Evaluation of bactericidal effect of three antiseptics on bacteria isolated from wounds

• **Objective:** Antiseptics are widely used in wound management to prevent or treat wound infections due to their proven wound healing properties regardless of their cytotoxicity. The objective of this study was to determine the bactericidal effects of three antiseptics on pathogens known to cause wound infections.

• **Method:** The study was carried out at a tertiary care hospital and a university microbiology laboratory in Sri Lanka in 2013. The three acids (acetic acid, ascorbic acid and boric acid) in increasing concentration (0.5%, 0.75% and 1%) were tested against bacterial suspensions equivalent to 0.5 McFarland standard. The Bacteria isolates used were isolated from wound and standard strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

• **Results:** There were 33 (68.8%) Coliforms, 10 (20.8%) *Pseudomonas* species, and 5 (10.4%) strains of *Staphylococcus aureus.* Acetic acid at concentration of 0.5% inhibited growth of 37 (77%) and 42 (87.5%) of tested isolates when exposed for 30 and 60 minutes, respectively. However 100% inhibition was achieved at four hours. At a concentration of 0.75%, 40 (83.3%) and 44 (91.7%) were inhibited when exposed for 30 and 60 minutes, respectively, with 100% inhibition at 4 hours. At concentration of 1%, 46 (95.8%) inhibition was seen at 30 minutes and 100% inhibition at 60 minutes.
Ascorbic acid, at 0.5% and 0.75 % concentrations, inhibited growth of 45(93.7%) and 47(97.9%) of isolates respectively when exposed for 30 minutes. At these two concentrations, 100% inhibition was achieved when exposed for one hour. At 1% concentration, 100% inhibition was achieved at 30 minutes. Boric acid did not show bactericidal effect at concentrations of 0.5%, 0.75 % and 1%. *Pseudomonas* species were inhibited at 30 minutes by 0.5% acetic acid. Bactericidal effect against all the standard strains was seen with three acids at each concentration tested from 30 minutes onwards

• **Conclusion:** Ascorbic acid was bactericidal for all organisms tested within the shortest exposure time at the lowest concentration compared to other two acids. Despite promising bactericidal effects, further studies warrant, as ongoing debates on toxicity of acids on tissue epithelialisation. Application of antiseptics for a shorter duration could overcome this problem without losing bactericidal activity.

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bactericidal effect; antiseptics; wound care; ascorbic acid; acetic acid; boric acid; wound healing

Microbial pathogens have several different mechanisms to delay wound healing, such as persistent production of inflammatory mediators, metabolic wastes, and toxins. Furthermore, they maintain the activated state of neutrophils, which produce catalytic enzymes and free oxygen radicals. Hence antiseptic therapy plays a role in control of wound infection. They are also effective against bacteria, fungi, viruses, protozoa, and even prions.[1,2] There is increasing evidence suggesting that healing of chronic ulcers is inhibited by bacterial infection, and reduction in bacterial numbers can reduce inflammation and enhance healing.[3] Various acids have been used on chronic wounds with a view of reducing the bacterial load infecting or colonising the wound.[4–6] Povidone iodine, chlorhexidine, alcohol, acetate, hydrogen peroxide, boric acid, silver nitrate, silver sulfadiazine, and sodium hypochlorite are widely used in wound management currently.

While the application of various acids has proven to be helpful in wound healing, regardless of their cytotoxic nature, it is unclear whether the beneficial effect is due to pH or chemical composition. The aim of this study was to assess the bactericidal effect of three antiseptics on stored isolates in the department of Microbiology, Colombo South Teaching Hospital, Sri Lanka. These isolates consisted of commonly encountered pathogenic bacteria causing wound infections.

Methodology

This study was carried out at Colombo South Teaching Hospital and Department of Microbiology, Uni-

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versity of Sri Jayewardenepura in 2013. Acetic acid, ascorbic acid and boric acid at in three concentrations (0.5%, 0.75% and 1%) were tested against a total of 48 bacterial isolates (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, coliforms) from wounds. Suspensions equal to 0.5 McFarland standards, a reference for bacterial turbidity used to standardise microbial testing, were prepared using pathogenic organisms. The suspensions (5ml) were dispensed into ten glass tubes. Equal volumes of three acids were added to nine tubes leaving the tenth tube as the growth control. Tubes containing isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and coliforms were incubated at 35^{0}C aerobically and subcultured on blood agar and incubated at time intervals of 30 minutes, one hour, four hours and 24 hours at 35^{0}C in ambient air. Plates were observed for growth after overnight incubation.

Standard strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* from American type Culture Collection (ATCC) 25923, 25922 and 27853, respectively and National Collection of Type Cultures (NCTC) 6571, 11560 and 10662, respectively were also tested as above.

