Efficacy of Acetic Acid for Home Care Procedures.

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Abstract

Background: Acetic acid has been used for many decades to clean and disinfect surfaces in the household. Disinfection in the household is particularly important for the group of YOPI (young, old, pregnant, immunocompromised), as they represent a risk group. In addition to known pathogens, viruses are also becoming a major problem not only for this group, but due to the new Sars-CoV-2 virus all people are affected.

The aim of this study is to show that acetic acid has a disinfecting effect against certain microorganisms and is able to destroy certain viruses. Furthermore, a disinfecting effect of laundry in a simulated washing cycle will be investigated.

Results: At a concentration of 10% and an addition of 1.5% citric acid according to the specifications of DIN EN 1040 and DIN EN 1275, acetic acid showed a reduction of > 5-log steps in the following microorganisms: *P. aeruginosa*, *E. coli*, *S. aureus*, *L. monocytogenes*, *K. pneumoniae, E. hirae and A. brasiliensis*. For MRSA a logarithmic reduction of 3.19 was obtained.

The results of the test according to DIN EN 13697 showed a complete reduction (> 5-log steps) for *P. aeruginosa*, *E. coli*, *S. aureus*, *E. hirae*, *A. brasiliensis* and *C. albicans* already from a acetic acid concentration of 5%.

The results of the tests according to DIN EN 14476 and DIN EN 16777 showed a reduction of ≥ 4-log-steps against the Modified Vaccinia virus Ankara (MVA) for the tested acetic acid concentrations of 5%, 7.5% and 10%. This means a virucidal effect of 5% acetic acid.

Furthermore, the results showed that acetic acid does not have a sufficient disinfecting effect on microorganisms in a dosage that is commonly used for cleaning. However, this can be achieved by increasing the concentration of acetic acid used, especially when combined with citric acid.

Conclusions: Our results show a disinfecting effect of acetic acid in a concentration of 10% and an addition of 1.5% citric acid against a variety of microorganisms. A virucidal effect against enveloped viruses could also be proven. Furthermore, the results showed a disinfecting effect of acetic acid in domestic laundry procedures.

Keywords: acetic acid, antimicrobial, antifungal, antiviral, domestic hygiene

Background

For decades people have been using natural products like vinegar to clean and sanitize surfaces in the domestic environment (1). However, there is little scientific evidence on the antimicrobial efficacy of these traditional cleaning methods.

Inter alia, an appropriate, yet effective use of antimicrobial active products must be considered important to prevent the spread of infections. At home, especially young, old, pregnant and immunocompromised persons (YOPIs) are at risk. Many potential pathogens such as *Pseudomonas aeruginosa*, members of the *Enterobacteriacea*e family or even methicillin-resistant *Staphylococcus aureus* (MRSA) have already been found on household surfaces (2–6). In order to achieve an adequate hygiene at home, many people use bleaching agents, as these are readily available, relatively inexpensive and have a very good antimicrobial effect (7–9). On the other hand, consumers do not want to use chemical cleaning agents and thus like to use "green" alternatives such as vinegar. Already in 2000, Rutala *et al.* were able to show that undiluted white distilled vinegar has a strong effect against *Salmonella spp.* and *P. aeruginosa* at an exposure time of 30 s, but does not work well against *S. aureus* and *Escherichia coli* (10). Vinegar is mainly comprised of acetic acid, a weak organic acid, for which an antimicrobial effect is mainly delivered by its undissociated form, by passive diffusion through the cell wall of the bacteria. The resulting change of the internal pH is believed to have an inhibitory effect on the bacteria by releasing protons (11).

Acetic acid has already been used in the food industry to inhibit food pathogens. Various studies have shown a protective effect on various types of meat (12), tomatoes (13), carrots (14) and some salads (15). Further studies were also able to proof an inhibitory effect against some microorganisms such as *Enterobacteriaceae* (13,14,16–18).

