The Role of Laundry Detergents in the Elements of Skin Pathogen

Erdong Wei
University Hospital, LMU Munich
Markus Reinholz (Markus.Reinholz@med.uni-muenchen.de)
University Hospital, LMU Munich
Lars E. French
University Hospital, LMU Munich
Benjamin M. Clanner-Engelshofen
University Hospital, LMU Munich

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Abstract

Background: The Corona pandemic fuelled up skin pathogen challenges in young and adults, the antimicrobial efficacy of laundry detergents could be considered particularly. However, no available data focusing on the form of laundry detergent, additives and conditions affect the antimicrobial efficacy. This study simulated washing procedures to investigate the antibacterial and antifungal activity of laundry detergents.

Methods and Results: Mimic laundry procedures were performed to treat Gram-positive bacteria, Gram-negative bacteria and fungus, colony counting and propidium iodide staining were used to assess the antimicrobial activity. Powder detergent A, NaBO3*4H2O with the tetraacetylethylenediamine, 2Na2co3.3H2O with tetraacetylethylenediamine could achieve a > 5-$\log_{10}$ reduction of three microbial colony generation. Anionic surfactant sodium dodecylbenzene sulphonate (SDBS) group had the strongest fluorescence intensity in three microbial propidium iodide staining.

Conclusions: Powder form laundry detergents are superior to liquid form, peroxide-based bleaches and bleach activator in solid form, the solid surfactants with matched PH and alkyl chain length showed a considerable antimicrobial effect.

Introduction

Skin is a large, complex organ with the essential function to form an effective barrier between the ‘inside’ and the ‘outside’ of the organism, protect the body against pathogens and excessive water loss [1]. In addition to its immunity role, skin has some other functions like insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates [2]. Besides, human skin also sustains microorganisms that influence human health and disease [3]. The microbiome is similar from person to person and there are over 1000 species of bacteria on and in the skin, while these bacteria are non-pathogenic for the biggest part [3]. For example, *Cutibacterium acnes* (*C. acnes*, formerly Propionibacterium acnes) exists on the skin of almost every person and can be found in regions rich in sebaceous glands. It has a role in skin health and has long been thought of as a commensal bacterium, its development as an opportunist pathogen is due to its participation in a variety of diseases [4]. When the follicle opening gets blocked, *C. acnes* will thrive and primarily break down the sebum triglycerides into skin-irritating substances like free fatty acids. It therefore is a significant factor in the pathophysiology of acne, which affects over 9% of the global population with a wide range of potential harms and associated costs [5]. Normally, acne is recognized as a self-limited disease that occurs in adolescence and affects nearly 90% of teenagers [6]. Teens with acne are perceived more negatively, e.g. more anxious, socially inhibited, and aggressive compared to teens without acne [7]. Furthermore, acne is also the only skin disease whose severity has been linked to a risk factor for suicide, especially in men [4]. In addition to its role in inflammatory acne, *C. acnes* has been identified in a variety of clinical settings, including inflammatory diseases and implant-associated infections. *C. acnes* as a superficial infectious

agent must be taken seriously, as we recently described that acneiform lesions could be aggravated due to inappropriate and prolonged use of face masks related to the COVID-19 pandemic[8].

However, not only face masks, but especially our clothes have prolonged contact with the skin. Consequently, the survival of bacteria on clothes during domestic laundering presents a potential risk of re-exposure, cross-contamination of other clothes within the same washing machine and transmitting potential pathogens to others [9]. Laundry detergents are not only used to make clothes look clean but also to have an antimicrobial effect. Eliminates *C. acnes* on clothes is an important strategy to prevent the recurrence and transmission. Nowadays, many markets across the world have switched from powder to liquid laundry detergent because it is more convenient and user-friendly [10]. In clinical work, dermatologists advise patients with acne to use powder laundry detergents rather than liquid detergents to clean their clothes, even though the powder and liquid detergents share similar ingredients. Surprisingly, patients benefit from advice (authors' observation) but there is little scientific evidence for the antibacterial efficacy of the individual commercially available detergents forms or their ingredients.

Hence, the present study aims to provide an extensive investigation of the antimicrobial efficacy of different laundry detergents, additives and conditions. For this purpose, common laundry detergents in the German market, anionic, zwitterionic, non-ionic surfactants, enzymes, peroxide-based bleaches with and without bleach activator and different temperatures have been evaluated.

