

The antimicrobial efficacy of silver on antibiotic-resistant bacteria isolated from burn wounds

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ABSTRACT

The antibiotic-resistant bacteria are a major concern to wound care because of their ability to resist many of the antibiotics used today to treat infections. Consequently, other antimicrobials, in particular ionic silver, are considered ideal topical agents for effectively helping to manage and prevent local infections. Little is known about the antimicrobial efficacy of ionic silver on antibiotic-resistant bacteria at different pH values. Consequently, in this study our aim was to evaluate the effect of pH on the antimicrobial efficacy of a silver alginate (SA) and a silver carboxymethyl cellulose (SCMC) dressing on antibiotic-resistant bacteria isolated from burn patients. Forty-nine antibiotic-resistant bacteria, including Vancomycin-resistant *Enterococcus faecium*, methicillin-resistant *Staphylococcus aureus*, multidrug-resistant (MDR) *Pseudomonas aeruginosa*, MDR *Vibrio* sp, MDR *Stenotrophomonas maltophilia*, extended-spectrum β -lactamase (ESBL) producing *Salmonella* sp, ESBL producing *Klebsiella pneumoniae*, ESBL producing *Proteus mirabilis*, ESBL producing *Escherichia coli* and MDR *Acinetobacter baumannii*, routinely isolated from burn wounds were used in the study and evaluated for their susceptibility to two silver containing wound dressings using a standardised antimicrobial efficacy screening assay [corrected zone of inhibition (CZOI)]. The mean overall CZOI for the Gram-positive isolates at a pH of 5.5 were very similar for both dressings. A mean CZOI of 5 mm was recorded for the SCMC dressing, which was slightly higher, at 5.4 mm for the SA dressing. At a pH of 7.0 both dressings, in general, showed a similar activity. However, at a pH of 8.5 the mean CZOI of the SCMC dressing was found to be significantly ($P < 0.05$) higher than the SA dressing for a select number of isolates. The mean overall CZOI for the Gram-negative bacteria followed a similar pattern as observed with the Gram-positive bacteria. Susceptibility to silver ions did vary significantly between genera and species of bacteria. Interestingly, when pH was changed from 8.5 to 5.5 antimicrobial activity for both dressings in general increased significantly ($P < 0.05$). Overall, all forty-nine antibiotic-resistant bacteria isolated from burn wounds showed susceptibility to the antimicrobial activity of both silver containing wound dressings over all pH ranges. In addition, the study showed that the performance of both dressings apparently increased when pH became more acidic. The findings in this study may help to further enhance our knowledge of the role pH plays in affecting both bacterial susceptibility and antimicrobial activity of silver containing wound dressings.

Key words: Antibiotic resistance • Burns • Microbiology • Silver • Wound dressings

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INTRODUCTION

The resistance of bacteria to antibiotics is a growing concern to public health worldwide (1). The antibiotic-resistant bacteria of most concern to wound care are the so called 'superbugs'. This is because antibiotic-resistant bacteria have the ability to resist many of the major last-resort antibiotics available for clinical usage. Antibiotic-resistant bacteria include meticillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-resistant enterococci and multidrug-resistant strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, which all present a management risk. Consequently, when human infections occur and the colonising bacteria, often in a biofilm, show resistance to antibiotics, our ability to manage these infections is limited (2,3). In these circumstances, sepsis in burn patients becomes a major concern (2). Wound infections are one of the most common surgical complications leading to significantly high levels of mortality and morbidity. Surgical site and burn infections are reported to be the most common form of hospital-acquired infections for surgical patients and reported in both the UK and USA to occur in 10–38% of patients (4,5).

Healing of an infected wound is a complex process and can be affected by, and cause changes in pH (6–9). Consequently, numerous researchers have suggested that pH could be used as a very good indicator of wound healing progression (7,6,10). Early studies have reported that the pH of a chronic wound exists in the range of 7.15–8.9 (6,11,12). However, a more recent study undertaken by Dissemond and colleagues (13) found that chronic wound pH values can range from 5.45 to 8.65.

Roberts and colleagues (10) have showed that wounds with a high alkaline pH have a lower healing rate when compared to wounds with a pH closer to neutral. Furthermore, Hoffman and colleagues (14) have reported that within alkaline conditions wound healing progression decreases. A recent review by Gethin (15) highlighted further on the role pH plays in wound healing and provided evidence that the acute and chronic wound environment progresses from an alkaline state, to a neutral and then acidic state when healing commences.

In addition to affecting wound healing pH is known to have an effect on antiseptic efficacy including popular wound antiseptics such as iodine and silver (16). The bioavailability of

active free metal ions specifically in a wound is affected by numerous factors including cationic exchange, ability to form complexes, precipitation and adsorption (17) with metal ion solubility known to increase when pH decreases (18).

With the concerns associated with antibiotic-resistant opportunistic and strict pathogenic bacteria in burn wounds ionic silver is considered an ideal antiseptic of choice for the treatment and prevention of local infections (19–21). This is because ionic silver has a rapid broad-spectrum bactericidal activity (19,21,16). However, as wounds are known to exist at different pH values it is particularly significant to know if the activity of silver is affected by pH as such a finding is clinically important (22,23).