Not only bacteria, but also viruses, such as the norovirus, which belongs to the *Caliciviridae* family (19,20) the annually occurring influenza virus (21) and above all the new coronavirus Sars-CoV-2 (22), must be considered important for domestic hygiene procedures. Norovirus is the leading cause of non-bacterial gastroenteritis in industrialised and developing countries (23). Here, infection usually occurs via the faecal-oral route, e.g. by ingesting contaminated food or water or via contact to droplets and aerosols of an infected person (24–27). Sars-CoV-2 , as a member of the *Coronaviridae* family, is an enveloped virus, which can cause a severe form of pneumonia and has impacted the global community in an unseen manner since its emergence in December 2019 (28). Apart from changing the daily life of billions of people, the corona pandemic has also led to a special perception for the proper inactivation of microorganisms in home care procedures and a fallback to traditional cleaning options with hygienic effects for lack of available disinfectants. As mentioned above, vinegar is widely believed to be an effective means for hygienic cleaning (29,30). However, there is little scientific evidence for the antimicrobial efficacy of acetic acid based products for domestic cleaning and laundering.

Hence, the present study aimed to provide data on the antimicrobial efficacy of acetic acid, especially when used in domestic cleaning and laundering procedures. For this purpose, antibacterial, antifungal and antiviral efficacy tests based on existing and adapted standard protocols have been conducted to evaluate the hygienic potential of acetic acid.(31–34)

Results

Bactericidal and fungicidal activity in suspension tests

To assess its possible use for hygienic cleaning, acetic acid in different concentrations and combined with citric acid, was first evaluated in suspension tests according to DIN EN 1040 and DIN EN 1275. The logarithmic reduction factors (LR) for an extended spectrum of test strains are summarised in Fig. 1.

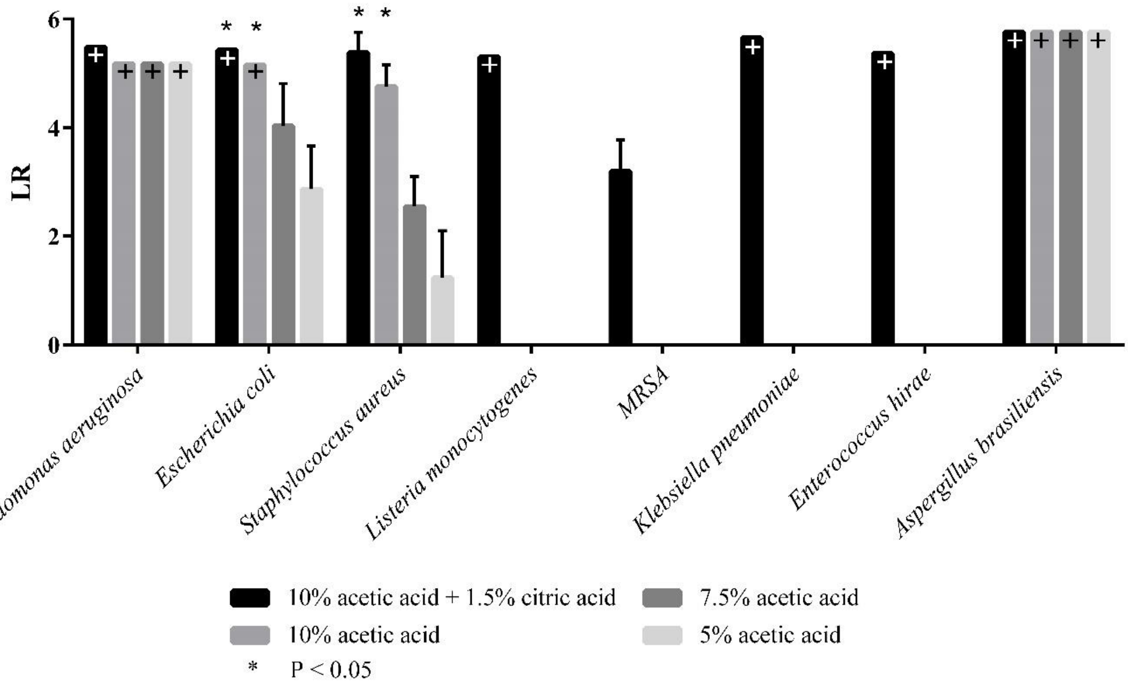

Fig. 1 *LR for Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Aspergillus brasiliensis, Listeria monocytogenes, Methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae and Enterococcus hirae (according to DIN EN 1040:2006-03 and DIN EN 1275:2006-03). The different values for LR max. were obtained due to different initial loads. [+] indicates a complete reduction of the microbial load*