Methods

Sample Preparation

Gram-positive, anaerobic *C. acnes* (ATCC 6919) bacteria were cultured by inoculating BD Bactec Anaerobic culture vials (Becton, Dickinson and Company, Bergen, USA) with a glycerol cryo-stock stored at -80°C and incubated for seven days at 37°C. *Trichophyton rubrum* (*T. rubrum*) was obtained from the mycology diagnosticsfacility of the department of dermatology at the Ludwig-Maximilian-University in Munich, Germany. It was cultured on dermatophyte agar at room temperature (RT) for 30 days. Gram-negative bacteria isolated from the author's skin, were cultured on blood agar plates for 24h at 37°C.

Potassium Hydroxide (KOH) Test

Potassium hydroxide solution was used to test the bacteria from the author's skin to be Gram-negative or Gram-positive; briefly, one drop of 3% KOH solution in water was placed on a glass slide. One loopful of bacteria obtained from a 24h culture on a blood agar plate was stirred into the KOH solution. Raising the loop allowed to check for the formation of strings due to selective lysis of Gram-negative bacteria [11].

Laundry Detergent Preparation

Five common brands of laundry detergent were purchased from a regular German supermarket: A (powder, general-purpose), B (liquid, color-detergent), C (liquid, wool detergent), D (powder, color-detergent), E (liquid, hygienic rinser). According to the labels, the detergents A to D share similar

components using anionic surfactants, non-ionic surfactants, soap, phosphonates, enzymes, optical brighteners, and fragrances. However, the concentration of each compound is supposedly different (German market regulations do not require manufacturers to disclose the specific composition). E contains non-ionic surfactants and cationic didecyldimethylammonium chloride. Anionic surfactant sodium dodecylbenzene sulphonate (SDBS), non-ionic surfactant Polyalkylene glycol ethers (Brij C10), peroxide-based bleach benzoyl peroxide (BPO), sodium perborate tetrahydrate ($NaBO_3*4H_2O$), sodium percarbonate ($2NaCO_3*3H_2O$), bleach activator tetraacetylethylenediamine (TAED), lipase from *Candida rugosa* (L1754) were purchased from Sigma-Aldrich (Steinheim, Germany). Zwitterionic surfactants cocamidopropyl betaine (CAPB) was purchased from Combi-block (San Diego, USA). Trypsin-EDTA was purchased from PAA Laboratories (Pasching, Austria).

All the compounds' concentrations have followed the products' description.

Colony generation after laundering-mimic procedures

Sterilized gauze patches (Lohmann & Rauscher GmbH & Co., Rengsdorf, Germany) were used as cloth-substitute for inoculating with bacteria. Gram-negative bacteria and *T. rubrum* were dispersed in phosphate buffered saline (PBS), *C. acnes* culture in the aforementioned liquid medium used for cultivation. For inoculation, 1ml of the bacterial suspensions diluted to approximately 0.5 MFU (McFarland units) equivalent to a bacterial density of 1.5×10^8 cells per ml were used. After 1h of incubation, gauze patches were washed with or without different laundry detergents dissolved in 100ml water in a 100ml threaded laboratory bottle. Inoculated gauze patches washed with laundry detergent were treated as the experimental group, and inoculated gauze patches washed with water only were treated as the control group. A shaker (Type MTS4, Ika-werke Gmbh & Co., Staufen, Germany) to emulate the washing machine with 700 rpm shaking and a duration of 1 hour were chosen to mimic a standard washing program, facultatively. A warming cabinet (Type BE 200, Memmert GmbH Co. City, Germany) was used to create washing cycles at elevated temperatures, three commonly used laundry temperatures RT, 40°C and 60°C were chosen for testing.

The experimental group and control group were performed in pairs. After washing, gauze patches were transferred to and pressed on blood agar plates and then the gauze patches were discarded. The agar plates with *C. acnes* were incubated in an anaerobe container system with an indicator (Becton, Dickinson and Company, Sparks Maryland, USA) and an anaerobic jar (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany), then incubated at 37°C for seven days. The Gram-negative group was incubated in the 37°C incubators directly for one day. At any point in the above steps, an open flame was employed to create an upwards stream of air to avoid contamination by external microbes. Each experiment was performed in triplicates.

