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Hygiene aspects in domestic laundry

Summary

The laundering of textiles as one major aspect of home hygiene is considered important by the consumer not only because of its role in the prevention of infections, but mostly due to esthetic reasons. In this context, the understanding of how microorganisms colonise fabrics and how the washing process can eliminate those contaminations, is a crucial step to prevent adverse secondary microbiological effects, such as malodour or infection risks. However, the factors influencing both microbial colonisation and subsequent removal during laundering are complex, substantiating the necessity for test methods that allow a realistic evaluation of the antimicrobial efficacy of the washing process.

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Introduction

When textiles that have been worn or used in the household are washed this is done for various reasons. On the one hand, it is done to remove visible soils and stains and, on the other hand, to eliminate in the washing process malodours accumulated while using the textiles. In addition to these two aspects the consumers' desire to assure hygienic processing of laundry has to be considered.

As per its definition, hygiene entails measures aimed at infection prevention and health promotion, and while elimination of bacteria contributes to the cleanliness of textiles, it is not the only factor implicated here. So what role does the antimicrobial performance of a washing process play in the household?

While in recent decades somewhat less emphasis has been put on the prevention of infections, in the future this could be ascribed even more importance in view of the

rising numbers of persons being cared for in domestic settings as well as because of the emergence of new pathogens. In any case, secondary hygiene effects, such as the elimination of malodours, is a key demand made by consumers on the washing process. In this respect consumers are aware that a reduction in the washing temperature will possibly affect the hygienic washing performance, but they expect modern detergents to rise to this task anyway [1].

Apart from the issue of the degree of antimicrobial activity that a domestic washing process actually needs to, first of all, reduce as far as possible any infection risk posed by contaminated textiles and, second, to solve aesthetic problems, such as elimination of malodours, the main emphasis must be on the mechanism of action. While the removal of pathogens from textiles and prevention of transmission of these microbes to other textiles during the washing process demands for a disinfectant action (i.e. reduction in the number of microorganisms present to a non-infectious level), microbistatic action is needed to eliminate malodorous substances and prevent their re-occurrence. Accordingly, for the antimicrobial mechanism of action to be able to meet these different demands it is not just special solutions that are needed (such as detergent formulations or specific features of the washing machine) but above all a suitable test methodology to verify antimicrobial efficacy.

With regard to the antimicrobial factors unfolding during the washing process, essentially two aspects must be borne in mind here: first, the growing market share of liquid, bleach-free detergents [1, 2] and, second, the ongoing trend towards low-wash temperatures [3]. The latter mean that not only is the physical component 'temperature', being assigned a minor role in reducing the microbial count, but they also prevent adequate activation of percar-

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bonate bleaching agents in powder and granulated detergent formulations, which normally have a good antimicrobial activity as from 40°C.

Microorganisms on textiles

In order to be able to take well-targeted measures for laundry hygiene, it is vital to identify and understand its basic concepts. In this respect microorganisms play a pivotal role both in terms of the way infections can be spread and malodours caused. In principle, there are two main sources of the microorganisms found on textiles: first, the human body with its resident and transient microbiota as well as possible pathogens and, second, the environment as a source of ubiquitous water- and airborne microbes. Besides, domestic pets are also a potential reservoir of the microorganisms found on textiles, to the extent that interaction with these animals can give rise to contamination of household textiles.

The microorganisms belonging to the human microbiota spread to textiles are primarily microbes that colonise the skin and mucosa, and the nature and quantity of such a microbial community can vary in accordance with the respective skin region. The principle genera found on large parts of the skin are *Propionibacterium*, *Corynebacterium*, *Micrococcus*, *Acinetobacter* and coagulase-negative species of *Staphylococcus* as well as the yeast *Malassezia* [4]. Other common transient bacteria include *Bacillus* species, pseudomonads, *Staphylococcus aureus* and *Enterobacteriaceae* [5]. The axillae (armpits) are regions with a particularly high microbial density, where the total microbial counts can be up to 10⁶ colony forming units (cfu) per cm² [6]. The predominant bacteria found in the axillae are staphylococci and coryneform bacteria, with the microbial count of the latter being correlated with the intensity of the odour emitted in axilla perspiration [7]. Apart from that, development of sweat odour is a thoroughly complex process that is not yet fully understood, with the bacterial species of the armpit involved in different ways in generating these diverse odour components.

