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ANTIMICROBIAL EFFECT OF SODIUM ACETATE AND OTHER HYGROSCOPIC SALTS

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ABSTRACT: A cotton textile dried with various hygroscopic salts then contaminated with *Staphylococcus epidermidis* and dried again, showed remarkable reduction in viable bacterial cells. Of the salts investigated, sodium acetate was found to have the greatest antibacterial effect. Calcium chloride, magnesium chloride and magnesium sulfate, also showed some antibacterial effect. For sodium acetate, drying for 24 h reduced the count of viable cells to 1.5%, and less, relative to the count in the control sample. For the other salts the count of viable cells after drying was more variable and ranged from 0.3% to 44% relative to the count in the control sample. It is inferred that the hygroscopic nature of the salts may enhance the effect of desiccation in killing bacteria during drying. The results indicate that salt-enhanced drying is a new class of sanitizing method warranting further investigation.

Keywords: Antimicrobial; Sodium acetate; Hygroscopic; Staphylococcus epidermidis; Textile

1. INTRODUCTION

There is on-going need for new methods to control bacteria as they develop resistance to antiseptics and disinfectants [1] and resistance to antibiotics [2]. Bacteria also affect more simple concerns of daily life such as the occurrence of body odor [3], [4], [5] and odors developed in clothing [6]. Laundering of textiles is not always effective in the removal of bacteria which cause odors [7]. The ability of bacteria to remain in textiles also affects their use as cleaning cloths [8]. Recent developments in antimicrobial research has included nano-structured materials on surfaces [9] and textiles [10]. Examples include the application of metal nanocomposites [11], functional polymers [12] and coating cotton fibres with silver nanoparticles [13], [14]. Such antibacterial approaches need not only demonstrate efficacy against microbes but also the absence of harmful impacts including inflammatory responses and cytotoxicity [15].

A wide range of naturally occurring materials have been investigated for antimicrobial properties. For example, naturally occurring and synthesised clay minerals have been found to have antimicrobial properties [16], [17]. Bathing in naturally occurring salts has been considered to have health benefits [18] including for skin complaints [19]. Common household substances such as acetic acid (vinegar) have a long history of use as antiseptics and preservatives. “The main reasons for the use of acetic acid as a food preservative are its toxicity value, commercial availability, and low cost. ... Acetic acid and certain other organic acids appear to have a toxicity in excess of that which could possibly be due to the pH alone.” [20]. Recent research confirms that, although pH can be the dominant effect, acetic acid does have inherent antimicrobial properties [21]. Antibacterial effects of other acid systems have been investigated [22]. Antimicrobial effects of sodium salts of organic acids including sodium acetate has been studied in food preservation [23]. In relation to acetic acid, it is noted that peracetic acid is a well known antiseptic [1], [24], [25].

It has long been known that bacteria are very sensitive to conditions of temperature and humidity [26]. One of the most effective methods of sterilization of surfaces is the application of dry heat [27], [24]. Research is being conducted into the mechanisms by which desiccation kills bacteria and the development of resistance to desiccation and ionizing radiation by some bacteria [28] including in extreme environments such as Antarctica [29]. Drying does not necessarily kill bacteria and can be used to keep microbes in a stable live state [30], [31]. Desiccation tolerance of prokaryotes including bacteria has been studied [32], [33] and has applications for health of human cells [34]. The impact of water stress on organisms is related to the osmotic role of salts [35]. Hygroscopic and deliquescent minerals may play a part in storing liquid water and facilitating life in hostile environments such as Mars [36]. Hygroscopicity can be determined and classified by observing mass changes at different relative humidity conditions [37]. This study investigates antimicrobial properties of some common salts associated with drying.

2. METHODOLOGY

2.1 Selection of salts

Four salt compounds were selected for use in the study, being sodium acetate, calcium chloride, magnesium chloride and magnesium sulfate. The four compounds are readily available substances known to have hygroscopic properties [38]. The four compounds have a range of uses in food preparation and are known by 'E' numbers: sodium acetate, E262(i); calcium chloride, E509: magnesium chloride, E511; magnesium sulfate, E518 [39], [40].