The results show that acetic acid in all tested concentrations lead to a complete reduction for *P. aeruginosa* and *A. brasiliensis*. For *E. coli,* a complete reduction could be achieved when using 10% acetic acid concentration, either alone or in combination with 2% citric acid. *S. aureus* an of the logarithmic reduction (LR) increased with increasing concentrations of acetic acid and reached a maximum when 10% acetic acid and 2% citric acid was used, without, however, being able to exhibit a complete reduction. Furthermore, a complete reduction was achieved with a test concentration of 10% acetic acid with the addition of 2% citric acid for the microorganisms *L. monocytogenes* and *K. pneumoniae*. For MRSA a maximum reduction of 3.19 was achieved. At an acetic acid concentration of 10%, a complete reduction was achieved for *P. aeruginosa*, *E. coli* and *A. brasiliensis*. For *S aureus* an LR of 4.75 could be detected. At acetic acid concentrations of 7.5% and 5% respectively, no sufficient reductions (LR 4.03 to 1.23) could be achieved for the microorganisms *E. coli* and *S. aureus*.

The microorganisms *L. monocytogenes*, MRSA, *K. pneumoniae* and *E. hirae* were only tested with 10% acetic acid + 1.5% citric acid, as this was the only concentration at which the other microorganisms achieved a LR of >5 log steps.

Bactericidal and fungicidal activity in surface tests

The antimicrobial efficacy of 5% and 10% acetic acid as well as a combination of 10% acetic acid and 1.5% citric acid was evaluated on surfaces using a protocol based on DIN EN 13697, since suspension tests are not reflecting this application very well.

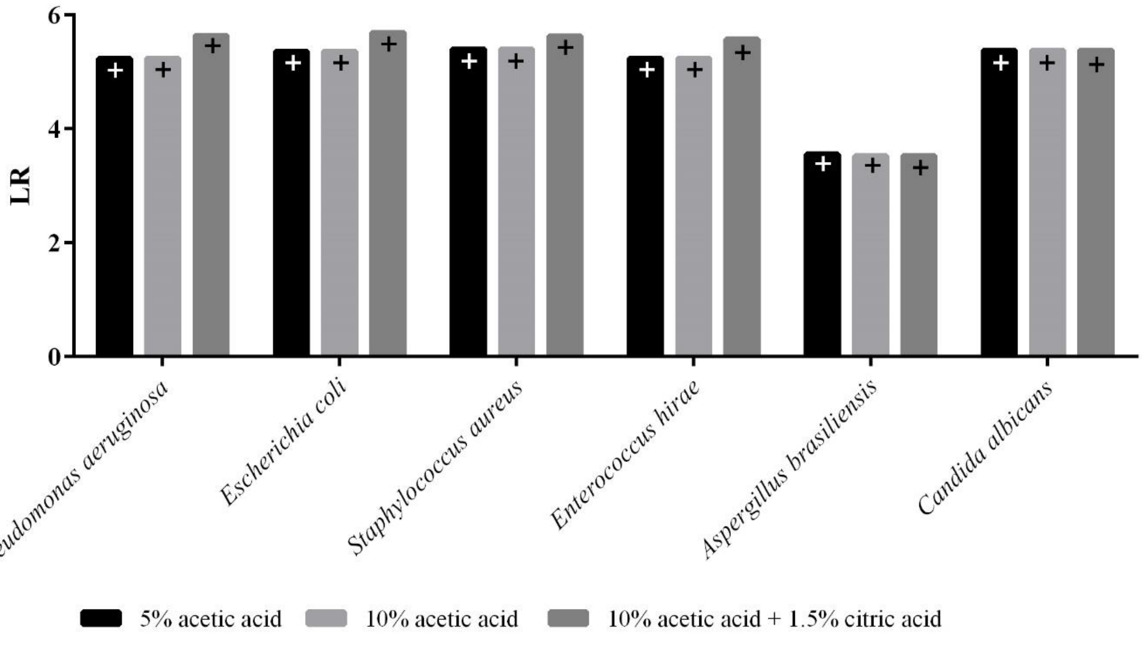

Fig. 2: *LR for P. aeruginosa, E .coli, S. aureus, A brasiliensis, C. albicans and E. hirae (DIN EN 13697:2015-06). The different values for LR max [+] (indicating a complete reduction of the microbial load) were obtained due to different initial loads.*

The results show that for all tested microorganisms in the three tested concentrations a complete reduction could be demonstrated.

Virucidal activity

In order to test the virucidal activity of acetic acid, the tests were carried out in accordance with the standards EN 14476 (35) and EN 16777 (36), where the effect against the Modified Vaccinia virus Ankara (MVA) was tested in suspension and on surfaces, respectively (Fig. 3).