Propidium iodide staining

C. acnes, T. rubrum and gram-negative bacteria were treated with the mimic standard washing program. Microbial cells (10^8 cellsml^{-1}) were plated on sterile slides and treated with PBS containing 1‰

propidium iodide (PI, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) at RT for 5 minutes. Fluorescence microscopy was carried out using EVOS M7000 microscope (Thermo Fisher Scientific Inc., Waltham, USA), 40x object and RFP light cube were chosen. Quantification of fluorescence was performed by ImageJ/ Fiji (https://imagej.net/ImageJ) [12], the integrated density (IntDen) value was measured and expressed as $\log_{10}$ change of their controls. Logarithmic increase (LI)= $\log_{10}$C-$\log_{10}$T, where C is the control group before laundering, T is the experiment group after laundering.

Colony Counting

Plates with distinct single colonies were counted depending on the number of colonies. Plates with approximately up to 100 colonies were counted completely, on plates with 100 up to 500 colonies one quarter of the plate was counted and multiplied by four and on plates with more than 500 colonies 3 to 5 squares of 1cm^2 in size were randomly selected to count, then the average of the counts was multiplied by 57 to yield the total number of colonies on the 57 cm^2 plate. The colony count directly resembles the number of viable bacteria on the surface of the gauze patch after washing.

Logarithmic reduction (LR) was performed to evaluate the antimicrobial activity in colonies generation, LR=$\log_{10}$C-$\log_{10}$T, where C is the control group before laundering, T is the experiment group after laundering.

Sequencing

The 16S rRNA gene was partially amplified by colony PCR with the HotStarTaq Master Mix Kit (Qiagen, Qiagen GmbH, Hilden, Germany) using the primer pairs 27f (5‘-AGA GTT TGA TCA TGG CTC A-3‘) / 1492r (5‘-TAC GGT TAC CTT GTT ACG TCT T-3‘) with standard conditions (Initial heat activation of 15 mins at 95°C, 3-step cycling: denaturation of 1 min at 94°C, annealing of 1min at 50°C, extension of 1min at 72°C, 35 cycles, final extension for 10mins at 72°C), and bacterial isolates were identified by Sanger sequencing using the 27f primer performed by Eurofins Genomics GmbH (Ebersberg, Germany). Obtained sequences were compared to 16S reference genomes using nucleotide BLAST[13, 14].

Statistics

All statistics were performed using SPSS version 26 software (IBM, Armonk, NY). The significance level was set at p=0.05 for all tests. Counts of the microorganisms after different detergents washing were compared to the water-washed negative controls using the paired T-test.

Results

Potassium Hydroxide (KOH) Test

To test for gram-negative bacteria, the KOH solution characteristically became very dense and mucous. A string of the mixture follows the loop when raised. This is a strong indicator the bacterium from the author’s face is Gram-negative.

Cutibacterium acnes

Colony generation

To assess the advantage of powder-form, no detergent group and the disinfectant group were acted as negative control and positive control (Table 1, Figure 1A).

Table 1
Detergents Clearance of *C. acnes* at different temperatures

	RT	40°C	60°C
A	2.82	3.10	**4.00**
B	1.66	1.91	1.98
C	2.05	2.36	2.54
D	3.15	3.15	**4.00**
SDBS	3.15	3.15	**4.00**
Brij C10	1.30	1.40	1.41
CAPB	1.26	1.38	1.50
BPO	2.66	3.15	**4.00**
2NaCO3.3H2O	1.91	3.05	3.15
TAED	0.30	0.48	0.49
NaBO3.4H2O	2.02	2.66	**4.00**
2NaCO3.3H2O+TAED	**4.00**	**4.00**	**4.00**
NaBO3.4H2O+TAED	**4.00**	**4.00**	**4.00**
Trypsin	0.00	0.02	0.00
Lipase	0.00	0.03	0.00
Bold texts mean to reach a completely reduction			

Compared to the negative control group with water only, all experimental conditions lead to a reduction for C. acnes. A complete reduction could be achieved when using $NaBO_3*4H_2O$ combined with TAED, $2NaCO_3*3H_2O$ combined with TAED at RT, powder A, powder D, anionic surfactant SDBS, BPO and $NaBO_3*4H_2O$ alone at 60°C.

A significant increase of the colonies could be observed in the liquid group (p=0.03), for liquid B, a LR of 1.66 at room temperature and increased to 1.98 at 60°C. A LR of 2.05 at RT, 2.36 at 40°C and 2.54 at 60°C

could be detected in liquid C.