While the majority of microorganisms colonising the skin, and in particular the potential contaminants belonging to the mucosa flora or faecal bacteria can be classified as potential pathogens and assigned to risk group 2, and some also to risk group

1 [8,9], the risk of infection posed by textiles contaminated with bacteria belonging to the resident microbiota is deemed to play only a minor role here since, after all, we are constantly in contact with these microorganisms. Accordingly, only those persons at increased risk for infection, e.g. because of an impaired immune system, face a real potential threat from contact with members of the resident cutaneous microbiota. While textiles can no doubt serve as a 'vector' by virtue of the fact that microbes colonise them and can even reproduce under favourable conditions, the health risks emanating from contamination with these microorganisms via textiles must be viewed as negligible. This hypothesis is also corroborated by the experimental findings of Lichtenberg et al., who concluded that textiles harbouring normal everyday soils can be rendered hygienically clean even at low temperatures when using a bleach-free detergent [10]. But things are different when it comes to facultatively or obligatorily pathogenic microorganisms of the transient microbiota and pathogens that can gain access to clothing and textiles through direct contact with the human body. Noteworthy in this respect are dermatophytes and other pathogenic fungi such as *Candida* or bacterial pathogens, such as *S. aureus* (in particular persons colonised with MRSA can be at special risk as regards laundry hygiene since especially *S. aureus* is not necessarily effectively inactivated under normal household conditions, as recently demonstrated by Linke et al. [11]), or the causative organisms of gastrointestinal infections, e.g. noroviruses. If such an infection occurs in a private household one must ensure that the implicated aetiological agents are reduced during the disinfectant washing process to a non-infectious level to prevent the possibility of reinfection of members of the household or cross-contamination of hitherto uncontaminated textiles. Lending credence to that belief, Ossowski and Duchmann demonstrated that dermatophytes are not effectively inactivated at temperatures below 60°C [12].

Despite the, in the meantime, accretion of knowledge of microbial colonisation, one can really only speculate on the actual microbial load, in a qualitative and quantitative sense, since there are few data available on this topic. Likewise, there has been little research into the relationship between the composition of textiles and the propensity of microbes to adhere to them. In this

respect, Teufel et al. demonstrated that growth of bacteria that had been isolated from human sweat on polypropylene was lower than on the other materials studied (cotton, polyamide, polyester) [13]. But since mixed cultures of bacteria were studied here, no conclusion can be drawn as regards the implications of different kinds of textiles on the adhesion and growth of different species. This is all the more true since the authors found, at least in the case of cotton, major differences between the perspiration samples from male and female subjects.

In another study McQueen et al. found evidence that the textile type played a role in the intensity of perceived perspiration odours, with the odour being more intense on polyester compared with cotton, but the odour intensity was not correlated with the number of adhering bacteria [14]. The challenge posed by these analyses, no doubt, derives from having to take account of multiple influence factors: on the one hand, different bacterial species generate different odour components, which, on the other hand, do not necessarily adhere to an equal extent to textiles. Moreover, it is unclear whether olfactory substances deemed to be unpleasant are always formed already in the armpits or whether bacterial metabolism of sweat components takes place on the textiles.

Microorganisms in the washing machine

Besides the microorganisms gaining access to clothing and textiles through direct contact with the human body, ubiquitous microorganisms represent the second important source of microbial contamination of textiles. Depending on how exactly these textiles are used, airborne microbes must first of all be taken into account, often mould spores in dust or Gram-positive micrococci, but also microorganisms from other sources, such as the earth, foodstuffs or body fluids [15]. Another, and possibly more important, reservoir is the washing machine itself where in the course of use microorganisms can accumulate in the water-conveying areas and can then be transmitted to textiles during the washing process. Investigations of domestic washing machines have shown that microbial biofilms can be formed at various sites, such as the rinsing chamber, seals or washing solution drain [16]. Bacte-

ria were found in virtually all biofilm samples, and fungi in around 60 % of cases. The latter consisted of both filamentous fungi (moulds) and yeasts. The main bacterial isolates were alpha-proteobacteria, a subgroup of Gram-negative bacteria, suggesting that the cleaning water used was the main source of the bacteria found in the machine. While no reliable data are available to elucidate to what extent textiles harbour microbial contamination from the washing machine after washing, when assessing the antimicrobial performance of a washing process it must be borne in mind that microbes gain access to textiles not only through direct contact but also within the washing machine.