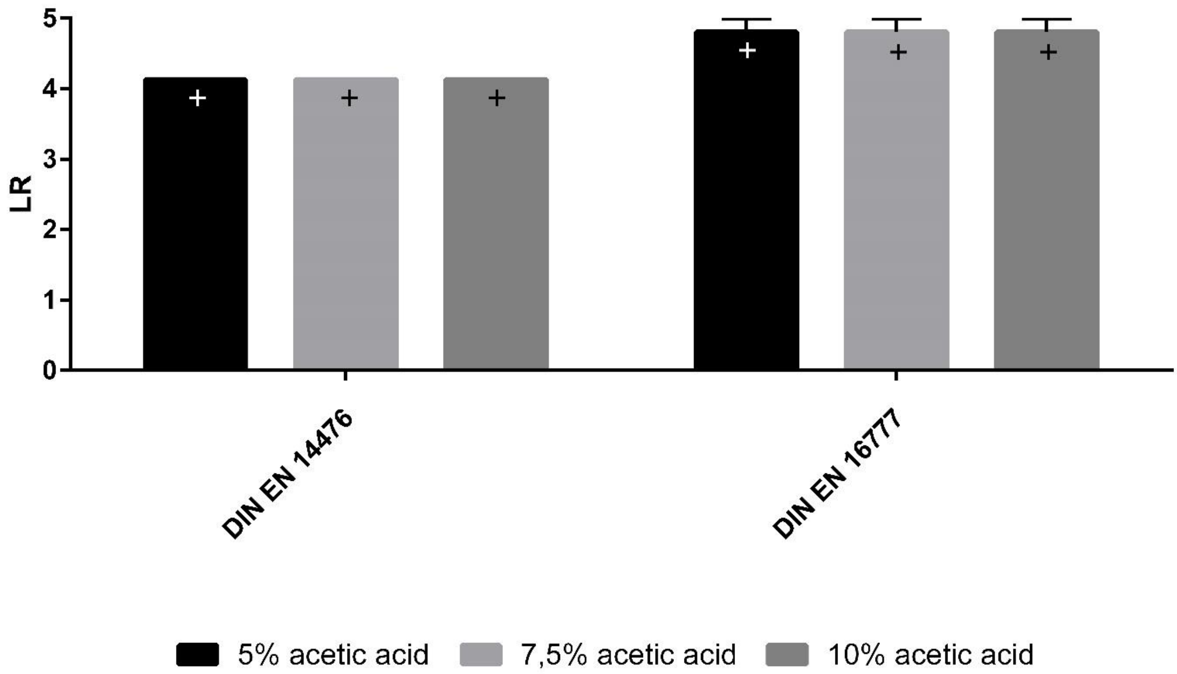

Fig. 3: *LR for acetic acid concentrations of 5%, 7.5% and 10% or 15% according to EN 14476 and EN 16777. The experiments were tested against the modified vaccinia virus Ankara (MVA). [+] means that the values are ≥ the value shown.*

The results of the virucidal tests according to DIN EN 14476 and DIN EN 16777 show that for all tested acetic acid concentrations 5%, 7.5% and 10% a complete reduction ≥ 4 log steps could be achieved. According to the standards, a product is considered virucidal as soon as it has achieved a reduction of ≥ 4 log steps.

Antibacterial activity in laundering procedures

To assess a putative effect of acetic acid in a laundry application, the LR of selected microorganisms was determined in a simulated main wash cycle using a lab-scale washing machine (Rotawash). In contrast to the previous tests, a total concentration of 0.3% or 0.75%

acetic acid was added to the wash liquor, alongside with a standard laundry detergent. The LR achieved in these tests are shown in Fig. 4.

