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Original Article

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Antimicrobial Effects of the Laundering Process Applied to Household Linens in the Hotels of Makkah City

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ABSTRACT

Public events are confronted with public-health challenges such as the risk of the spread of infectious diseases. Laundering should make clothing visibly clean and reduce the risk of transmission of infectious illnesses, especially during mass-gathering events. The present study evaluates the effectiveness of the laundering process for household linen using detergent either with or without chlorine bleach or with both. The study was conducted on 30 household linen samples of cotton clothes collected from 10 buildings. The antibacterial activity of these textile samples was determined after frequent laundering using detergent, bleach, or both. Results showed that bleach inhibition achieved a higher reduction in the bacterial count when compared to that of the detergent. For Staphylococcus aureus and Escherichia coli, the maximal percentage of bacterial-count reduction was 99.7% and 99.6%, respectively, when both detergent and bleach were used with five-time washes. The bacterial count of S. aureus and E. coli showed a significant difference ($p > 0.05$) versus the frequency of washing. It was concluded that laundry is important in reducing the transmission of bacterial infections via household linen. The inhibition results were more effective when bleach was used with detergent. The best hygienic cleaning of laundering is achieved by a combination of rinsing, detergent, and chemical action.

Key words: *Laundry, Linen, Detergent, Bleach, Bacterial count*

INTRODUCTION

A public event with more than 1000 people during a particular period is called a mass gathering [1]. Sanitation is a key area where strict and prompt actions are required. Annual events are confronted with public-health challenges, such as the risk of the spread of infectious diseases [2]. During these events, buildings should be well-furnished, containing the basic facilities for human habitation. Rooms should have good air conditions and adequate toilets. Linen, blankets, towels, personal clothing, fabrics, and uncleaned washrooms should remain clean and free of contamination [3]. Household linen such as sheets, pillows, and towels may act as carriers for the spread of infection and could be a contact site and component to transmit infection during mass gatherings. This occurs when room members are sharing bed linen or towels. However, reports of accommodation-associated diseases linked to contaminated fabrics are rare [4]. The most commonly found contaminants (microorganisms) on building surfaces and textiles are *Actinobacteria*, coagulase-negative staphylococci, *Bacillus* sp., typical skin flora, *Cladosporium, and Penicillium* [5]. Viruses and fungi are sometimes isolated. These risks need to be appropriately managed following the level of risk. Cleaning and germ control are important in resident rooms. Laundering should make clothing visibly clean and be able to reduce the risk of transmission of infectious illnesses and to control the spread of antibiotic-resistant strains such as methicillin-resistant *Staphylococcus aureus* (skin flora), or multidrug-resistant Gram-negative bacilli such as extended-spectrum beta-lactamase producing bacteria. During laundering, the extent of the microbial contamination of fabrics is determined by three mechanisms: the first is the physical removal by which a substantial proportion of microorganisms is removed during rinse and spin cycles, referred to as dilution. The second mechanism is thermal inactivation by which microorganisms can be killed by heat. The third mechanism is the chemical treatment (inactivate) during which inactivation can be performed by chemical treatment such as detergents (bleach) [6]. The main laundry processes are washing with water and detergents (or chemicals), followed by agitation, rinsing, drying, pressing (ironing), and folding. Washing is often performed at a temperature higher than room temperature to increase the activities of detergents. Many studies reported the effectiveness and synergistic effect of detergency, heat, and chemical inactivation, and how to reduce microbial loads through detachment and dilution for fabrics [7-10]. The present study aimed to evaluate the effectiveness of the laundering process for household linen using either detergent with or without chlorine bleach or with both.

MATERIALS AND METHODS

The study was conducted on 30 household linen samples of cotton clothes collected from 10 buildings during mass gatherings in the city of Makkah, Saudi Arabia. Each sample was collected in isolation in sterile polythene bags to avoid cross-contamination. Each sample was 5 × 5 cm and sterilized in an autoclave (under pressure at 121 ^{o}C for 20 min). Bacterial suspensions of *Staphylococcus aureus* ATCC 25,923 and *Escherichia coli* ATCC 25,922 were prepared to be used in the tests. Bacterial-cell suspensions were prepared in physiological saline with the concentration of 6 x 10^8 CFU/mL using McFarland's standards. The suspensions of bacterial strains were placed in the samples in the amount of 1 mL per textile square and spread over the surface using a glass spreader. The antibacterial activity of these textile samples was determined using the OECD textiles method [11]. Inoculation with 0.2 mL for each sample was performed from a suspension of either *S. aureus* or *E. coli* mixed in the nutrient broth (1/500; [12]. Samples were then incubated for 24 h at 37 °C. Samples were washed 1–5 times using either detergent (commercial Tide detergent) with or without chlorine bleach (antimicrobial agent), or both **(Table 1)**. Laundering temperature was fixed at 40 °C, with the addition of 125 mg/L chlorine bleach after 20 min of each laundry cycle. After drying, individual samples were transferred to an aliquot of sterile distilled water. Six control samples were similarly treated and left unwashed. All samples were then vigorously agitated, and the resulting suspension was cultured for counting colony-forming units using a dilution plate count with trypticase soy agar. Plates were incubated for 24 h at 37 °C, and colonies were then counted. Growth reduction in each bacterial inoculum was measured.

