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The Practice of the Dilution of Cleaning Solutions: Motivations and Risks

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***Abstract*—** *The habit of diluting cleaning solutions is routinely practiced in many homes and facilities around the world every day. The aim of the present work was to analyze the antimicrobial effect on six pathogenic microorganisms transmitted by hand on the two most commonly diluted cleaning solutions used in the state of Paraíba (Brazil). This practice was identified by means of a questionnaire. The cleaning solutions were diluted in sterile water (1:2, 1:4, 1:8 and 1:16) and suspensions of Acinetobacter baumanii, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes and Candida albicans were exposed to each of these solutions for 20 minutes. The yeast remained viable when the solutions had the highest concentrations of water. More than two-thirds of the 395 respondents admitted to having diluted detergent solutions because it was cheaper while believing that the dilution promoted antiseptic action, but not understanding the risk that this poses to health*

***Keywords*— Household practices, Handwashing, Candida albicans, Paraíba.**

I. INTRODUCTION

Handwashing is an essential routine activity related to the reduction of cross-contamination of different human activities, such as meals, food handling, toilet use, and health care. However, this concept is relatively modern and coincides with the increased use of soaps in the early 20th century (Draelos 2018; Robinson et al. 2016).

Historically handwashing is related to soul purification, or ablution, to the detriment of simple hygiene practice (Allegranzi et al. 2009). Only in the 19th century, motivated by germ theory, the importance of transmission of infectious disease by hands was demonstrated and doctors began to accept handwashing as a global standard of health, considering it a crucial measure for the control of infectious diseases (Ataee; Mehrabi and Salesi 2017).

Handwashing is a procedure that promotes the removal of debris, chemicals and pathogens by using water and with soap or detergent, thereby reducing the risks of food poisoning and gastric and respiratory diseases (Rabie 2006). The simple habit of washing hands with soap is associated with the healthy growth of children under 5 years of age (Dangour 2013), reducing diarrhea and pneumonia mortality rates by up to 50% (Cairncross et al. 2010; Curtis and Cairncross 2003). Handwashing is considered to be the most efficient and cost-effective way to prevent diseases (Adams and Marie 1982), as well as reducing expenses for antibiotic treatments (Webster; Faoagali and Cartwright 1994).

Hand care products are used to enhance the efficiency of water in removing stains and dirt. The use of surfactants is based on the principle of reducing the surface tension between the debris and sebum on a contact surface, favoring its scattering and subsequent removal by friction (Bhamla et al. 2017).

Cleaning the hands using either warm or cold water is inefficient due to the insolubility of fats. Hot water is also uncomfortable for the hands and not suitable for the elimination of transient pathogenic microorganisms. Use of soaps or detergents together with hot water makes fat removal more efficient. The reduction of microbial concentration, however, is independent of temperature when soaps and detergents are used (Laestadius and Dimberg 2005; Michaels et al. 2002).

In different parts of the world, the habit of diluting cleaning solutions used for handwashing has been observed. This practice is disseminated informally through websites and blogs available on the world wide web and most webpages recommend the dilution of the original product with tap water, in a ratio of 1:3.

It is important to note that many cleaning products are formulated for use without prior dilution; moreover, it is a very subjective act for the consumer to perform the dilution with the correct proportion of water. In addition, the variability and lack of knowledge of the

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microbial density present in the water does not guarantee the activity of the diluted components against the microbiota, and may indeed favor microbial development in the bottles, representing risk of infections to users. Given this, the present study aimed to evaluate the antimicrobial activity of the two diluted cleaning solutions most consumed in the state of Paraíba (Brazil) on six pathogens transmitted by hand. In addition, a questionnaire was developed to verify the practice of dilution and the main factors that encourage it.

II. MATERIAL AND METHODS

Microorganisms and cleaning solutions

Six representative pathogens of the resident and transient microbiota were used: *Acinetobacter baumanii, Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*. A detergent dishwashing solution and a bathroom liquid soap were evaluated. The criteria used to select the tested brands were: popularity, market value and lack of recommendations or information concerning dilution. The products were tested to ensure absence of cultivable heterotrophs prior to being employed in the assays.