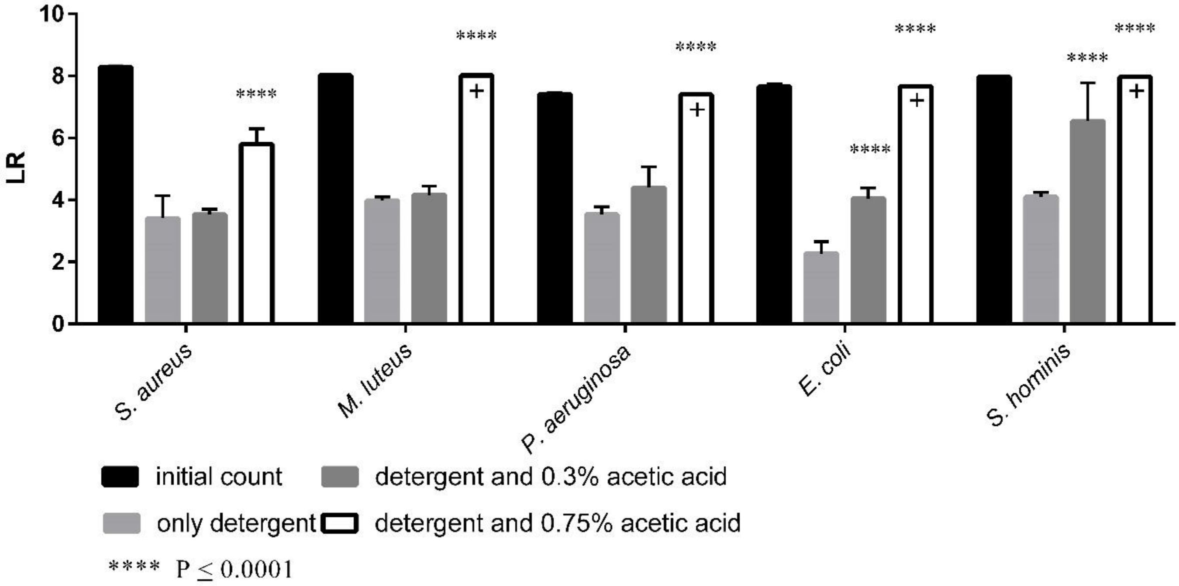

Fig. 4: *LR of S. aureus, M. luteus, P. aeruginosa, E. coli and S. hominis after a simulated main wash cycle (60 min at 30 °C) in the Rotawash using liquid detergent. A washing cycle without addition of acetic acid, a wash cycle with addition of 0.3%L acetic acid and a wash cycle using 0.75% acetic acid. The different values for LR max [+] (indicating a complete reduction of the microbial load) were obtained due to different initial loads. (n=3)*

The results show that for *S. aureus*, *M. luteus* and *P. aeruginosa* there was no significant difference in the LR between a wash cycle where only detergent was used or a cycle where 0.3% acetic acid was added to the wash liquor including the detergent. In contrast, a significant increase of the LR could be demonstrated for the *E. coli* and *S. hominis* when 0.3% acetic acid was added. Furthermore, a significant increase in LR could be observed for all tested microorganisms when 0.75% acetic acid was added to the wash liquor. Here, a complete reduction could be observed for all bacterial test strains, except for *S. aureus*, for which a LR of 5.8 was determined.

Discussion

The current study aimed to investigate the antimicrobial, antifungal and antiviral effects of acetic acid for domestic cleaning and laundering based on different standard procedures and comprehensive tests. Although there are many studies that have investigated the antibacterial and antifungal effects of acetic acid (15,37–42) there is no available data on how acetic acid does perform in standard procedures for the testing of disinfectants in suspension or on surfaces. Likewise, the potential of acetic acid for laundry procedures has not been investigated before, although it is known that consumers sometimes use this substance as an additive to increase the hygiene performance of laundering (1). Finally, it turned out that the Corona pandemic in 2020 lead to an increased demand for pragmatic, yet effective solutions to improve domestic hygiene, particularly with regards to viruses.

The results of this study showed that formulas containing an10% acetic acid and 1.5% citric acid are able to meet the standard requirements for disinfectants (i.e. a LR of >5), for all tested bacterial and fungal strains except for MRSA, which fits well with the findings of numerous other studies (38–40,43–47). In addition to the suspension tests (DIN EN 1040 and DIN EN 1275), DIN EN 13697 was used to test the disinfectant effect on a surface. Here, the results obtained clearly show that a complete reduction could achieved for all tested microorganisms even at lower concentrations of acetic acid.

Ayhan and Bilici could show that acetic acid disrupts the cell wall structure and thus causes a loss of ATP in the cell (43). Another study suggests that polyphenol compounds may also play a role in the antimicrobial effect of acetic acid. It was proven that polyphenols combine with the peptidoglycan structure of the cell wall and the phospholipid bilayer in the outer membrane of gram negative bacteria and thus impair the integrity of the cell. Furthermore, polyphenols were shown to interfere with the activity of the intracellular bacterial enzymes by inhibiting the formation of amino and carboxyl groups of proteins (44). This supports the findings that polyphenols present in the acetic acid possess antimicrobial activity against a broad spectrum of microorganisms (48,49).

