Antimicrobial activity of EAW water

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Summary: We tested the microbial activity of EAW water against methicillin-sensitive Staphylococcus aureus, methicillin-resistant Staphylococcus aureus, Staphylococcus epermidis, Serratia marcencens, Escheria coli, Pseudomonas aeruginosa and Burkholderia cepacia. The number of bacteria was reduced below detection limit following incubation in EAW water for 10s. The bactericidal activity of EAW water was similar to that of 80% ethanol, but superior to that of 0,1 chlorhexidine and 0,02% povidine iodone. We conclude that EAW water is a low cost but powerful disinfectant.

Introduction

Appropriate use of various disinfectants is necessary for the prevention of hospital-acquired infections. EAW water is a strong acidic and colourless solution with a high oxidation-reduction potential. The solution is prepared by mixing a small amount of salt with tap water in an electrolyser. In this study, we report the properties of EAW water as a new disinfectant. Our results indicate that EAW water is a powerful bactericidal disinfectant against a variety of Gram-positive and Gram-negative bacteria.

Materials and Methods

EAW water and disinfectants

EAW water was prepared by electrolysis of tap water using a EAW electrolyser. In this system tapwater is added, together with NaCl, to the electrolyser. Electrolysis yields EAW water of pH ranging from 2.3-2.7 and oxidation-reduction potential of 1000-1100mV, containing about 30 ppm of dissolved chlorine (Anolyte).

We evaluated the bactericidal properties of EAW water with three conventional disinfectants, including 0,1% chlorhexidine (Hebitane solution, ICI-pharma, Osaka, Japan), 0,02% povidine iodine (Isodine solution, Meiji Seika, Tokyo) and 80% ethanol (ethanol for disinfection, Maruisha Pharmaceutical Co. Ltd, Osaka). The selected concentrations represent those commonly used in solutions prepared for handwashing. All disinfectant solutions were mixed with sterile distilled water at the time of their use. Sterile distilled water was used as a control.

Bacterial strains

Methicillin-sensitive Staphylococcus aureus (MSSA, ATCC 25923), methicillin-resistant Staphylococcus aureus (MRSA, clinical isolate), Staphylococcus epermidis (ATCC 12225), Escheria coli (ATCC 25922), Serratia marcencens (ATCC 8100), Pseudomonas aeruginosa (ATCC 27853), Burkholderia cepacia (clinical isolate) were used as test bacteria.

Determination of bactericidal activity

Testbacteria were culture in brain-heart infusion broth (Becton Dickinson, Cockeysville, MD, USA) for 24 h at 37^{o} C. in air. A volume of 1mL of the bacterial solution (Concentrations: 10^{7} cfu/mL and 10^{9} cfu/mL) in saline was added to 5mL of the test disinfectant solution. The concentrations used were 0,1 ; 0,02 80% and full strength for chlorhexidine, povidone iodine, ethanol and EAW water, respectively. Following incubation for 10, 60 or 180 s at room temperature, we transferred 0,1 mL of the mixture into tubes each containing 0,9mL of neutralising agents. A 0,1 mL sample of this neutralised suspension was transferred to a brain-heart infusion agar plate. The plates were incubated overnight at 37^{o}C.and the number of colonies enumerated. The neutralising agents consisted of 10% Tween *), 3% lecithin and 0,5% sodium thiosulphate. The neutralising agents were confirmed through a serie of preliminary experiments to have inactivating effects against 0,1% chlorhexidine, 0,02% povidone iodine, 80% ethanol and EAW water. Used in the study. The results of bactericidal activity were expressed as described by Haley et al. As colony forming units of recovered bacteria/0,1 mL after indicated contact time with the disinfectant (10, 60 or 180 s)

Results

The results are summarised in Table 1 and Table 2.

When initial concentration of bacteria was 10^{7} cfu/mL (Table 1)
EAW water reduced the viable count below the limit of detection within 10 s. of contact as did 80% ethanol and 0,02% povidone iodine. Following contact with 0,1% chlorhexidine for 10 s the viable count was more than 500 cfu/0,1mL for all strains except P. aeruginosa and E. coli.

When initial concentration of bacteria was 10^{9}cfu/mL (Table 2)
The viable count of all strains except B. cepacia was reduced below detection limit within 10 s. of contact with EAW water. In contrast, the number of MSSA, MRSA, E.coli, B. cepacia was not reduced below the detection limit within 10 s. of contact with 0,02% povidone iodine. EAW water killed B. cepacia faster than 0,02% povidine iodine. The viable count of all strains tested was more than 500 cfu/0,1 mL after contact with 0,1% chlorhexidine for 10 s. 80% ethanol killed all bacteria within 10 s. of contact.
Our results indicated 80% ethanol solution was the most effective disinfectant. This was followed by EAW water. The bactericidal activity of EAW water was superior of that of 0,1% chlorhexidine and 0,02% povidone iodine.

