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The University Of Sheffield.
Acetic Acid, the Active Component of Vinegar, Is an Effective Tuberculocidal Disinfectant

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ABSTRACT Effective and economical mycobactericidal disinfectants are needed to kill both Mycobacterium tuberculosis and non-M. tuberculosis mycobacteria. We found that acetic acid (vinegar) efficiently kills M. tuberculosis after 30 min of exposure to a 6% acetic acid solution. The activity is not due to pH alone, and propionic acid also appears to be bactericidal. M. boletii and M. massiliense nontuberculous mycobacteria were more resistant, although a 30-min exposure to 10% acetic acid resulted in at least a 6-log10 reduction of viable bacteria. Acetic acid (vinegar) is an effective mycobactericidal disinfectant that should also be active against most other bacteria. These findings are consistent with and extend the results of studies performed in the early and mid-20th century on the disinfectant capacity of organic acids.

IMPORTANCE Mycobacteria are best known for causing tuberculosis and leprosy, but infections with nontuberculous mycobacteria are an increasing problem after surgical or cosmetic procedures or in the lungs of cystic fibrosis and immunosuppressed patients. Killing mycobacteria is important because Mycobacterium tuberculosis strains can be multidrug resistant and therefore potentially fatal biohazards, and environmental mycobacteria must be thoroughly eliminated from surgical implements and respiratory equipment. Currently used mycobactericidal disinfectants can be toxic, unstable, and expensive. We fortuitously found that acetic acid kills mycobacteria and then showed that it is an effective mycobactericidal agent, even against the very resistant, clinically important Mycobacterium abscessus complex. Vinegar has been used for thousands of years as a common disinfectant, and if it can kill mycobacteria, the most disinfectant-resistant bacteria, it may prove to be a broadly effective, economical biocide with potential usefulness in health care settings and laboratories, especially in resource-poor countries.

Mycobacteria are important agents of human infections. While the most well-known infections are tuberculosis and leprosy, infections with nontuberculous mycobacteria are an increasing problem in soft tissues after surgical or cosmetic procedures (1) or in the lungs of cystic fibrosis and immunosuppressed patients (2). Killing mycobacteria in health care settings is important because Mycobacterium tuberculosis strains can be multidrug resistant and therefore potentially fatal biohazards, and environmental mycobacteria must be thoroughly eliminated from surgical implements and respiratory equipment.

Disinfection of mycobacteria can be a problem, as mycobacteria are the most disinfectant-resistant bacteria (4). Quaternary ammonium compound (QAC) disinfectants are regarded as only mycobacteriostatic (4), and QAC tolerance is a problem with mycobacteria of the Mycobacterium abscessus complex (5). Some of the more effective disinfectants (6), such as chloramine bleach and peracetic acid, can be relatively toxic, unstable, or expensive (4), and the emergence of glutaraldehyde-resistant strains has caused extensive outbreaks of nontuberculosis infections (7). While using acetic acid as a solvent, we fortuitously found that it had significant mycobactericidal activity. Vinegar has been used as a disinfectant for thousands of years (8, 9) and is today commonly used for, among other things, eliminating bacteria from fresh produce (10, 11) or curing acute otitis externa (12, 13). There is a description of its use in the Middle Ages for treating infected wounds and scrofula, which generally means tuberculosis of the lymph nodes in the neck (http://en.wikipedia.org/wiki/Medieval_medicine_of_Western_Europe). In this study, we quantitated the significant mycobactericidal activity of acetic acid and suggest that it may be a useful, low-cost, and effective disinfectant.

Acetic acid or vinegar was added to aliquots of bacterial cultures in proportions to create the concentrations shown in Table 1. Commercial white vinegar was used whenever possible. The cultures were then inverted several times and incubated at room temperature without agitation for the specified periods (20 to 30 min). They were then centrifuged, and the pellet was resuspended in Middlebrook 7H9 medium supplemented with oleic acid-albumin-dextrose-catalase (OADC). Aliquots of serial dilu-
TABLE 1  Bactericidal effect of increasing exposure times and acid concentrations

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<th>Acid and/or conditions</th>
<th>BSA and RBC concn (%)*</th>
<th>pH with M. abscessus*</th>
<th>Exposure time (min)</th>
<th>Final concn (%)</th>
<th>E. coli DH5α survivors</th>
<th>M. smegmatis</th>
<th>M. tuberculosis mc²7700</th>
<th>M. tuberculosis H37Rv</th>
<th>MDR M. tuberculosis</th>
<th>XDR M. tuberculosis</th>
<th>M. abscessus</th>
<th>M. bolletii R</th>
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a High-level disinfectant activity is indicated in bold.