Like anionic surfactant SDBS peak at 60°C, non-ionic surfactant Brij C10 and zwitterionic surfactant clearance reached a maximum when temperature increased to 60°C, LR of 1.41 and 1.50 were achieved in Brij C10 and CAPB each.

In the test of bleach, without bleach activator TAED, antibacterial efficacy of $2NaCO_3*3H_2O$ peak at 60°C with a LR of 3.15. Single-use of TAED have a LR of 0.30 at RT and 0.49 at 60°C.

When enzyme using alone, a slight difference could be observed between lipase, trypsin-EDTA and the control group at various temperatures.

Viability

As can be seen on quantitative images (Fig. 2A), after PI staining SDBS group had the highest LI of 2.99, as the representative image (Fig. 2B, C), followed by the disinfectant group E with a LI of 1.90. The powder groups A and D had PI of 1.79 and 1.54, while the liquid groups B and C had PI of 0.37 and 0.98.

BPO achieved a LI of 1.19 in *C. acnes* at RT, significantly higher than treatment with other antimicrobials.

$2NaCO_3*3H_2O$ combined with TAED, $NaBO_3*4H_2O$ combined with TAED achieved a LI of 1.54 and 1.75c, while single-use $2NaCO_3*3H_2O$, $NaBO_3*4H_2O$ had a LI of 1.26 and 0.70.

Trypsin-EDTA had a LI of 0.78, whereas TAED, lipase using alone, slight changes can be observed compare to the negative control ((Fig. 2D, E).

Gram-negative bacteria

Colony generation

A complete reduction could be achieved when using NaBO3.4H2O combined with TAED at RT $2NaCO_3*3H_2O$ combined with TAED at 40°C, powder A, $NaBO_3*4H_2O$ alone at 60°C (Table 2, Fig. 1B).

Table 2
Detergents Clearance of *K. aerogenes* at different temperatures

	RT	40°C	60°C
A	3.13	3.43	**4.00**
B	1.42	1.87	1.92
C	0.42	0.42	0.45
D	0.34	0.41	0.61
SDBS	1.72	1.76	1.76
Brij C10	0.26	0.35	0.74
CAPB	0.11	0.25	0.33
BPO	1.49	1.65	1.78
2NaCO3.3H2O	2.70	2.77	2.96
TAED	0.19	0.26	0.40
NaBO3.4H2O	2.66	2.66	**4.00**
2NaCO3.3H2O+TAED	3.13	**4.00**	**4.00**
NaBO3.4H2O+TAED	3.43	**4.00**	**4.00**
Trypsin	0.15	0.45	0.16
Lipase	0.15	0.37	0.15
Bold texts mean to reach a completely reduction			

The LR of liquid B, liquid C, powder D at RT are 1.42, 0.42 and 0.34 respectively at RT, then increased to 1.92, 0.45 and 0.61 with temperature increased to 60°C.

Anionic surfactant SDBS could have a LR of 1.72, then increased to 1.76 at 60°C. Non-ionic surfactant Brij C10 achieved a LR of 0.26 at RT and peak of 0.74 at 60°C. Zwitterion surfactant CAPB achieved a LR of 0.11 at RT and reached the peak of 0.33 at 60°C.

Single-use of $NaBO_3*4H_2O$ or $2NaCO_3*3H_2O$ was inferior to combination with activator TAED, $2NaCO_3*3H_2O$ could be observed a LR of 2.70 of Gram-negative bacteria at RT and maximum 2.96 at 60°C. In comparison, $NaBO_3*4H_2O$ could have a LR of 2.66 at RT and achieve the complete clearance at 60°C.

When enzyme using alone, LR of trypsin-EDTA and lipase reached a maximum of 0.45 and 0.37 at 40°C, no statistically significant difference was observed between the control group and RT group, either did

60°C.

Viability

As can be seen on the representative image (Fig. 2F, G), the SDBS group achieved the highest LI of 2.45. Then, TAED combined with 2Na2co3.3H2O with a LI of 1.99, superior to $2NaCO_3*3H_2O$ alone with a LI of 1.13. Similarly, in combination with TAED, $NaBO_3*4H_2O$ achieved a LI of 1.20, higher than 0.66 when single-use of NaBO3.

The powder A could have a LI of 1.27, while powder D showed a slight difference of 0.02. The liquid B and C had LI of 0.51 and 0.41 each.