Table 1. Bacterial Count (Log Reduction) After Laundry Washing Using *S. aureus* and *E. coli*

Number of Washes	Log CFU/25 cm^2					
	S. aureus			***E. coli***		
	Detergent	**Bleach**	**Detergent and Bleach**	**Detergent**	**Bleach**	**Detergent and Bleach**
1	5.60	4.30	4.23	5.54	4.04	4.13
2	5.40	4.26	3.93	5.49	3.90	3.80
3	5.28	3.70	3.20	4.70	3.60	2.95
4	4.18	3.04	2.70	4.36	3.00	2.93
5	3.01	2.78	2.17	3.54	2.87	2.0
Control	5.61	4.46	4.70	5.68	4.26	4.43

Statistical data analysis

All experiments were independently carried out. Linear regression analysis was performed to compare the bacterial count of each species with the number of washing times using SPSS 20.0. F statistic and *p* values were calculated (significance was considered when the *p*-value was less than 0.05).

RESULTS AND DISCUSSION

In this study, the antimicrobial effects exerted by laundering 30 household-linen samples were investigated against *S. aureus* and *E. coli*. Generally, using either detergent or bleach, or both, repeating washing could reduce the bacterial count for both bacterial types as seen in **Table 1**, and **Figures 1 and 2**. Results showed that, after one wash, *E. coli* count was reduced to log reduction 5.54, while that of *S. aureus* to 5.60, as shown in **Table 1**, and

Figures 1 and 2. Similarly, five-time washing revealed a higher reduction in *E. coli* log reduction (5.68) concerning the control, while *S. aureus* was reduced to 5.61, as seen in **Table 1**, and **Figures 1 and 2**. In all study groups, the results of bleach inhibition showed a higher reduction in the bacterial count when compared to that of the detergent. For *S. aureus*, the maximal percentage of the reduction in bacterial count (99.7%) when both detergent and bleach were used with five-time washing, while the least (2.4%) was seen when the only detergent was used for one-time washing **(Table 2, Figures 3 and 4)**. For *E. coli*, the maximal percentage of the reduction in bacterial count (99.6%) was when both detergent and bleach were used with five-time washes, while the least (27.1%) was seen when the only detergent was used for one-time washing **(Table 2, Figures 3 and 4)**. The linear regression analysis of the bacterial count of *S. aureus* and *E. coli* versus the number of washes showed a significant difference in the two slopes ($p = 0.001$), and the F statistic value was 7.181.

Table 2. Reduction Percentage in Bacterial Count after Repeated Laundry Washing Using Detergent, Bleach, or Both

Number of Washes	Reduction in CFU/25 cm^2 (%)					
	S. aureus			*E. coli*		
	Detergent	Bleach	Detergent and Bleach	Detergent	Bleach	Detergent and Bleach
1	2.4	31.0	66.0	27.1	38.9	50.0
2	39.0	37.9	83.0	35.4	55.6	77.0
3	53.7	82.8	96.8	89.6	77.8	96.7
4	96.3	96.2	99.0	95.2	94.4	96.9
5	99.7	97.9	99.7	99.3	95.9	99.6

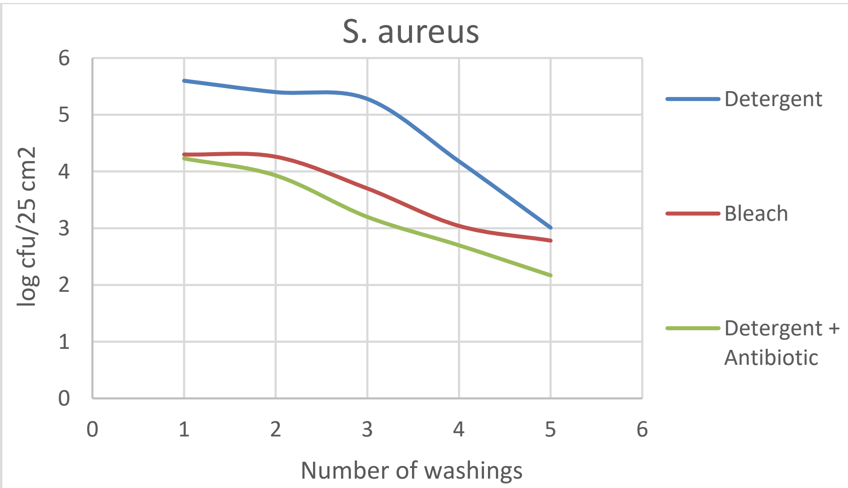

Figure 1. Log Reduction in Bacterial Count of *S. aureus* (Log CFU/25 cm^{-2}) after Repeated Washes Using Detergent, Bleach, or Both