2.1 Salt application

A plain white cotton textile was selected as a substrate for the experiments. The textile was washed twice to remove any dressing agents and cut into 15 mm diameter circular sample discs (Figure 1 A). A solution of each of the four salts was prepared (1 M). Each solution (100 μL) was applied to separate textile sample substrate followed by drying for one hour at 60°C.

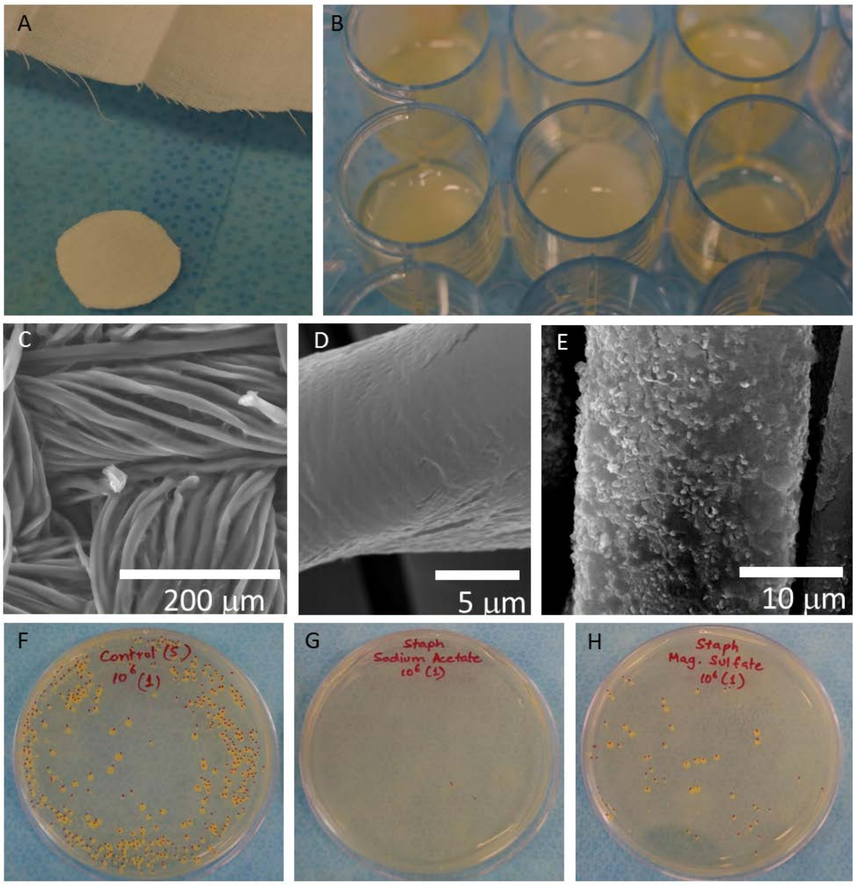

Fig. 1 (A) Cotton textile cut into a 15 mm diameter disc. (B) Wells used for conducting experiments. SEM images of (C) untreated cotton textile showing woven threads comprised on individual fibers, (D) an individual untreated cotton fiber and (E) an individual fiber with sodium acetate precipitated in the aseptic drying phase of the experiment. Gold coated, voltage 30 kV, pressure 0.5 Torr. (F-H) Examples of plate counting results (identified in Table 1)

At this stage scanning electron microscope (FEI Quanta 200 ESEM at RMIT University) imaging was conducted to observe the distribution of the precipitated salts. Samples of textile were gold coated and observed at a voltage 30 kV under low pressure (0.5 Torr). The textile comprises woven threads approximately 200 μm in diameter (Figure 1 C). Each thread is composed of fibers approximately 20 μm in diameter (Figure 1 D). The sodium acetate showed formation of sub-micron scale grains on the cotton fibers (Figure 1 E).