Lipase had a LI of 0.68, and Trypsin-EDTA had a 0.92, while BPO and CAPB could hardly observe the difference with the control group (Fig. 2H, I).

Trichophyton rubrum

Colony generation

Powder A and D, liquid B and C, SDBS, $NaBO_3*4H_2O$ and $2NaCO_3*3H_2O$ with or without TAED could achieve a complete reduction at RT, while TAED alone had a LR of 0.22, lipase had a LR of 0.26, BPO had a 0.15, CAPB had a 0.10 at RT (Table 3, Fig. 1C).

Table 3
Detergents Clearance of *T. rubrum* at RT

	RT
A	**4.00**
B	**4.00**
C	**4.00**
D	**4.00**
SDBS	**4.00**
Brij C10	0.00
CAPB	0.10
BPO	0.15
2NaCO3.3H2O	**4.00**
TAED	0.22
NaBO3.4H2O	**4.00**
2NaCO3.3H2O+TAED	**4.00**
NaBO3.4H2O+TAED	**4.00**
Trypsin	0.00
Lipase	0.26
Bold texts mean to reach a completely reduction	

With the increase of temperature, the colonies in the negative control group decreased significantly then close to 0, so that it could not tell the changes in the experimental groups had a statistically significant. Then only RT group was kept in the test of *T. rubrum.*

Viability

According to the most significant image (Fig. 2J, K), E group achieved the LI of 1.88, followed by SDBS with a LI of 1.74.

The powder groups A and D achieved LI of 1.31 and 1.65. In contrast, the liquid groups B and C had 1.58 and 0.74. The LI of $NaBO_3$*$4H_2O$ combined with TAED is 1.49, while without TAED is 1.07. Similarly, The $2NaCO_3$*$3H_2O$ with TAED had a LI of 1.40 while $2NaCO_3$*$3H_2O$ alone 1.17.

The LI of Lipase is 0.46, and the Trypsin-EDTA, BPO, Brij C10 had no evident fluorescence increasing compare to negative control (Fig. 2L, M).

Sequencing data

Compare to the nucleotide BLAST 16S reference genomes, the obtained 16S sequence (GenBank: SUB10460522 gramneg OK350364, Supplementary file 1) had 99.91% sequence identity with the 16S partial gene sequence of *Klebsiella aerogenes (K. aerogenes)* strain 18-2341.

Discussion

The current study was designed to investigate the antimicrobial, antifungal effects of laundry detergents, additives and conditions. Although many studies have investigated the antimicrobial and antifungal effects of laundry detergents [15], there is no available data focusing on the form of laundry detergent. Likewise, the powder-form detergents clear *C. acnes* during laundry procedures to prevent the acne from recurring and transmission has not been investigated before, although it is known that the dermatologists ask the patients to do so to increase the hygiene performance of laundering. Finally, the COVID-19 pandemic since 2019 has resulted in a rising demand for practical solutions to improve skin protection.

Generally, Gram-negative bacteria have an outer membrane containing lipopolysaccharides and lipids, a thin peptidoglycan layer, and an inner phospholipid membrane, while Gram-positive bacteria are distinguished by a thick peptidoglycan layer and an inner membrane [16]. The difference in structure could explain that *C. acnes* did not respond to lipase but trypsin, while *K. aerogenes* could be reduced by both enzymes. Lipase acted against the *K. aerogenes* better than trypsin supposedly because of the thickness of the cell wall, but the EDTA in Trypsin-EDTA could chelate the iron in *K. aerogenes* outer membrane.

BPO is mainly used to treat acne, topically, the effects of BPO include sebostatis, comedolytsis, and inhibition of the growth of *C. acnes* [17]. Okamoto et al. [18] showed that without the protection of the outer membrane, the cell wall of *C. acnes* was disrupted partly after exposure to BPO, and cytoplasmic materials were released to the extracellular space. In our study, BPO could only get a complete reduction in *C. acnes* at 60°C, and the *C. acnes* BPO group had significantly stronger effect than *K. aerogenes* and *T. rubrum*. In Gram-negative bacteria, the outer membrane could block the BPO unless EDTA damaged the membrane [18].