Antimicrobial influence factors

While cumulative effects cannot be ruled out, it can essentially be concluded that the effects unfold in the course of a circuit that begins and ends with the wearing of the clothing (or when using the textiles in another way, e.g. as bed linen, towels, etc.). The components in this cycle and their exact role in reducing the number of microorganisms should be explored separately in order to be able to better estimate the overall hygienic quality of textiles.

Textiles

Apart from their role as the carriers of microbial contamination, as outlined above, textile materials can also be endowed with more or less antimicrobial activity. In this respect, attention must be paid first of all to the material properties, which, in particular based on the water binding capacity, depend on the type of fibres and how they are processed to form surface constructions such as fabrics and knitwear. This also depends on the chemical structure of polymer. It must be borne in mind that a hydrophilic molecule can possibly bind more water, but that actual adhesion of microorganisms to surfaces often takes place through hydrophobic interactions, hence no direct conclusion can be drawn from hydrophilic properties and microbial adhesion. But it is possible that the textile type exerts not only a quantitative effect on adhesion of microorganisms, since in the already mentioned study by Teufel et al. there is evidence that the diversity of adherent bacterial species is markedly less on

cotton and the cotton-like material Tencel® (Lyocell) [13].

However, a far greater impact can be exerted on the antimicrobial activity through subsequent refinement of a textile material compared with any effect arising from its inherent properties. Thanks to their claim of being able to protect against the generation of odours, such refined textiles are used to manufacture textile products for the end consumer as well as for use in the medical setting, e.g. to prevent the spread of pathogens via the clothing of healthcare workers or in wound dressings to prevent infection.

Myriad substances are used to endow textiles with antimicrobial properties, e.g. the most commonly used in the meantime is silver in solubilised or colloidal form, but also other substances such as quaternary ammonium compounds, biguanides, chitosan or triclosan [17]. As regards the overall antimicrobial activity it is important to take account of the specific mechanism of action of the chemicals involved, which tend to more be microbiostatic than microbicidal. However, antimicrobial textile refinement can continue to be effective even after the textiles are washed, thus conferring a certain 'long-term protection', something that should be borne in mind when evaluating microbial reduction.

Detergent agents

Unlike the substances used for refinement of textile materials, the constituents of detergent agents are generally active only for the duration of the wash programme. The most important antimicrobial agent is the percarbonate-based bleaching agents used in powder or granulate form in standard full detergents, which in most cases are not used because of their microbicidal efficacy but rather primarily to remove stains amenable to bleaching. Nonetheless, this percarbonate bleach is the reason why modern bleach-based full detergents are virtually endowed with disinfectant action as from 40 °C [18]. In many countries in which the custom is to use bleach-free detergent formulations, chlorine bleach added at a later stage fulfils the same purpose. Even without bleach the microbial count can be reduced during the washing process thanks to the detergent chemical agents, and this reduction is thought to be in the region of between two and three orders of magnitude [10, 19]. However, under these conditions transfer of microorganisms to previously uncontaminated textiles has been ob-

served, hence the use of bleach-free detergents is not recommended at low temperatures in cases where hygienic laundry processing is needed to prevent the spread of infections in private households (such as in the case of dermatophyte infections or diarrhoea of microbial origin). Otherwise, the antimicrobial activity generated by detergents varies, of course, in accordance with the microorganisms implicated. In this respect there are some bacterial species that merit special attention because of their high resistance to a bleach-free washing process at low temperatures and which, because of their virulence properties, are theoretically capable of giving rise to infection in such a situation. These include fungal species such as dermatophytes and *Candida* [12, 20], and especially non-enveloped viruses. Whereas enveloped viruses such as influenza virus can be effectively inactivated by surfactant action, only high temperatures and bleach can inactivate non-enveloped viruses [21, 22, 23]. Here, heat-resistant bacteria such as enterococci or *S. aureus* must of course also be taken into account. This holds true in particular in the case of highly infectious pathogens such as noroviruses, which are difficult to inactivate using other disinfectants and which pose a real risk of infection if harboured by inadequately decontaminated textiles [24].