Results

Among the tested isolates there were 33 (68.8%) Coliforms, 10 (20.8%) *Pseudomonas* species, and 5 (10.4%) *Staphylococcus aureus*.

Acetic acid at a concentration of 0.5 % inhibited growth in 37 (77%) and 42 (87.5%) of the tested isolates when exposed for 30 and 60 minutes, respectively. However 100% inhibition was achieved when exposed 4 hours or more. At concentration of 0.75% 40 (83.3%) and 44 (91.7%) were inhibited when exposed for 30 and 60 minutes, respectively. While 100% inhibition was achieved when exposed for 4 hours or more. At concentration of 1%, 46 (95.8%) inhibition was seen at 30 minutes and 100% inhibition after 60 minutes (Table 1).

Considering ascorbic acid, at 0.5% and 0.75 % concentrations, 45 (93.7%) and 47 (97.9%) inhibition of growth was achieved respectively when exposed for 30 minutes. Both concentrations produced 100% inhibition when exposed to one hour or more. The 1% concentration produced 100% percent inhibition after 30 minutes. (Table 1)

Boric acid did not show any bactericidal effect irrespective of concentrations tested and time of exposure (Table 1).

The effect of the acids on different bacteria

When coliforms were considered, 0.5% acetic acid showed inhibition of growth in 23 (70.0%) and 28 (84.8%), when exposed for 30 and 60 minutes respectively (Table 2). At four hours and longer 100% inhibition was achieved. At a concentration of 0.75%, 26 (78.8%) and 29 (87.9%) were inhibited when exposed for 30 and 60 minutes, respectively. Similarly 100% inhibition was achieved at four hours or more. At a concentration of 1%, 31 (93.9%) inhibition was seen at 30 minutes and 100% inhibition after 60 minutes or more. When exposed to ascorbic acid 30 (90.9%) and 32 (96.9%) of the coliforms were respectively inhibited when exposed to ascorbic acid at 0.5% and 0.75 % concentrations, for 30 minutes (Table 2). Both concentrations resulted in 100% inhibition following an exposure of one hour or more. The 1% concentration of ascorbic acid caused a 100% inhibition at all the point times assessed. Boric acid did not show any bactericidal effect at any concentrations tested.

When *Pseudomonas* species were considered, 100% of the isolates showed inhibition of the growth after 30 minutes at all concentrations of acetic acid and ascorbic acid. None of the isolates were inhibited by boric acid (Table 2).

Among the *Staphylococcus aureus*, 4 (80%) isolates showed inhibition of growth at concentration of 0.5% acetic acid when exposed for 30 and 60 min-

Table 1. Number of organisms inhibited by different acids at different concentrations and time

Type of acid	Time interval / Concentration	30 minutes (%)	One hour	Four hours	24 hours
Acetic acid	0.5%	37 (77%)	42 (87.5%)	48 (100%)	48 (100%)
	0.75%	40 (83.3%)	44 (91.7%)	48 (100%)	48 (100%)
	1%	46 (95.8%)	48 (100%)	48 (100%)	48 (100%)
Ascorbic acid	0.5%	45 (93.7%)	48 (100%)	48 (100%)	48 (100%)
	0.75%	47 (97.9%)	48 (100%)	48 (100%)	48 (100%)
	1%	48 (100%)	48 (100%)	48 (100%)	48 (100%)
Boric acid	0.5%	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	0.75%	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	1%	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 2. The bactericidal effect of acid on different bacteria (%)

		Coliforms				*Pseudomonas*				*Staphylococcus aureus*			
		30 min	1 hr	4 hr	24 hr	30 min	1 hr	4 hr	24 hr	30 min	1 hr	4 hr	24 hr
Acetic acid	0.5%	70	84.8	100	100	100	100	100	100	80	80	100	100
	0.75%	78.8	87.9	100	100	100	100	100	100	80	100	100	100
	1%	93.9	100	100	100	100	100	100	100	100	100	100	100
Ascorbic acid	0.5%	90.9	100	100	100	100	100	100	100	100	100	100	100
	0.75%	96.9	100	100	100	100	100	100	100	100	100	100	100
	1%	100	100	100	100	100	100	100	100	100	100	100	100
Boric acid	0.5%	0	0	0	0	0	0	0	0	0	0	0	0
	0.75%	0	0	0	0	0	0	0	0	0	0	0	0
	1%	0	0	0	0	0	0	0	0	0	0	0	0

utes. Further 100% inhibition was achieved when exposed 4 hours or more. At concentration of 0.75%, 4 (80%) were inhibited when exposed for 30 minutes and 100% inhibition was achieved when exposed for 1 hour or more. At concentration of 1%, 5 (100%) inhibition was seen at 30 minutes (Table 2). When exposed to ascorbic acid, 100% isolates were inhibited at 30 minutes at all concentrations tested. Boric acid again did not show any bactericidal effect at any concentrations tested.