***In vitro* antimicrobial activity test**

The test was based on the methodology described by Medeiros; Vasconcelos and Calazans (2007). Initially, the cleaning solutions were aseptically diluted in sterile distilled water in the following ratios: 1:2, 1:4, 1:8 and 1:16, making up to a final volume of 10 mL. Then, a suspension of each pathogen from fresh culture incubated at 37±2°C was standardized by the turbidity indicated on the McFarland scale tube #1 (≈$3x10^8$ CFU/mL) and 1 mL of this suspension was transferred to the tube containing solution diluted 1:2. After a gentle mixing in a vortex mixer, aliquots of 1 mL were serially transferred up to the 1:16 dilution. The tubes were allowed to stand for 20 minutes at laboratory temperature and thereafter, aliquots of 1 mL of each tube containing the diluted solutions were transferred to tubes containing a nutrient broth. After incubation at 37±2°C for 48 hours, the viability of the microbial cells was determined by turbidity visualization and the addition of 1 mL of 1% resazurin solution. This assay is based on the reduction of the resazurin, observed by the color change, from blue to pink, in up to 2 hours, indicating the presence of products from microbial metabolism (O'Brien 2000).

Questionnaire on the dilution of household cleaning products

A multiple-choice questionnaire was developed with 10 questions, aiming to identify the habit of diluting cleaning solutions as well as the reasons why people do it. The questionnaire was published in Portuguese on the internet on March 16, 2018 and was available for a period of 15 days on three social networks: Facebook, Instagram and Twitter.

III. RESULTS

Cleaning solutions

Neither type of cleaning solution had instructions for use regarding dilution in large quantities of water. In the composition reported on the labels, both presented more than one surfactant and preservative solutions in their formulation.

***In vitro* antimicrobial activity test**

The dilutions in water of the two cleaning solutions were efficient for all bacterial strains tested. In the other hand, C. albicans remained viable in the diluted solutions 1:8 and 1:16, as shown in Tab. 1.

Users profile on the cleaning solutions dilution

A total of 395 people answered the questionnaire. The practice of diluting dishwashing detergents with tap water was admitted by about 50% of respondents; less than 30% stated that they diluted bathroom liquid soap. Interestingly, when asked if they know someone who did this with both liquid soap and detergent, the percentage for the "yes" answers were higher.

Almost half of the respondents believed that dilution of dishwashing detergent (44.9%) or bathroom liquid soap (49.1%) could reduce their antiseptic action but surprisingly disagreed that this practice could increase the risk to the user's health for both products. The data are presented in Tab. 2.

Table. 1: Cell viability of hand pathogens (at least two replicates)

Pathogen	Cell viability							
	Dishwashing detergent				bathroom liquid soap			
	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16
Acinetobacter baumanii	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa	–	–	–	–	–	–	–	–
Staphylococcus aureus	–	–	–	–	–	–	–	–
Escherichia coli	–	–	–	–	–	–	–	–
Enterobacter aerogenes	–	–	–	–	–	–	–	–
Candida albicans	–	–	+	+	–	–	+	+

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Table. 2: Percentage of user evaluation on the dilution of cleaning solutions (n = 395)

Responses	**Dishwashing detergent**			**Bathroom liquid soap**		
Have already practiced dilution	48.6			29.6		
Meet people who practice	**Yes**	**No**	**Not sure**	**Yes**	**No**	**Not sure**
	23.5	26.3	50.1	32.2	34.2	33.7
Believe in the anti-germ protection after the dilution of the product	**Yes**	**No**	**Not sure**	**Yes**	**No**	**Not sure**
	36.7	44.8	18.5	30.9	49.1	20.0
Believe that dilution may pose a health risk	**Yes**	**No**	**Not sure**	**Yes**	**No**	**Not sure**
	6.3	64.8	28.9	7.8	63.5	28.6

Tab. 3 reveals the desire to save on household expenses as the main motivation in all the declarations presented by the interviewees to justify the practice of the dilution of dishwashing detergents and bathroom liquid soaps. Other reasons also mentioned by the interviewees, especially with reference to the use of dishwashing detergents, included: avoiding damage to the skin, facilitating the cleaning of objects such as jewelry, the need to perceive the formation of foam and the desire to reduce viscosity of the product, making it easier to pour, as well as ensuring greater yield. In the bathroom liquid soap, the concern about damage to the skin was not mentioned, however foam formation was mentioned by the majority of the interviewees.