Nastou *et al.* tested the effects of household washing treatments to control *L. monocytogenes* from lettuce. It was shown that application of 1% acetic acid resulted in a reduction of microorganisms by 1 log. According to the results of Nastou *et al.*, which were able to disrupt an inhibitory activity of acetic acid, this effect is proportional to the concentration used (45). Medina *et al.* also showed that vinegar (acetic acid) led to a complete reduction of *L. monocytogenes* and killed a high number of *E. coli* and *S. aureus* (39). These results support the data obtained in this study, as the maximum LR of *L. monocytogenes* of 7.31 was achieved with a 10% acetic acid concentration and a citric acid concentration of 1.5%. Furthermore, LRs of 5.43 and 5.39 for *E. coli* and *S. aureus* were achieved, respectively.

Gopal *et al.* showed in 2017 that an acetic acid concentration of 25% led to a complete reduction of *B. subtilis*, *E. coli* and *P. aeruginosa* from. These results are consistent with some pre-tests of the work presented here (data not shown). Furthermore, the study of Gopal et al. indicated that a 10% acetic acid led to a complete reduction for *Aspergillus niger* (now: *A. brasiliensis* (50)) and a reduction of more than 2 log steps for *Candida albicans* (*C. albicans*) (40). The results obtained in the present study largely agree with these findings. The study at hand also obtained a complete reduction for *A. brasiliensis* already at an acetic acid concentration of 5%. The results for *C. albicans*, however, are different in the current study, since were also able to achieve a complete reduction of *C. albicans* at a low acetic acid concentrations (5%). These differences might be explained by the vinegar used, since Gospal *et al.* used an apple cider vinegar, whereas in the current study vinegar made from acetic acid diluted with water and purified to a high degree of purity was used.

Ryssel *et al.* investigated whether acetic acid might be used as an alternative for common local antiseptics. They mixed 0.1 mL bacteria solution (bacterial count approx. 10^7 – 10^8 cfu/mL) with 9.9 mL acetic acid (3%) and incubated the mixture for 5, 30 and 60 min at a temperature of 37 °C. They showed that at 3% acetic acid concentration no colonies of *P. aeruginosa*, of *P. vulgaris*, of *A. baumanii* and of β-haemolytic Group B *Streptococci* could be detected after

5 min incubation. Furthermore *E. coli*, *E. faecalis* and MRSA were eliminated after a exposure time of 60 min (47). Our experimental design used an incubation time of 5 min and also showed a complete reduction of *P. aeruginosa*. In contrast to the attempt of Ryssel *et al.* the current study also tested up to a concentration that would be required to pass disinfection tests, which for most observed microorganisms was 10% acetic acid and 1.5% citric acid. At this concentration, a complete reduction for *E. coli* and a LR of 3.19 for MRSA could already be demonstrated with an incubation time of 5 min.

Overall, there has been little research in the literature on the virucidal effect of acetic acid against enveloped viruses. In 2010 Greatorex *et al.* were able to show in a study that acetic acid in a concentration of 10% is effective against the influenza virus A/H1N1 (51). This result agrees with those of the present study, which showed that acetic acid is effective against the MVA at a concentration of 5%. This could be demonstrated on the basis of the standards DIN EN 14476 and DIN EN 16777, which apply to disinfectant tests with regard to virucidal activity. The present study could confirm the virucidal effect of acetic acid on the basis of existing standards. However, no tests were carried out on the virucidal effect in washing machines, because Heinzel *et al.* were able to show in 2010 that conventional household washing detergents achieve a complete reduction of enveloped and non-enveloped viruses already at 40°C (52).

The results of the simulated washing process using the Rotawash showed that a complete reduction of four microorganisms (*M. luteus*, *P. aeruginosa*, *E. coli* and *S. hominis*) could be achieved by adding an acetic acid concentration of 0.75% to the wash liquor. Likewise, a high LR of 5.8 could be achieved for *S. aureus*. Thus, a disinfecting effect of acetic acid was proven for all tested microorganisms at an effective concentration of 0.75% acetic acid. The acetic acid concentration of 0.3% was used since it corresponds approximately to the dosage of commercially available laundry sanitizers (53). Assuming that a common washing machine uses approx. 10 L of water for each wash step, a final concentration of 0.3% would equal a dosage of 120 mL of a commercially available vinegar essence containing 25% acetic acid. Consequently, a final concentration of 0.75% would require the use of 300 mL vinegar essence, which is still in the range that can be considered to be applied by consumers. The results showed that for *S. aureus* and *M. luteus* no additional antimicrobial effect was detected for the lower concentration of acetic acid compared to a simulated wash cycle with detergent alone. However, a significant difference (2- way- ANOVA) for an additional dosage of 0.3% acetic acid could be demonstrated for *E. coli* and *S. hominis*, which also exhibits a disinfecting effect with an LR of 6.5. These findings suggest that a considerable antibacterial effect may be expected, when acetic acid is used a hygiene additive for laundry.