Bactericidal effect against all six standard strains was seen with each three acids at all time intervals.

Discussion

Acetic acid is bactericidal against many Gram-positive and Gram-negative organisms, especially *Pseudomonas aeruginosa*.[7] It is frequently used in wounds as a 0.25–0.5% solution, however several researchers have studied higher concentrations.[8] In our study, acetic acid showed bactericidal effect against coliforms and *Staphylococcus aureus* at 0.5% with 4 hours of exposure and at 30 minutes of exposure for *Pseudomonas* species. This is the most frequently used concentration of acetic acid when managing chronic wounds using rotational antiseptics in Sri Lanka.

Although there have been concerns regarding delayed epithelialisation and wound healing with use of acetic acid, no significant effect has been found in animal and human models.[9] Several studies have found acetic acid to be cytotoxic *in vitro*, however the cytotoxicity depends on the concentration of the solution used.[10,11] These findings necessitate further studies to determine the *in vivo* effects.

Our results show bactericidal activity with 0.5% acetic acid against *Pseudomonas* species at 30 minutes of exposure. In practice antiseptics are usually kept in contact with the wound for about 24 hours. As prolonged exposure time may increase the cytotoxicity, a shorter period of contact time with the wound may be adequate in achieving bactericidal effect.

In our study, ascorbic acid at 0.5% concentration has shown bactericidal effect at 30 minutes of contact time against *Pseudomonas* species and *Staphylococcus aureus* and at 60 minutes against coliforms. Studies evaluating the bactericidal properties of ascorbic acid are sparse in literature. Ascorbic acid is well known for its antioxidant and wound healing properties.[12,13] However, it is not among the commonly used rotational antiseptics in our setting. As our results show excellent bactericidal effect of it against all the commonly isolated wound pathogens, this agent warrants consideration as an antiseptic subjected to further evaluation by *in vivo* studies.

Among the tested acids in our study, boric acid has not shown any bactericidal effect at any concentration against clinical isolates we have tested but studies done elsewhere have shown efficacy at higher concentrations.[14]

Pseudomonas aeruginosa is known to colonises burn wounds. Studies have shown that wounds with greater than 10^5 colony forming units are associated with higher risk of infection and failure of skin grafts.[15,16] In our study it was seen that *Pseudomonas aeruginosa* was 100% inhibited by 0.5% acetic acid at 30 minutes exposure. Sloss *et al*.[8] determined that 5% acetic acid was effective in eradicating *Pseudomonas aeruginosa* when wounds were dressed twice daily. However, McKenna and coworkers[17] found that 0.0025% of acetic acid had a moderate inhibitory effect on of growth but was not bactericidal against *Pseudomonas aeruginosa*. In yet another study however, the number of *Pseudomonas* was not reduced significantly ($p>0.05$) by 0.25% acetic acid.[7]

Studies carried out with *Staphylococcus aureus* have shown varying results. The work of Lineaweaver *et al*.[11,18] detected that 78% of *Staphylococcus aureus* survived 24 hours of exposure to 0.25% acetic acid. Another study showed a slight inhibition of staphylococcal growth using 0.0025% acetic

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acid solution.[17] Our study concludes that bactericidal effect of acetic acid against *Staphylococcus aureus* is best at 0.5% concentration in a short duration such as 4 hours.

Hansson and Faergemann in 1995 showed that 0.25% acetic acid has a significant bactericidal effect on Gram-negative rods in venous leg ulcers.[7] A literature survey done did not reveal any studies done on bactericidal effects of boric acid or ascorbic acid on individual organisms, as such this is the first demonstration of the effectiveness of ascorbic acid on as a bactericide.

Limitations

A broad range of concentrations of acids could not be tested due to limitations of funds. Furthermore, the pH value of the antiseptics could be a confounding factor which affects the bactericidal activity. pH of the solution depends not only on the chemical itself, but also on the growth of the organism. Therefore it was not possible to assess the effect of solution's pH as the sole factor affecting the bactericidal effect.

Conclusion

Despite promising bactericidal effects, further studies are warranted due to ongoing debate on the toxicity of the acids on tissue epithelialisation. Application of antiseptics for a shorter duration could overcome this problem without losing bactericidal activity. Acetic acid 0.5% used for 4 hours and ascorbic acid 0.5% from 1 hour can be recommended for wound management in a resource poor settings.

Ascorbic acid had a greater bactericidal effect for all the organisms tested within the shortest exposure time at the lowest concentration compared with the other two acids. ■

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