2.1 Bacterial inoculation and drying

Staphylococcus epidermidis (*S. epidermidis*, otherwise known as *S. albus*) was selected as it is a common bacteria associated with humans which has known tolerance to saline conditions. While *S. epidermidis* is a common bacteria found on human skin, it can be pathogenic, particularly in medical situations [41], [42], [43]. The textile circles (including untreated control samples) were placed in the sample vials. Bacterial culture of known volume (100 µL) and cell abundance (from 10^4 to 10^8) of bacterial culture was poured over the textile samples. The samples were incubated in the vials at 37°C for 24 h and allowed to dry gradually during this time. A growth media of peptone water (100 µL) was poured into each vial and gently agitated for an hour at 37°C before plating. The plates were kept in an incubator at 37°C for 24 to 48 h and colonies were counted manually (Figure 1 F-H). The experimental methodology is shown schematically in Figure 2.

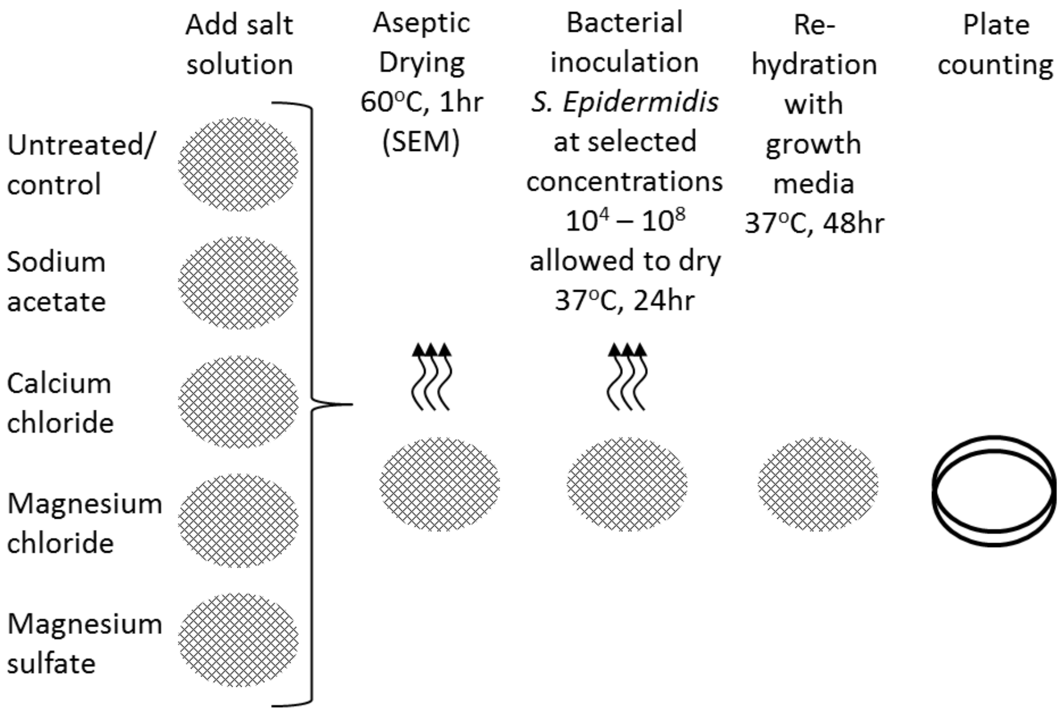

Fig. 2 Schematic representation of the stages of the experimental methodology outlined in the text

3. RESULTS

The first experiment applied initial abundances of bacterial cells of 10^4, 10^6 and 10^8. In this experiment the plate counting of cells (average of duplicates) was 46.5, 170 and over 500, respectively. In the repeat experiment the applied initial abundances of bacterial cells was reduced to 10^4, 10^5 and 10^6 to remain in a countable range. In the second experiment the plate counting of cells (average of duplicates) was 4, 57 and 282.5, respectively. The experiments demonstrate that viability of *S. epidermidis* cells was significantly reduced where drying was conducted in the presence of each of the four precipitated salts (Table 1, Figure 3). A number of the experiments showed zero viable cells present after treatment. The experiments with no zero counts after treatment and with a countable number of cells in the control were those with initial abundance of bacterial cells of 10^6. These results, including the percentage of treatment relative to control, are summarised in Table 1. The most notable decrease was observed for sodium acetate where the count of viable cells was 0.5% and 1.5% of the count in the control sample. The other salts showed more variable results ranging from 0.3% to 44% in comparison to the control (Table 1).