In addition to EDTA could chelate the outer membrane of gram-negative bacteria [19], surfactants with a good match alkyl chain length to cell lipid structure in efficacy. In 1997 [16], non-ionic surfactants were found to have an effect against gram-negative bacteria. Non-ionic surfactants with C10 (alkyl chain with 10 carbon atoms) could adsorb on the cell wall, while C12 (alkyl chain with 12 carbon atoms) material entered the membrane bilayer. The viability of gram-negative bacteria could be interfered with by C10 and C12, but not non-ionic surfactants with other alkyl chain length. In our test, Brij C10 as a non-ionic surfactant with C16, neither inhibited colony generation nor interfered with microbial viability.

Unlike non-ionic with C10 or C12 alkyl chain length, zwitterionic surfactant 12-16 alkyl chain length have been shown to have the highest antimicrobial activity [20]. CAPB is a medium-strength zwitterionic

surfactant widely used in cosmetics [21], but in the antimicrobial experiments, CAPB lacks the ability to kill in all tests. According to A. Krasowska et al.'s study [20], CAPB and alkyl dimethylamine oxide could achieve synergy in antimicrobial performance but still inferior to benzalkonium chloride (a cationic surfactant). No cationic surfactant are used in laundry detergents because the anionic surfactants are most commonly used in detergents and would lead to precipitates together with the cationic ones. For that reason, we did not test the antimicrobial activity of benzalkonium chloride.

Similarly, anionic surfactants with 12-16 alkyl chain length could get a good match with the cell wall lipid structure, which could improve the efficacy of antimicrobials. SDBS as the most widely used synthetic detergents, could be found in numerous cleaning supplies [22], with C12 alkyl chain length and strong acids, has a good affinity for microbial proteins [23]. In our test, all the SDBS groups perform well in inhibiting colony generation and viability.

Besides surfactants, bleaches are typical constituents in detergents that could remove color and have broad-spectrum bactericidal properties. Peroxide-based bleaches NaBO3*4H2O and 2NaCO3*3H2O could dissolve in water then generated hydrogen peroxide. Hydrogen peroxide is inefficient when used under 60°C. C. acnes as an aerotolerant anaerobic bacterium, possesses the cytochrome D oxidase gene, enabling it to grow in the limited amounts of oxygen that are present and to tolerate oxygen for a few hours [4]. Moreover, C. acnes is aerotolerant, then detergents could not achieve a complete reduction of C. acnes at RT. The observed increase in the TAED group could be attributed to TAED and hydrogen peroxide synthesize peroxyacetic acid, which has antibacterial, antifungal properties under 60°C [24]. TAED as a commonly used ingredient is widely used in laundry detergents currently, it is stabilized by granulation and not stable in water-based liquid detergents.

As mentioned in the literature [25], T. rubrum could be removed from the fabric at a normal washing process at RT, but later research noted that T. rubrum could be detected in the rinsing wastewater [26]. In our test, all the regular detergents could completely reduce colony generation, and fluorescence showed that the compounds made a different damage level of T. rubrum cells. It is a limitation that we did not check the microbial load in rinsing water. Still, with the development of laundry, all the rinsing water would leave into the wastewater pipe, the microbial go the cloth and human, back in nature is plausible.

Overall, the powder A and D had better antimicrobial performance than the liquid B and C in clearance of microbe. By analysing the ingredients in detergent, a possible explanation for this is that the compounds like bleach activator TAED are only stable in powder form. Besides, Peroxide-based bleach would also be affected by the form [26]. Like NaBO3*4H2O, the different hydrations cause the different PH, and an appropriate PH could significantly impact the antimicrobial of surfactant. For example, the acidic PH could make the zwitterionic surfactant take on a positive charge, the affinity for cell membranes will be stronger if the cell membrane isoelectric point is not achieved. Furthermore, isoelectric points of many proteins are in acidic PH, the PH may cause a switch in cell charge from negative to positive, resulting in increased affinity for anionic surfactants.

There are some limitations in this study. We just picked one kind of gram-positive bacterium, one gram-negative and one fungus. There could be some difference in other types of bacteria, but we do not have to eliminate all the microbes, it is impossible, and lots of the microbes are symbiotic bacteria, many are beneficial. In further research, it might be possible to determine what part of microbial or genes affects the clearance. We provide a preclinic basis for the dermatologist, could help patients with diseases like acne improve quality of life, and give good advice in domestic laundry processes.