Because of extensive dilution during the washing process, few substances are in principle able to exert an antimicrobial activity in the wash liquor. Apart from the already discussed bleaching agents, other agents of note are in particular quaternary ammonium compounds which, however, are rarely used in detergent formulations because of their lack of compatibility with anionic surfactants. But this substance group is commonly used to confer antimicrobial activity on additive agents used for laundry ('hygienic rinse agents'), whereby they are added after the main wash and, as such, do not come into contact with the anionic surfactants. While the performance of these products cannot be compared with that of bleach-based formulations, they are an alternative solution for textiles that may only be washed at low temperatures and without bleach, but are microbially contaminated. Here the benefits should be carefully pondered against possible intolerances caused by residues of ammonium quaternary compounds on the textiles.

Washing machines

Even without the chemical action of a detergent, the temperature effect unfolding during the washing process and exerting a physical influence on microorganisms, in addition to any bleach-related activity, must be taken into consideration. Mechanical forces are also generated on the microbial cell in the washing machine, which for example by means of a high rinse rate can further reduce the microbial count. Terpstra et al. demonstrated something along those lines by showing that certain country-specific effects were attributable to the physical characteristics of the machines used in the various countries [19]. The plethora of machine types in use, together with their variety of programmes, as well as the amount of water used and the actual temperatures reached make it difficult to obtain comparable data on the antimicrobial performance of a washing process. This is all the more challenging when one considers the possible combinations of different detergents. In terms of the machine-specific characteristics endowed with a microbe-reducing effect, in addition to the temperature, mechanical action and water quantity, there are other peripheral features that deserve to be mentioned, such as the use of steam or the release of silver ions. However, such machine types are niche products and as such will not be further considered here.

Drying

When assessing the antimicrobial action of a washing process, sampling is done in general from moist laundry from the machine. While in scientific terms this constitutes a logical approach when trying to compare the performance of detergents, this method does not really reflect the reality. Unfortunately, there is a paucity of studies to demonstrate how the drying process affects contaminated textiles. But one would expect that in principle many Gram-negative bacteria are inactivated by drying, but that the reduction in water activity alone is not enough to reduce sufficiently the microbial count of all relevant microorganisms, including fungi. But things are different if the textiles are tumbled, where the temperature effect is exerted in addition to water removal.

In any case for a realistic estimate of the infection risk emanating from washed laundry, all factors exerting an effect on the textiles during the washing process should be

borne in mind. These include, in addition to the detergent effect, in particular the type of drying and the mechanical effects.

Methods for detection of antimicrobial efficacy

The numerous factors that can contribute to reduction of microorganisms during the washing process and additional treatment steps, such as drying the laundry, inevitably raises the question as to how one can predict these effects as precisely as possible using experimental methods. To gain an insight into the problems related to the topic of the mechanism of action of domestic washing processes, only the microbicidal aspect of the washing process shall be explored in the following. A more detailed account of the methods used to determine the antimicrobial action and the requirements addressed to disinfectants are given in [27].

When testing disinfectants in accordance with the pertinent standards, in general a three-phase approach is used, as can also be employed in principle for assessment of the antimicrobial performance of detergents (Table 1).

Phase 1 comprises orientation tests during which a microbial suspension is incubated over a particular period of time together with the disinfectant. At the end of this period, the number of viable microorganisms is determined in the form of colony forming units (cfu) and a (logarithmic) reduction factor is ascertained by comparing this with the baseline microbial count.

The suspension tests conducted in Phase 2, Level 1, are essentially performed in the same manner, but now using a broader spectrum of microbes and an additional organic challenge substance (bovine serum albumin). Besides, at this juncture the standard stipulates that extensive controls be used to elucidate the validity of the method.

To assure compatibility with everyday practice, other tests are carried out in Phase 2, Level 2 which, for example in the case of surface disinfectants and detergents, help to identify the microbicidal action on surfaces. This is done by using stainless steel plates as germ carriers, to which the disinfectant is then added. Like the suspensions tests, the germ carrier method also yields a logarithmic reduction factor, with the reduction advocated for bacteria in the suspension test being 5 log levels and 4 log

levels in the germ carrier test. The reduction required for fungi is one log level lower in each case.