Table 1. Results of two experiments on effects of drying with hygroscopic salts on *Staphylococcus epidermidis* counts. Average of duplicates shown in italics. Representative percentage values, relative to control, shown in brackets.

Exp.	Initial Abund.	Control	Sodium acetate	Calcium chloride	Magnesium chloride	Magnesium sulfate
1A	10^4	48	0	0	2	7
1B		45	0	3	0	4
Av.		*46.5*	*0*	*1.5*	*1*	*5.5*
1C	10^6	194	4	0	5	44
1D		146	1	1	6	105
Av.		*170*	*2.5* (1.5%)	*0.5* (0.3%)	*5.5* (3.2%)	*74.5* (44%)
1E	10^8	>500	7	31	30	366
1F		>500	8	49	26	534
Av.		*>500*	*7.5*	*40*	*28*	*450*
2A	10^4	7	0	0	2	0
2B		1	0	3	0	0
Av.		*4*	*0*	*1.5*	*1*	*0*
2C	10^5	54	0	5	5	6
2D		60	0	6	6	3
Av.		*57*	*0*	*5.5*	*5.5*	*4.5*
2E	10^6	387(*)	3(*)	31	30	54(*)
2F		178	0	49	26	50
Av.		*282.5*	*1.5* (0.5%)	*40* (14%)	*28* (9.9%)	*52* (18%)

(*) Plate photographs in Figure 1 F-H

4. DISCUSSION

Staphylococcus epidermidis is a Gram positive bacteria with a thick cell wall and has tolerance to dry aerobic conditions. These features probably contribute to the survival observed in the control sample (drying without the presence of salt). The results indicate that the presence of salts during drying has made a significant negative impact on bacterial viability. The mechanism by which this occurs is not known. It is inferred that the hygroscopic nature of the salts may enhance the effect of desiccation in killing bacteria during drying. In the control sample a bacterium would be in contact with the cotton fiber and air. The effectiveness of drying would be limited to the efficiency of water transfer across these two boundary conditions. In the treated samples, a bacterium would also be in contact with precipitated grains of salt. It is inferred that additional osmotic pressure can occur at the interface of bacteria and salt grains and that the hygroscopic nature of the salt can increase the loss of water through the bacterial cell wall. A similar effect of hygroscopic salts has been suggested as a mode of enhanced impact of desiccation on plant leaves [44].

The observation that sodium acetate was the most consistent and effective of the salts is especially notable. This may be related to the size and distribution of precipitated grains or may be related in some way to the antimicrobial properties of peracetic acid [1], [24].