Abbreviations

BPO	Benzoyl peroxide
Brij C10	Polyalkylene glycol ethers
C. acnes	*Cutibacterium acnes*
CAPB	Cocamidopropyl betaine
IntDen	Integrated density
K. aerogenes	*Klebsiella aerogenes*
LI	Logarithmic increase
LR	Logarithmic reduction
MFU	McFarland units
PBS	Phosphate buffered saline
PI	Propidium iodide
RT	Room temperature
SDBS	Sodium dodecylbenzene sulphonate
T. rubrum	*Trichophyton rubrum*
TAED	Tetraacetylethylenediamine

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data set used/or analyzed during the current study are available from the corresponding author on reasonable request. The raw sequence data reported in this paper is available in the Genbank, accession number SUB10460522 gramneg OK350364.

Competing interests

Not applicable

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Not applicable

Authors' contributions

EW: planning of experiments, manuscript preparation. MR and BC: planning of experiments and correction of the manuscript. LF: Support with scientific advice. All authors have read and approved the manuscript.

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Figures

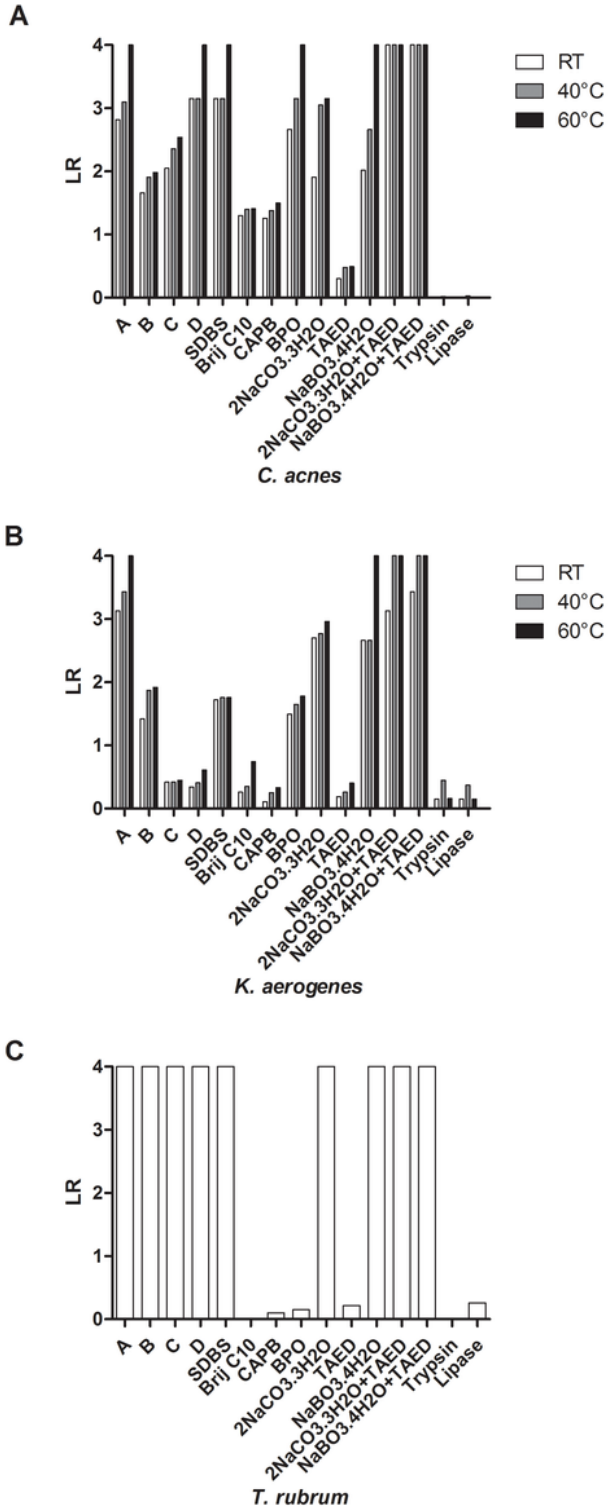

Figure 1

LR of three microbial at different temperatures A: LR of C. acnes treated with different detergents at RT, 40°C and 60°C. B: LR of K. aerogenes treated within different detergents at RT, 40°C and 60°C. C: LR of T. rubrum treated within different detergents at RT.