Table 1. Bactericidal effect of Anolyte (EAW water) inocolum 1.7 x 10^4 cfu/mL

Bacteria	Disinfectant	10 s	60 s	180 s
MSSA methicillin sensitive Staphylococcus aureus	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500
MRSA methicillin resistant Staphylococcus aureus	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500
Staphylococcus epermidis	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	0
	Control: distilled water	>500	>500	>500
Pseudomonas aeruginosa	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	41	0	0
	Control: distilled water	>500	>500	>500
Escheria coli	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	0	0	0
	Control: distilled water	>500	>500	>500
Serratia marcencens	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	27	0
	Control: distilled water	>500	>500	>500
Burkholderia cepacia	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500

Table 2. Bactericidal effect of Anolyte (EAW water) inocolum 1.7 x 10^6 cfu/mL

Bacteria	Disinfectant	10 s	60 s	180 s
MSSA methicillin sensitive Staphylococcus aureus	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	8	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500
MRSA methicillin resistant Staphylococcus aureus	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	15	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500
Staphylococcus epermidis	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500
Pseudomonas aeruginosa	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500
Escheria coli	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	71	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	1	0
	Control: distilled water	>500	>500	>500
Serratia marcencens	Anolyte (EAW-water)	0	0	0
	0,02% Povidine iodine	0	0	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500
Burkholderia cepacia	Anolyte (EAW-water)	3	0	0
	0,02% Povidine iodine	>500	237	0
	80% Ethanol	0	0	0
	0,1% chlorhexidine	>500	>500	>500
	Control: distilled water	>500	>500	>500

Discussion

Disinfectants play an important role in the prevention of nosocomial infection, and the appropriate use of disinfectants is particularly important for handwashing. Disinfectants can be classified as high grade, medium grade or low grade according to their bactericidal characteristics. The most popular disinfectants used for handwashing are in the low-grade category, but several problems related to the use of such infectants remain to be resolved, such as the use against resistant strains of bacteria and skin irritation after frequent use.

Anolyte (EAW-water) has a pH of 2.3-2.7, an oxidation-reduction potential of 1000-1100 mV, and about 30 ppm of dissolved chlorine. Although the present study did not examine the exact mechanism of action, we believe that the bactericidal effect of Anolyte against various strains of

bacteria was due to the combined action of hydrogen ion concentration, oxidation-reduction potential and dissolved chlorine. Anolyte is a strong acid, but it is different to hydrochloric acid or sulphuric acid. These acids have a strong degree of ionisation, and when oxidation occurs, H^+ is used and new H^+ is generated. In case of Anolyte, no new H^+ is generated because it is produced by electrolyses only of the saline solution. Thus the full-strength solution is not corrosive to skin and organic material.

In this study, we tested seven strains of bacteria, which are important pathogens. Anolyte produced an immediate bactericidal effect against six strains of bacteria except for B. cepacia. In particular, the bactericidal activity of Anolyte was superior to that of 0,1% chlorhexidine against all tested bacteria and to that of 0,02% povidone iodine against MSSA, MRSA, E. coli and B. cepacia. However, mixing Anolyte with 0,5 mg/mL albumin (from bovine serum), resulted in a reduction of the antibacterial activity of Anolyte (preliminary data). Thus the activity of Anolyte may be reduced in the presence of organic substances and sufficient bactericidal activity might not occur by a simple wipe. Therefore, for total eradication of bacteria, the item to be disinfected should be rinsed or immersed in Anolyte.
EAW water is prepared using salt and tap water. Therefore, it could be useful as a low cost disinfectant in the prevention of acquired infections.
Although Anolyte may be corrosive to metals, especially aluminium, it is not corrosive to titanium, glass, ceramics, vinyl chloride and polyethylene. Therefor, we believe that anolyte could be useful as a general disinfectant for handwashing, room cleaning (e.g. floor, wall, bed frames), machinery cleaning (e.g. portable toilets, stretchers), linen and whit coat washing and to disinfect shoes and slippers. Furthermore, it could also be used clinically for disinfecting open wounds, mouth gargle and as a general skin disinfectant. Obviously further studies are required to establish the safety of Anolyte . In this regard Iwasawa et al demonstrated that the cytotoxic effect of the solution on cultured cells was less than that of convential disinfectants. In addition skin irritation was minimal after the use of EAW water for handwashing.
Handwashing by healthcare personnel is important to prevent the spread of hospital-acquired infections. Our study demonstrated that Anolyte showed bactericidal activity almost equal to disinfectants used routinely for handwashing, such as povidone iodine, and ethanol. (the bactericidal activity of Anolyte was superior to that of 0,02% povidone iodine against four strains of tested bacteria). Moreover, Anolyte showed greater bactericidal activity than 0,1% chlorhexidine against all strains tested.
In summary, our results using anolyte in vitro indicated that Anolyte has powerful bactericidal activity, although further studies are needed to explore the use of the new disinfectant in clinical settings.

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