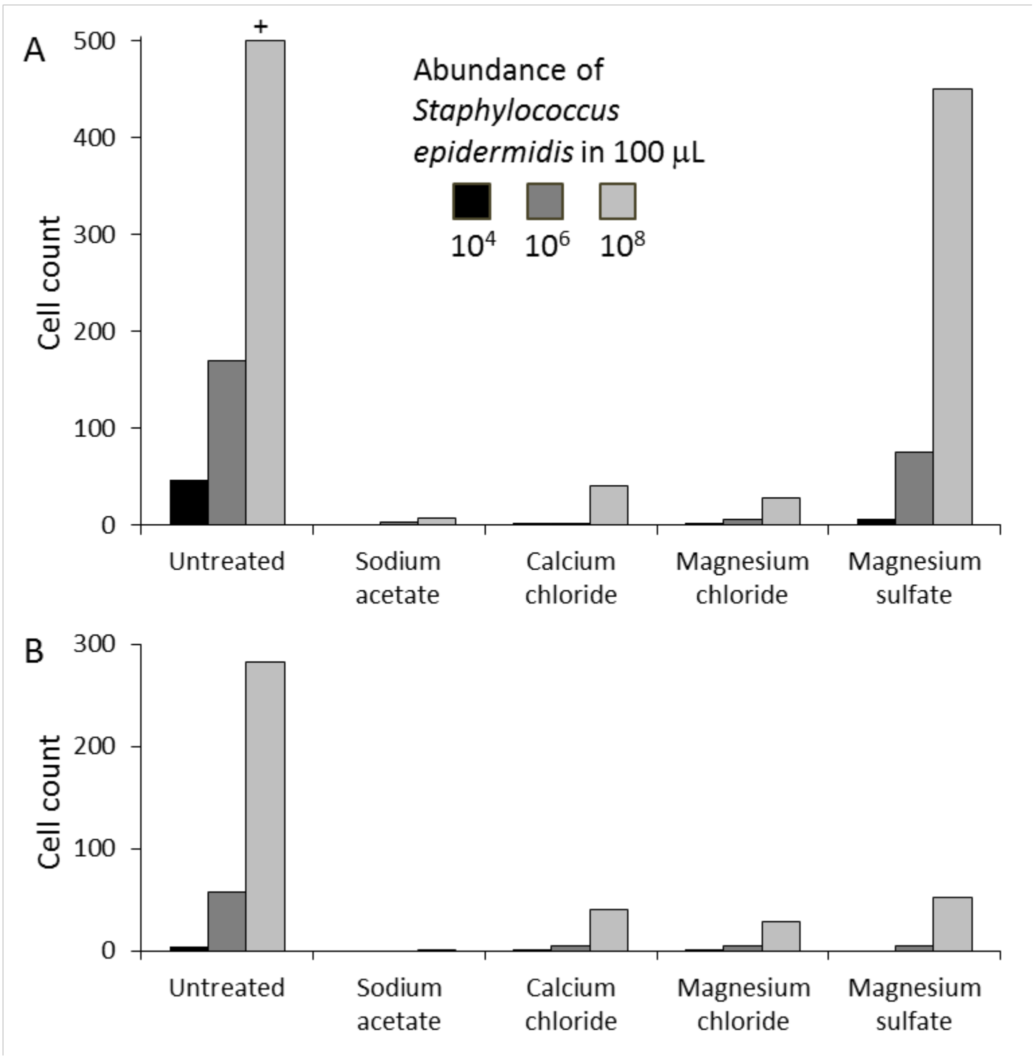

Fig. 3 (A and B) Results (average of duplicates) of repeated experiments on effects of drying with hygroscopic salts on *Staphylococcus epidermidis* counts (Table 1)

Additional experimentation is required to investigate the mechanism by which salts enhance the effect of desiccation in killing bacteria. For example, other microbes should be investigated, in particular those associated with resistance to antibacterial agents such as *Staphylococcus aureous*. If a similar efficacy of bacterial reduction were achieved for that bacteria with the method outlined in this study the result could be significant for improving sanitation of hospital linen and improved control of the resistant bacteria in general. It is also necessary to study the many other inorganic and organic compounds that have hygroscopic properties. Other drying regimes regarding various levels of humidity, rate, temperature range also need to be investigated. The role of the substrate also needs to be investigated, in particular hard surfaces such as medical instruments and floors and even on human skin. Although many bacteria are adapted to the saline conditions of the skin it is possible that precipitation of other salts on the skin may have antibacterial properties, perhaps partly accounting for claimed benefits of treatments such as bath salts.

Home remedies for removing odors from clothing often include soaking in household products such as baking soda (sodium bicarbonate) and vinegar (acetic acid) [45]. Sodium bicarbonate (baking soda) can be combined with acetic acid (vinegar) to form a solution (with release of carbon dioxide gas) that will precipitate sodium acetate during drying. The laboratory studies reported here show that drying of textiles with sodium acetate can be highly effective at reducing the presence of a common type of bacteria.

5. CONCLUSION

The results of this study indicate that salt-enhanced drying is a new class of antibacterial sanitizing method warranting further investigation. In particular, sodium acetate has been found to be the most effective of the four salts trialled in this study. Sodium acetate reduced viable bacteria (*Staphylococcus epidermidis)* to 1.5% or less compared to the control after 24 h drying. For calcium chloride, magnesium chloride and magnesium sulfate, the count of viable cells after drying was more variable and ranged from 0.3% to 44% relative to the count in the control sample.

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