A'^{,\lambda 2} - (\varepsilon_{red})^{\lambda 2}A'^{,\lambda 1}} \times 100 \quad (1)$$

where:

- $(\varepsilon_{ox})^{\lambda 1}$ - molar absorption coefficient of the oxidized from of AlamarBlue™ at 570 nm (= 80.586)
- $(\varepsilon_{ox})^{\lambda 2}$ - molar absorption coefficient of the oxidized from of AlamarBlue™ at 600 nm (= 117.216)
- $(\varepsilon_{ox})^{\lambda 1}$ - molar absorption coefficient of the reduced from of AlamarBlue™ at 570 nm (= 155.677)
- $(\varepsilon_{ox})^{\lambda 2}$ - molar absorption coefficient of the reduced from of AlamarBlue™ at 600 nm (= 14.652)
- A - sample absorbance
- A' - absorbance of the control sample (AlamarBlue™ in a PBS buffer solution)
- λ^1 - 570 nm
- λ^2 - 600 nm

Based on the percentage of the reduced dye calculated in such a way, a concentration of bacteria in the samples was determined. It was read from the reference curves of bacterial concentration depending on the percentage of AlamarBlue™ reduction. The curves for reference bacterial suspensions were prepared in a PBS saline buffer solution. They were determined in the previous studies using the linear regression model [22]. It must be

emphasised that individual bacterial species are characterised by various degrees of dye reduction at the same reference suspension concentration. Therefore, the concentration of a given bacterial species in the suspension was specified on the basis of the calibration curve equation determined for this particular species [22].

3. RESULTS AND DISCUSSION

The studies presented herein aimed at determining the number of bacteria with the use of AlamarBlue™ dye. The spectrophotometric method used in the study is based on the penetration of the dye to bacterial cells where it undergoes a reduction reaction with reductants produced in the metabolic processes that only take place in living microorganisms. The degree of AlamarBlue™ reduction was specified, based on which the concentration of living bacteria was calculated. The assay results for the species tested are presented in Tables 1-3.

As can be seen in the tables, the subsequent stages of the washing process reduced the numbers of all bacteria but in different ways in different species. The number of bacteria detected with the use of spectrophotometry in the control sample suspensions ranged from 35 - 54 x 10^6 CFU/ml for *Escherichia coli* and *Staphylococcus aureus*, but for *Enterococcus faecalis* the number was considerably higher and amounted to 1098 x 10^6 CFU/ml.

The main wash of the samples caused a decrease in the number of detected microbes. This predominantly concerns *Enterococcus faecalis* which showed a nearly 10-fold reduction in bacterial count in the washed samples (Table 3). In the case of *Staphylococcus aureus,* a noticeable reduction was noted (approximately 3-fold reduction), and after washing the samples with *E. coli,* the level of bacteria was comparable to the control samples (see Tables 1 and 2). In the last case mentioned, only after the full washing cycle, an approximately two-fold decrease in the level of bacteria was observed, judging by the dye reduction (Tables 1-3). However, their presence was noted only in the water after rinsing and the concentration of bacteria extracted from the rinsed textiles was too low and the determination of their exact number from the calibration curve based on the degree of dye reduction was not possible (Table 2).

The full washing cycle in *Enterococcus faecalis* caused further decrease in the number of microbes (as compared to the condition after the main wash) (Table 3). The extracts from the rinsed textiles revealed a two-fold decrease in the number of microorganisms compared to the washed textiles. It appears that during rinsing, large amounts of microbes pass directly to the water, in which, even after the last rinsing, approximately 141 x 10^6 CFU/ml of bacteria was detected. Such a level is comparable to the level of bacteria observed on the washed textiles and twice as high as in the rinsed ones.

As for *Staphylococcus aureus,* no influence of rinsing on the decrease in the number of microbes was observed. A reduced number of this microorganism was observed in the extracts from both washed and rinsed textiles. Moreover, in the water following rinsing, the number of bacteria that passed from the textile to the water was below the quantitation level (Table 1). This happened only in the case of *Staphylococcus aureus.*

A spectrophotometric analysis of the washing baths revealed a considerable number of microbes in all tested species. These concentrations were markedly higher than bacterial levels assayed in the control samples, i.e. in extracts from the microorganism-inoculated textiles that were not washed. This high level of dye reduction in the samples of the

Table 1. Concentration of *Staphylococcus aureus* in extracts from washed textiles determined with the use of spectrophotometry

Sample type	Degree of dye reduction [%]	Bacterial count [CFU/ml]
control (sample without washing)	7.6	54 x 10^6
textile following the main wash	3.4	20 x 10^6
washing bath	15.6	116 x 10^6
textile following the full washing cycle	2.6	14 x 10^6
water after rinsing	0.4	–*

** - beyond the limit of quantitation*

Table 2. Concentration of *Escherichia coli* in extracts from washed textiles determined with the use of spectrophotometry

Sample type	Degree of dye reduction [%]	Bacterial count [CFU/ml]
control (sample without washing)	6.2	35×10^6
textile following the main wash	5.3	27×10^6
washing bath	15.1	112×10^6
textile following the full washing cycle	1.9	–*
water after rinsing	4.2	18×10^6

** - beyond the limit of quantitation*

Table 3. Concentration of *Enterococcus faecalis* in extracts from washed textiles determined with the use of spectrophotometry

Sample type	Degree of dye reduction [%]	Bacterial count [CFU/ml]
control (sample without washing)	29.6	1098×10^6
textile following the main wash	4.7	149×10^6
washing bath	33.4	1242×10^6
textile following the full washing cycle	2.8	74×10^6
water after rinsing	4.5	141×10^6

washing bath may result from high density of the suspension, which apart from the bacteria, was caused by the presence of the washing agent solution. In comparison to the remaining analysed samples, this might have caused false readings of the results from the absorbance-measuring device. In this case, the results obtained cannot be accepted as reliable, particularly because the signal recorded by the device coming from the washing bath samples was very intensive in the entire radiation range tested (450 nm – 670 nm). For instance, it amounted to 1.089 at 450 nm whereas in the remaining samples, it was only 0.354 at the same wave length. The intensive signals for the remaining samples were recorded only for maximum dye absorption values, i.e. at 570 nm and 600 nm.

4. CONCLUSION

The quantitative analyses confirmed the possibility of using spectrophotometric measurements in determining the levels of bacterial species in the extracts from the laundered textiles. It was demonstrated that laundering textiles contaminated with bacteria results in a decrease in their count irrespective of the species. The greatest reduction of bacterial count was observed in *Enterococcus faecalis* following the main wash and this effect was intensified after the full washing cycle. However, the spectrophotometric analysis of the washing baths raises certain doubts as the number of microbes was considerably higher than in the control samples. Among others, this might result from the density of the suspension caused by the presence of the washing agent in the analysed samples.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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