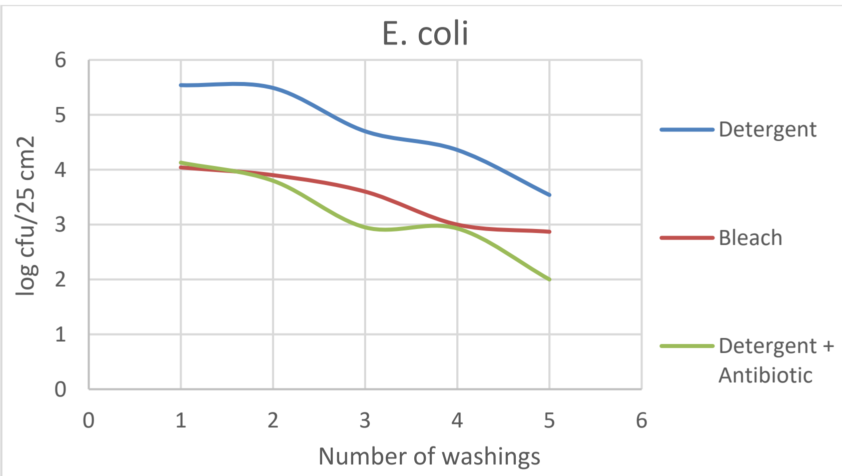

Figure 2. Log Reduction in Bacterial Count of *E. coli* (Log CFU/25 cm^{-2}) after Repeated Washes Using Detergent, Bleach, or Both

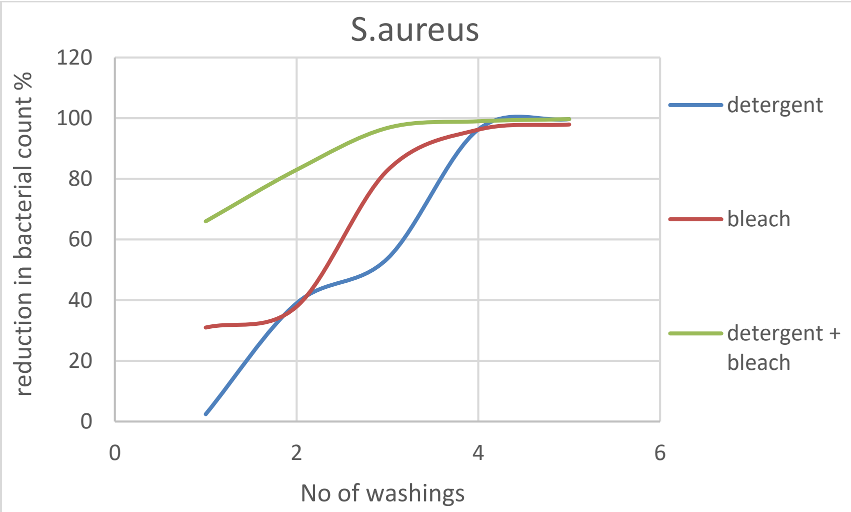

Figure 3. Reduction Percentage in Bacterial Count of *S. Aureus* after Repeated Washing Using Detergent, Bleach, or Both