IV. DISCUSSION

In vitro antimicrobial activity test

Humans harbor more microorganisms than the total number of cells in their bodies. Most of this microbiota is beneficial, commensal or neutral, whereas a minor number is represented by pathogens (Rosenthal et al. 2011). On the hands, microbial populations occur that vary in concentration depending on their location. In the palm region, for example, the amount is approximately 10^3 CFU/cm^2, while under the nails they can reach about 10^5 CFU/cm^2 (Blaser and Falkow 2009).

Table. 3: Consumer reasons for diluting cleaning solutions (%)

Responses	**Dishwashing detergent**	**Bathroom liquid soap**
Reduce costs	64.8	69.9
Both savings and avoiding skin damage	18.5	0.0
Only to reduce skin damage	1.9	0.0
Other reasons	5.9	3.3
Not sure	8.9	26.8

The microbiota present on the hands may be classified as resident or transient, and may sometimes be composed of symbiotic organisms as well as pathogens. So-called resident microbiota is installed from birth, existing in the deeper layers of the skin, more difficult to remove with water and soap, and may vary according to diet, lifestyle, environmental factors, age and gender (Schommer and Gallo 2013; Grice et al 2008). On the other hand, the transient microbiota varies in number and diversity as a function of time and generally constitute non-pathogenic or opportunistic microbes, originating from the contact of the hands with the environment. They rarely multiply on the skin and can be easily removed by washing and rubbing the hands using either liquid soap or an effective detergent (Mathur 2011).

The most critical periods during a day in the context of hand hygiene for the reduction of oro-fecal transmission of diseases are after defecation, before handling or preparation of food and before meals. For people with children at home, two critical periods can be added: feeding after a child who defecated and manipulating instruments related to infant feeding (Luby et al 2011).

The anionic surfactants present in formulations of dishwashing detergents and bathroom liquid soaps, such as sodium lauryl sulfate, attribute antimicrobial activity to these products, especially against bacteria. Although they may promote more skin irritations compared to similar cationic ones, the microbial density is significantly reduced during hand washing (Jensen; Rogers and Schaffner 2017).

The literature reports on some multidrug-resistant microorganisms as well as those resistant to preservatives present in health care and cosmetic products; however, most of these microorganisms are bacteria (Martins et al 2018). Few studies have also identified multidrug-resistant fungi (Elmorsy and Hafez 2016; Shaqra et al 2012). The present study highlights *Candida albicans* as the only resistant pathogen to the conditions applied in the in vitro assay.

Similar results were obtained by Bloomfield et al (1991). When testing different concentrations of active chlorine, 70% ethanol and 13 other sanitizing products, the authors observed sensitivity of *S. aureus* and *P. aeruginosa* and resistance of *C. albicans* under two

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distinct contact times, 1 minute and 60 minutes for hands and surfaces, respectively.

C. albicans has been described as partially resistant to vinegar solution and some antiseptic agents (Lafleur; Kumamoto and Lewis 2006). Resistance can be attributed to the presence of lipid rafts, that is, membrane microdomains with amounts of saturated fatty acids and sterols larger than those present in the rest of the cell membrane (Insenser et al 2006). This arrangement also ensures greater resistance to detergent solutions, also contributing significantly to the formation of biofilms by the yeast (Lattif et al 2011).

On the other hand, in cases of the occurrence of mutant cells, with failure of ergosterol synthesis and consequent destabilization of microdomain membrane interactions, hypersensitivity to antiseptics has been observed, as well as sensitivity to fuminisin, a mycotoxin that inhibits the synthesis of sphingolipids (Mukhopadhyat et al 2004). Proteomic analyzes suggest that polarization of domains rich in sphingolipids and cholesterol are involved in cellular processes for example cell signaling, cytokinesis, and morphogenesis, and have important proteins for the formation of hyphae that favor *C. albicans* adhesion on surfaces, increasing its virulence (Martin and Konopka 2004).