To elucidate the antimicrobial activity of detergents there is now a practice-oriented standard for Phase 2, Level 2 under development. Alternatively, there is the German Society of Hygiene and Microbiology (DGHM) method for testing chemothermal laundry disinfection [26] and the method published by Block et al. [18]. The latter is based on the DGHM methodology but does take account of the special conditions prevailing in a domiciliary setting. The principle underlying both methods is based on inoculation of cotton germ carriers, which are then exposed to the washing process in the machine together with sterile germ carriers and ballast laundry. After washing, the germ carriers are withdrawn and the number of remaining microorganisms determined. Besides, the extent of cross-contamination of sterile germ carriers can be determined as well as whether microorganisms can be detected in the washing solution. To that effect, various species are used as representatives of the Gram-negative and Gram-positive bacteria as well as of fungi (Table 2). In addition to the method presented in Table 2, there are others, such as the DGHM method for testing the chemothermal wash disinfection using an immersion method or certain the US regulations, which will not be further elaborated on here.

The test procedures already mentioned, and possible modifications of these, help to estimate the microbicidal activity of the washing process for bacteria and fungi, but they do not yield any data on antiviral activity. After a long time of confining the methodology for estimation of the virucidal activity of a washing process to suspension tests, two studies were recently published proposing methods for application-based testing of the antiviral action of washing processes [21, 22]. Both methods are based on the use of a washing machine, similar to the DGHM methodology for chemothermal laundry disinfection [26], but the test organisms used are different, since Gerhardt et al. [22] worked with the bacteriophages MS2, whereas Heinzel et al. [21] in line with the Guideline formulated by the German Society for Control of Viral Diseases (DVV) and the Robert Koch Institute (RKI) used polioviruses to evaluate the virucidal action of chemical disinfectants [25].

Table 1: Testing the disinfectants contained in detergents.

			Standard methods		Alternative methods
			Detergents	Detergents	Detergents
Phase 1	Orientational suspension tests	Bacteria	DIN EN 1040		–
		Fungi	DIN EN 1275		–
		Viruses			–
Phase 2 Level 1	Advanced suspension tests	Bacteria	DIN EN 1276		–
		Fungi	DIN EN 1650		–
		Viruses	DIN EN 14476		DVV/RKI method [25]
Phase 2 Level 2	Practice-oriented tests	Bacteria	DIN EN 13697	Standard under preparation	DGHM method [26]
		Fungi			Block et al. [18]
		Viruses	–		Heinzel et al. [21] Gerhardts et al. [22]

An important aspect when assessing the infection risk (or its prevention) is that the test method used should be able to demonstrate as realistic as possible a way the antimicrobial performance of the washing process, as assured by all the methods listed in Table 2 under Phase 2, Level 2. Since the methods are carried out in a washing machine, they also reflect a combination of chemicals, temperature and mechanical action, which in their entirety are responsible for the hygienic activity. Apart from these comprehensive test methods there are also protocols designed to compare the antimicrobial performance of various types of washing machines. These include, in particular, the method used by the US National Sanitation Foundation (NSF) for determination of the antimicrobial performance of domestic washing machines, using three bacterial strains (*S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). A similar standard is currently under preparation at European level.

In a general sense it would be of interest to know just how effective an antimicrobial household washing process should be. The DGHM guideline on chemothermal laundry disinfection stipulates a 6 log level reduction for *Candida albicans* as well as for mycobacteria and a reduction by 7 log levels for the remaining test organisms. However, this demand is based on the needs of hospitals and cannot necessarily be extrapolated to the domestic setting.