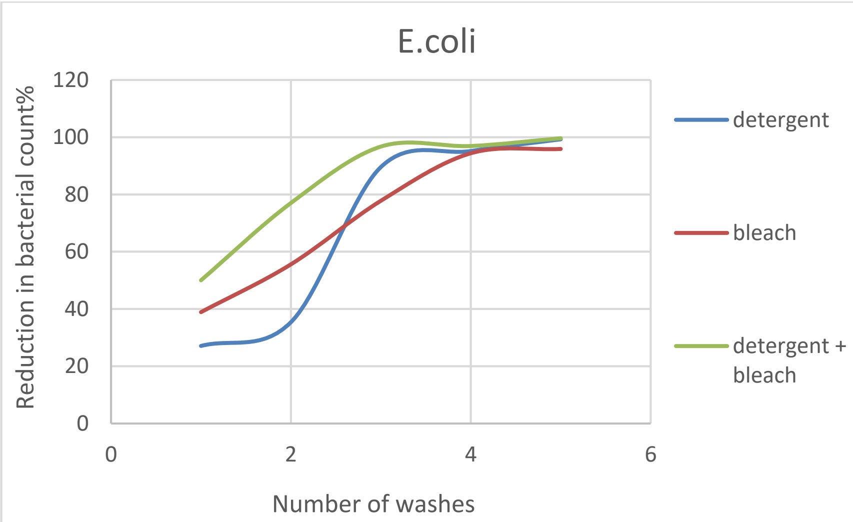

Figure 4. Reduction Percentage in Bacterial Count of *E. coli* after Repeated Washing Using Detergent, Bleach, or Both

A significant method for reducing the spread of infectious diseases is the re-evaluation of current practices, and hygiene improvement in home and everyday-life settings. To avoid infection transmission via clothing and household linen, it is very important to assess the microbial contamination on household linen during use. The present study evaluated the decontaminating efficacy of a laundering process in pilgrim buildings in Makkah. About 30 samples of cotton clothes were collected from 10 pilgrim buildings in Makkah, Saudi Arabia during Hajj 1438 (September 2017). The present study showed that by using detergent, bleach, or both, repeated washing could reduce the bacterial count after one wash; *E. coli* count was reduced to 5.54 (log), while that of *S. aureus* to 5.60 (log). Five-time washing revealed a higher reduction in *E. coli* count (log) 5.68 concerning the control, while the bacterial count (log) of *S. aureus* was reduced to 5.61, as seen in **Table 1**, and **Figures 1 and 2**. Linear regression analysis of the bacterial count of *S. aureus* and *E. coli* versus the number of washes showed a significant difference in the two slopes (p = 0.001), and the F statistic value was 7.181. The antimicrobial action of washing is controlled by the frequency of washes (dilution), detergents, water heat, and chlorine bleach. Excessive washing (dilution) reduces contamination on fabrics during laundering [13, 14]. Gibson and colleagues estimated a 90% reduction in Gram-negative bacteria through "normal" laundering compared with a 99% reduction through the use of a sanitizing detergent [15]. Hygienic quality improvement can be achieved through detergents with bleaching agents. The present study revealed that, in all study groups, bleach inhibition achieved better bacterial reduction than that of the detergent. This result is similar to those of many researchers, who proved that the improved effectiveness of laundering can be significantly increased by the inclusion of a detergent [16-18].

Chlorine bleach has been used for a long time in laundering on both bleaching action and disinfectant activity. One of the negative effects of bleach is that it can trigger allergies and be toxic to the environment [19, 20], which is why bleach treatment is sometimes avoided. Insufficient laundering, on the other hand, cannot eliminate contamination, and pathogen transmission may then occur [21]. A previous study showed that two-thirds of the tested samples (68%) had no antibacterial effect after 10 washing cycles, and 28% of the samples did not show any effect before washing [12]. Bockmuhl and colleagues concluded that low concentrations of hypochlorite used for a short amount of time did not affect flammability after 20 washes, while high concentrations of hypochlorite used for a long time resulted in a loss of flame retardancy after 6 washes [22]. The addition of chlorine bleach to machine laundry cycles was successful in reducing bacterial counts in both hot and cold water, and its addition during laundering similarly reduced bacterial counts in the two temperature processes [23].

The maximal percentage of the reduction in *S. aureus* was 99.7% when both detergent and bleach were used with five-time washes, while the least (2.4%) was seen when the only detergent was used for one-time washing **(Table 2, Figures 3 and 4)**. The maximal percentage of the reduction in *E. coli* count was 99.6% when both detergent and bleach were used with five-time washes, while the least (27.1%) was seen when the only detergent was used for one-time washing **(Table 2, Figures 3 and 4)**. A similar study indicated that effective laundering processes are key to preventing the spread of all staphylococcus strains, including Methicillin-resistant Staphylococcus Aureus (MRSA), in crowded households [24]. Using a range of bacterial species, including both Gram-positive and Gram-negative bacteria, many studies showed that log reduction after laundering at a low temperature (22 °C) could be increased by five or six times after the addition of 114–125 mg/L chlorine [6]. The data presented in this study indicate the importance of laundry in reducing the transmission of bacterial infections via household linen. The results of inhibition were more effective when bleach was used with detergent. The best hygienic laundry cleaning is achieved with a combination of rinsing, detergent, and chemical action.

CONCLUSION

The results of inhibition are more effective when bleach is used with detergent. The best hygienic laundry cleaning is achieved with a combination of rinsing, detergent, and chemical action.

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