Although the results obtained by our work suggest that the practice of diluting dishwashing detergent or bathroom liquid soap does not alter the effectiveness of the products against about 85% of the tested pathogens, especially bacteria, it is noteworthy that the study was performed under limited laboratory conditions, not reflecting the use in a domestic routine, that is, dilution in water captured directly from the tap which may contain a certain microbial density, including the presence of pathogens. Aside from this, the volume of water used in the dilution of a given volume of cleaning solution may be even greater than that investigated in this study.

On the habit of diluting cleaning solutions

Personal opinion questionnaires guarantee a more accurate and close-to-the-scene result when respondents are asked to express the opinion of others and can reveal their own habits without running the risk of judgment (Graefe 2014; Rothschild and Wolfers 2012). Therefore, most interviewees assumed that they practiced or knew someone who dilutes cleaning solutions, especially dishwashing detergents. However, dilution with excess of water can compromise the product quality, reducing the activity of the active compounds as well as the preservatives, favoring the microbial development in the recipient washed, thus increasing the risk of dissemination of pathogens (Campana et al 2006).

Another reason for dilution that may reflect microbial proliferation concerns the consumer's desire to alter undesirable organoleptic characteristics such as high viscosity and non-foaming, characteristics of which the consumer associates with the quality of the product (Santa Bárbara et al 2007). The viscosity of detergents and liquid soaps is a result of the addition of salts which function as additives, having among them the prevention of liquid leakage during manufacturing, transportation and use processes. The addition of water may also generate a foaming effect because of the surfactants present in the formulation. The role of foam is a visual indicator of the need to rinse; however, foaming, for most consumers has a more stimulating effect of the feeling of cleanliness and freshness than a real antiseptic action (Tang et al 2015; Cobirman 2012).

Disregarding the economic factor, the practice of diluting cleaning solutions such as dishwashing detergent, bathroom liquid soap, and even shampoo was seen to be common when the product needed to be used but the container was nearly empty. More people resorted to dilution of the dishwashing detergent but preserving the bathroom liquid soap for some reason that was not investigated by the questionnaire but which seems to be answered by the preconceived notion that bathroom liquid soap is for cleaning hands rather than utensils. This status probably occurs due to certain organoleptic characteristics of the product, such as odor, foam and moisturizing sensation, which guarantees the hygienic sensation for the user by associating the product with beauty and cleanliness (Martins et al 2018).

Hand skin protection was also identified as a motivating factor for the practice of dilution. Kein; Gubauer and Fitsch (1992) demonstrated the correlation between the constant use of dishwash detergents and non-allergic skin lesions that remain for weeks. Some studies have already indicated that skin irritation when using soaps and detergents can be aggravated by dry climates and the influence of hard water as a rinse (Baranda et al 2002). They also reported that glycerinated soaps and other cosmetic products made from glycerin, vegetable oils and petrolatum prevent dehydration of the stratum corneum, i.e. the outer layer of keratin on the skin, minimizing irritation (Cornwell 2018; Wilson; Berardesca and Maibach 1988). However, the literature consulted contained no data that correlates the dilution of these products with the amelioration of skin damage. Ours was the first study reported in Brazil that sought to understand the practice of dilution by consulting users through a questionnaire. The results suggest that the label contain instructions on how to properly dilute dishwashing detergents and toilet liquid soaps when appropriate, however, the health risks related to the practice as well as

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the number of dilutions permitted and the source of the water to be used for this purpose should be clear.

V. CONCLUSION

The habit of diluting cleaning products is disseminated and routinely worldwide practiced by a large number of consumers, especially motivated by the reduction of costs and the increase in the domestic consumption time of the product. Under the conditions used in this study, dilutions between 1:2 and 1:16 did not interfere with the antiseptic action of the active compounds against bacterial pathogens. However, the result was inefficient for *C. albicans* which remained viable when both solutions presented higher percentages of water.

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