Scientific studies of the antimicrobial performance of washing processes

The question what level of microbial reduction should be achieved by a normal domestic washing process to assure adequate hygienic performance is difficult to answer, since it is not at all clear just what is meant by ‘adequate hygienic performance’. As explained above, protection against malodours is a key demand made by consumers on laundry hygiene [1], even though odour-generating microorganisms do not necessarily pose a risk to health. To assess the infection risk emanating from household textiles it is, however, important to first of all know how high is the actual typical microbial load and how the washing process affects this load. As pointed out, there is a lack of comprehensive studies for the private-households, but studies in the hospital setting suggest that the number of microorganisms on textiles vary by several orders of magnitude. For example, Christian et al. found on hospital laundry up to 10⁵ cfu/cm² aerobic bacteria and up to 10³ cfu/cm² for both coliforms and staphylococci, which could be fully eliminated by a washing process of at least 48 °C and the use of a bleaching agent [29]. Smith et al. found on towels used in a hospital 10⁴ to 10⁶ cfu/cm² and a microbial reduction by the washing process of more than 3 log levels, likewise using chlorine bleaching agents [30]. In a household-related study conducted in several European countries Terpstra et al. found contamination loads of more than 10⁷ cfu/cm² on diapers and kitchen towels and up to 10⁴ cfu/cm² on handkerchiefs and

socks [19]. That effective killing of microorganisms in the washing process could in fact have an effect on the infection risk is suggested by a study by Larson et al., who citing epidemiological studies in private households, demonstrated that dispensing with chlorine bleaching agents for washing led to a significantly lower rate of infections in the respective families [31]. These studies, presented in a realistic environment, supplement investigations conducted using laboratory methods and artificially contaminated textiles, painting a similar picture. Already in 1975, Walter and Schilling showed that in an everyday laboratory experiment with *S. aureus* and *K. pneumoniae* reductions of around 4 log levels can be achieved at 38 °C. Furthermore, it was demonstrated by this study that drying exerted an additional effect on bacterial contamination and that after drying only low counts of *S. aureus* and no residual activity of *K. pneumoniae* were found [32].

More recent laboratory tests, which in particular entailed bleach-free detergent formulations at low temperatures, do not lend themselves to comparison because of the detergent formulations, machine types and variety of other parameters, but did permit general conclusions [10, 12, 18, 19, 20, 21, 22, 23]:

- even at 30 to 40 °C granulate or powder detergents with bleaching agent had a good antimicrobial activity.
- as from 60 °C bleach-free formulations also achieved similar reduction rates.
- the extent of antimicrobial performance depends essentially on the machine type used.
- the drying method must be taken into account when assessing the antimicrobial

Table 2: Test organisms used to test for antimicrobial activity of detergents.

	Method	Source	Test Organisms
Phase 2 Level 1	Quantitative suspension test – bactericidal activity	DIN EN 1276	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Enterococcus hirae</i>
	Quantitative suspension test – fungicidal activity	DIN EN 1650	<i>Candida albicans</i> , <i>Aspergillus niger</i>
	Quantitative suspension test – virucidal activity	DIN EN 14476	Poliovirus, Adenovirus, Bovine Parvovirus
	Quantitative suspension test – limited virucidal activity	DVV/RKI [25]	Bovine Viral Diarrhea Virus, Vacciniavirus
	Quantitative suspension test – complete virucidal activity	DVV/RKI [25]	Bovine Viral Diarrhea Virus, Vacciniavirus, Poliovirus, Adenovirus, Bovine Parvovirus, Polyomavirus
Phase 2 Level 2	Chemothermal laundry disinfection	DGHM [26]	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Enterococcus hirae</i> , <i>E. faecium</i> , <i>Mycobacterium terrae</i> , <i>M. avium</i> , <i>Candida albicans</i>
	Antimicrobial performance of domestic washing processes	Block et al. [18]	<i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Enterococcus faecium</i> , <i>Candida albicans</i> , <i>Trichophyton mentagrophytes</i>
	Virucidal performance of low-temperature washing processes	Gerhardts et al. [22]	Bacteriophage MS2
	Virucidal performance of domestic washing processes	Heinzel et al. [21]	Poliovirus

performance of the washing process.

— pathogenic fungi and non-enveloped viruses are particularly difficult to inactivate and because of their virulence properties and pose a possible infection risk with regard to domestic laundry.

Taking account of the microbial counts expected on normally soiled domestic laundry, low-wash temperatures are thus sufficient; in situations where household members have an infection the use of bleach-based products at high temperatures (at least 60°C) is recommended.

Conflict of Interest

The author declare that there is no conflict of interest as understood by the International Committee of Medical Journal Editors.

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