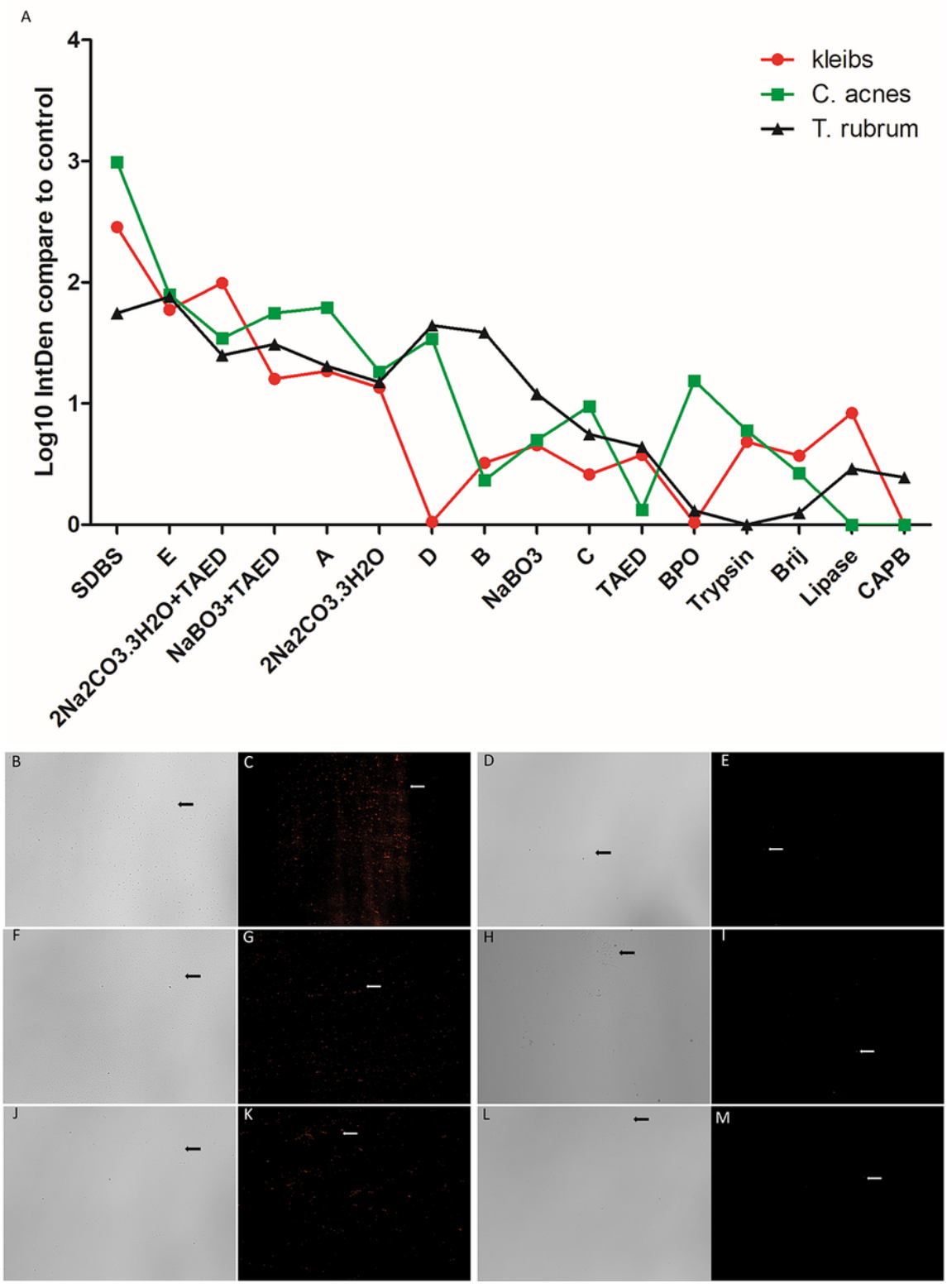

Figure 2

Qualification of fluorescence and representative photo Brightfield and fluorescent (RFP) pictures of microbes, arrows show the microbial cells and PI staining cells. A: Qualification of fluorescence. B: SDBS group of C. acnes under normal light. C: SDBS group of C. acnes under RFP light cube. D: Control group of C. acnes under normal light. E: Control group of C. acnes under RFP light cube. F: SDBS group of K. aerogenes under normal light. G: SDBS group of K. aerogenes under RFP light cube. H: Control group of

K. aerogenes under normal light. I: Control group of K. aerogenes under RFP light cube. J: E group of T. rubrum under normal light. K: E group of T. rubrum under normal light. L: Control group of T. rubrum under normal light. M: Control group of T. rubrum under RFP light cube.

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