Conclusion

The results of this study show that acetic acid in a concentration of 10% and an addition of 1.5% citric acid has a disinfecting effect against a variety of microorganisms. In addition to the typical pathogens *E. coli*, *S. aureus* and *L. monocytogenes*, also *P. aeruginosa, K. pneumoniae, E. hirae, A. brasiliensis* and *C. albicans* are among the microorganisms that achieve a reduction of > 5-log steps against acetic acid in the concentration mentioned. Furthermore, this study was able to show that acetic acid in a concentration of 5%, 7.5% and 10% is also effective against enveloped viruses.

Moreover the present study showed that acetic acid above a certain concentration also has disinfecting properties on the laundry in a washing machine. It could be shown that an above-average dosage of the acetic acid *S. aureus*, *M. luteus*, *P. aeruginosa*, *E. coli* and *S. hominis* > 5 log- steps are reduced.

Methods

Determination of the bactericidal and fungicidal activity in suspension tests

The suspension tests were performed according to the standards DIN EN 1040:2006 and DIN EN 1275:2006 (31,32) for bacteria and fungi and to the standard DIN EN 14476 (35) for viruses. Unlike described in DIN EN 1040 and DIN EN 1275 1 mL was used in the experiments instead of 10 mL. All products were tested at room temperature.

The virucidal tests were carried out strictly according to DIN EN 14476. The calculation of the reduction factors was done as described in chapter 'Microbiological and statistical analysis'.

All strains were purchased at the German Collection of Microorganisms and Cell Cultures (DSMZ, Brunswick, Germany), except from the Methicillin resistant *Staphylococcus aureus* (MRSA), which was derived from the Culture Collection of the University of Gothenburg (CCUG). The corresponding strain code of the American Type Culture Collection (ATCC) is provided in Table 1 for information only.

Table 1: Fungal, bacterial and viral test strains

Strain	Code
Fungal strains:	
Aspergillus brasiliensis	DSM 1387, ATCC 16404
Candida albicans	DSM 1386, ATCC 10231
Bacterial strains:	
Enterococcus hirae	DSM 3320, ATCC 10541
Escherichia coli	DSM 682, ATCC 10536
Klebsiella pneumoniae	DSM 26371, ATCC 700603
Listeria monocytogenes	DSM 20600, ATCC 15313
Micrococcus luteus	DSM 1790, ATCC 10240
Pseudomonas aeruginosa	DSM 939, ATCC 15442
Staphylococcus aureus	DSM 799, ATCC 6538

Staphylococcus aureus, Methicillin resistant	CCUG 35601
Staphylococcus hominis	DSM 20329, ATCC 27845
Viral strains:	
Modified Vaccinia Ankara virus (MVA)	ATCC VR-1508

Determination of the bactericidal, fungicidal and virucidal activity in surface tests

The determination of bactericidal an fungicidal activity on surfaces was performed according to DIN EN 13697 (33) and for virucidal activity according DIN EN 16777 (36). All tests using bacterial strains were executed at Rhine-Waal-University of Applied Sciences; for virucidal tests, an external lab (Dr. Brill und Dr. Steinmann Institute for Hygiene and Microbiology, Hamburg, Germany) was commissioned by the funder of this study.

All tests were performed in presence of an organic challenge (0.3 g * L^{-1} albumine, 0.1 L A. dest.) at room temperature. For the tests according to DIN EN 13697 *P. aeruginosa*, *E. coli*, *S. aureus*, *E. hirae*, *A. brasiliensis* and *C. albicans* were used; for the tests according to DIN EN 16777 the Modified Vaccinia Ankara virus (MVA) was used. The calculations of the reduction factors were performed as described in chapter 'Microbiological and statistical analysis'.