b The numbers represent the reductions, expressed in log₁₀, in the number of colonies recovered after the acid exposures, compared to that of controls exposed to sterile water alone under the same conditions.

c BSA, bovine serum albumin; RBCs, human red blood cells.

d M. abscessus with optical density at 600 nm of 1 was diluted 1:10 in the acetic acid solutions.

e Limit of detection (no colonies recovered from exposed bacteria).

f R and S refer to the rough and smooth morphotypes of M. massiliense.
nontuberculous mycobacteria, although the 20- to 30-min exposure time required to obtain optimal killing is longer than the 5 min recommended for some commercial bactericides. Exposure to 6% acetic acid for 30 min resulted in an 8-log_{10} reduction of viable M. tuberculosis bacteria, including XDR and MDR strains. In tests with the M. abscessus complex, which are emerging as the most pathogenic of the nontuberculous mycobacteria (17), the same conditions produced a 8-log_{10} killing only with M. abscessus sensu stricto, while M. massiliense and M. bolletti were more resistant in some assays. However, the generally accepted definition of an effective mycobactericide is one that has the ability to reduce viable bacteria by 4 to 5 log_{10} (18), while the 6-log_{10} reduction of both these bacteria achieved with 10% acetic acid for 30 min would be classified as high-level mycobactericidal activity (19). In some assays in which 10^7 M. smegmatis bacteria were exposed to 6% acetic acid for 20 min and 3×10^7 M. massiliense were exposed to 10% acetic acid for 30 min, there were no survivors. Although we did not attempt carrier tests, the effective mycobactericidal activity was maintained even under “dirty” conditions that simulate contamination with organic material. While this level of killing may not be adequate for all critical uses, such as sterilizing surgical instruments, it is likely that higher levels of killing of these highly resistant strains could be achieved with higher concentrations of acetic acid and/or longer exposure times than the ones tested here. Preliminary studies, however, suggest that 10% acetic acid for 30 min does not kill Bacillus subtilis spores (data not shown), so it cannot be classified as a high-level general disinfectant (20). We have not assessed the activity of acetic acid against viruses and fungi.

Although the disinfectant properties of organic acids such as acetic acid, propionic acid, and butyric acid—a component of sweat (21)—are well known, they are not usually included in reviews of bactericides (4). However, in the early part of the 20th century, they were fairly extensively studied for disinfectant properties, as reviewed by Reid in 1932 (22), and their tuberculocidal activities, as reviewed by Barker in 1964 (23). It was found that they had broad bactericidal activity that increased with increasing carbon chain length through caprylic acid (C = 8), although the longer-chain lactic acids were less soluble. Bactericidal activity was also found to increase with decreasing surface tension of the organic acid solutions and appeared to be due to the undissociated acid rather than the hydrogen ion concentration. It was therefore suggested that the bactericidal effect might be related to the ability of the acids to pass through the bacterial membrane (23).

Acetic acid is not very toxic, although prolonged exposure will produce corrosive effects, both on skin and metals. In reports from nearly 100 years ago, it was found that the topical application of a 1% acetic acid solution in saline cured Pseudomonas aeruginosa (Bacillus pyocyaneus) wound infections (24, 25). It might be worthwhile testing its effectiveness as a topical agent on mycobacterial ulcers (26, 27).

Acetic acid is relatively inexpensive—2.5 liters of 99% acetic acid costs less than US$100 and could effectively disinfest up to 20 liters of M. tuberculosis cultures or sputa. Commercial vinegar bought in supermarkets was used wherever possible in the experiments described here, but the concentrations vary from country to country. Commercial vinegar could be used at effective concentrations for M. smegmatis or M. tuberculosis in France, where it is sold as 8% acetic acid, but not in the United States or Venezuela, where vinegar is sold as 5% acetic acid. While longer-chain organic acids may have better bactericidal activity, acetic acid (vinegar) is relatively nontoxic, inexpensive, and available, which could make it an effective, economical biocide for disinfecting M. tuberculosis from clinical specimens, cultures, and laboratory surfaces, and it would be particularly useful in low-income countries. The high-level capacity of acetic acid in killing mycobacteria, regarded as the most disinfectant-resistant bacteria due to the structure of their lipid-rich cell walls (4), suggests that perhaps it should be revived as a broadly effective bactericide that can be used as a general sanitizer.

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REFERENCES