Determination of the antibacterial activity in laundering procedures using a laboratory washing machine

To assess the antimicrobial performance of products containing acetic acid for use in laundry detergents, a laboratory washing machine (Rotawash M228C, SDL Atlas, Rock Hill, SC, USA)) was used as described in Schages *et al.* (34). To simulate a normal household washing machine all quantities were downscaled adequately, i.e. a 1 L vessel was filled with 0.5 L of water in addition to the ballast load textiles, the soil ballast, the detergent and eight steel beads (to simulate the mechanics of a washing machine) as described below.

In this study, cotton (wfk 10 A, 170 g/m^2, wfk testgewebe, Brüggen, Germany) was used as the ballast load. In addition to the ballast load, SBL2004 (SBL2004, wfk testgewebe, Brüggen, Germany) was used as a source of organic soil. All materials used are calculated based on the volume of water in a vessel of the laboratory washing machine:

Ballast load (100 g/vessel) consisted of 96.5 g textile ballast of standard cotton and of 3.5 g textile comprised by the SBL2004 swatches equalling approx. 1.2 g standard soil. A liquid heavy duty detergent (Ariel Actilift, Procter & Gamble, Germany) was dosed according to the detergent manufacturers' instructions (120 mL/ 10 L) and adjusted to the volume of one vessel of the Rotawash (6 mL/0.5 L).

The duration of the wash cycle in the Rotawash was 60 min, and the temperature was adjusted to 30 °C, at a water inlet temperature of approx. 15 °C – 20 °C. In every test run five artificially contaminated biomonitors (one swatch per microorganism) are added to one vessel. In this series of experiments *S. aureus, M. luteus, P. aeruginosa, E. coli* and *S. hominis* were tested. All tests in the Rotawash were run in triplicates. The evaluation is performed as described in chapter 'Microbiological and statistical analysis'.

Microbiological and statistical analysis

The microbial count on each contaminated biomonitor was quantified by extraction with 1 mL TSB-TLH-thio (TSB with 30 g*L^{-1} Tween 80, 0.3 g*L^{-1} lecithin, 1 g*L^{-1} histidine, 5 g*L^{-1} sodium-thiosulfate) followed by investigating the colony forming units (cfu/mL) on surface culture on TSA (Oxoid, Wesel, Germany; incubation at 37 °C for 24 h). Rotawash-tests were carried out in a 1.5 mL reaction tube (Sarstedt, Nürmbrecht, Germany) for 10 min at 15 °C and 1000 rpm in an orbital incubating shaker (Thermomix comfort, Eppendorf, Hamburg, Germany).

The colony forming units (cfu/mL) were investigated in surface culture either on TSA for bacteria (incubation at 37 °C for 24 h) or MEA for *C. albicans* and *A. brasiliensis* (incubation at 30 °C for 48 h). After laundering, the microbial count on the test swatches is determined similarly. The number of colony forming units (cfu/mL) on plates was used to calculate the microbial load in the extraction liquid (c_{wei}) (Eq. (1)):

$$C_{wei} = \frac{\sum C}{(n_1 * 1) + (n_2 * 0.1)} * d \quad (1)$$

C_{wei} = weighted arithmetic average

$\sum C$ = sum of viable cell count of all agar plates, used for calculation

n_1 = count of agar plates with the lowest evaluable dilution

n_2 = count of agar plates of the next higher dilution stage

d = dilution factor of the lowest evaluable dilution stage

Plates with less than 10 cfu or more than 300 cfu were not considered.

To calculate the LR, the logarithmic cfu value of the biomonitors was subtracted from the logarithmic mean of the initial microbial count of the respective biomonitors.

$$LR = K_0 - K_S \quad (2)$$

LR = logarithmic reduction factor

K_0 = common logarithmic of the microbial count per mL of the initial load on the swatches before laundering

K_S = common logarithmic of the microbial count per mL of the initial load on the swatches after laundering

Unless otherwise stated, the tests were performed in triplicates and statistically evaluated in the case of a non-Gaussian distribution using Students *t*-test, Kruskal-Wallis or in the case of a Gaussian distribution using a 2-way ANOVA.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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This work was financially supported by Speyer & Grund GmbH & Co. KG. The funding body had no role in designing the study, sample collection, analysis, and interpretation of data or writing the manuscript.

Authors' contributions

MKZ performed antibacterial and antifungal tests, analysed the data and wrote the manuscript. DB designed the study, analysed the data and edited the manuscript.

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