Consequently, the goal in this article was to evaluate the affect of pH on the antimicrobial efficacy of a silver alginate (SA) dressing and a silver carboxymethyl cellulose (SCMC) dressing on antibiotic-resistant bacteria isolated from burn patients.

MATERIALS AND METHODS

Test microorganism

Forty-nine antibiotic-resistant bacteria routinely isolated from burn wounds at West Virginia University Hospital (Morgantown, WV) were used in this study. The test isolates included 25 Gram-positive isolates namely Vancomycin-resistant *Enterococcus faecium* (VRE – 8 isolates), meticillin-resistant *S. aureus* (MRSA – 14 isolates), *S. aureus* (3 isolates) and 24 Gram-negative isolates including multidrug-resistant (MDR) *P. aeruginosa* (2 isolates), MDR *Vibrio* sp (2 isolate), *Enterobacter aerogenes* (1 isolate), MDR *Stenotrophomonas maltophilia* (2 isolate), extended-spectrum β -lactamase (ESBL) producing *Salmonella* sp (3 isolates), ESBL producing *Klebsiella pneumoniae* (2 isolates), ESBL producing *Proteus mirabilis* (3 isolates), ESBL producing *Escherichia coli* (5 isolates) and MDR *A. baumannii* (4 isolates).

Test dressings and materials

The wound dressings tested included a SA dressing (Advanced Medical Solutions Ltd, Cheshire, UK) and a SCMC (ConvaTec, Skilman, NJ). All dressings were aseptically divided into 1 × 1 cm test squares and stored (in the dark) until use. A non-antimicrobial alginate dressing was used as a control (Advanced Medical Solutions Ltd).

Key Points

- the resistance of bacteria to antibiotics is a growing concern to public health worldwide
- when human infections occur and the colonising bacteria, often in a biofilm, show resistance to antibiotics, our ability to manage these infections is limited
- surgical site and burn infections are reported to be the most common form of hospital-acquired infections for surgical patients and reported in both the UK and USA to occur in 10–38% of patients
- numerous researchers have suggested that pH could be used as a very good indicator of wound healing progression
- in addition to affecting wound healing pH is known to have an effect on antiseptic efficacy including popular wound antiseptics such as iodine and silver
- the goal in this article was to evaluate the affect of pH on the antimicrobial efficacy of a silver alginate (SA) dressing and a silver carboxymethyl cellulose (SCMC) dressing on antibiotic-resistant bacteria isolated from burn patients
- forty-nine antibiotic-resistant bacteria routinely isolated from burn wounds at West Virginia University Hospital were used in this study

Antibiotic and silver efficacy testing

Each test isolate was added to 5 ml of blood bank saline (0.85%). The inoculated (1×10^6 CFUs/ml) saline was swabbed onto each Mueller Hinton agar (MHA) plate according to clinical and laboratory standards institute guidelines (24). All wound dressings (1 cm²) were strategically placed onto each MHA plate that had been inoculated with each test isolate. All plates were then incubated at 37°C for 24 hours. To show the effects of pH on the antimicrobial activity of silver agar plates were made to a pH of 5.5, 7.0 or 8.5. The adjustment in the pH of the agar was achieved by the addition of hydrochloric acid or sodium hydroxide during media preparation. After incubation the corrected zone of inhibition (CZOI) around each wound dressings was recorded.

All experiments were performed in triplicate.

Measurement of the corrected zone of inhibition (CZOI)

The zone of inhibition around both the control and test wound dressings was measured both vertically and horizontally (mm). The CZOI was then calculated by subtracting the dimensions of the dressing (vertically and horizontally, in mm) from the zone of inhibition around the dressing thus obtaining a CZOI value.

Statistical analysis

A student's *t*-test was used to compare the CZOI between pH values for each silver wound dressing and also between each wound dressing. All data was analysed using Microsoft™ Excel software.

RESULTS

The effect of pH on the activity of silver (Gram-positive bacteria)

A zone of clearing around the SCMC dressings and the SA dressing was recorded for all Gram-positive antibiotic-resistant bacteria evaluated. Activity against MRSA strains differed between dressings. For example, at pH 8.5 the mean CZOI for the SCMC dressing was significantly larger ($P < 0.05$) when compared with the SA dressing (Figure 1). However, at a pH of 7.0 and 5.5 the mean CZOI were very similar for both dressings. Interestingly when the mean CZOI at pH 8.5 and 5.5 were

compared for each dressing the zones were significantly ($P < 0.05$) larger in the more acidic than alkaline environment.

Activity against VRE was found to be very similar for both dressings at pH 7.0. Although at a pH of 5.5, the SA dressing showed enhanced activity ($P < 0.05$). At pH 8.5, the mean CZOI for the SCMC dressing was larger ($P < 0.05$) than the mean CZOIs observed with the SA dressing. At pH 8.5 and 5.5, the mean CZOI was compared for each dressing and the zones were found to be significantly ($P < 0.05$) larger in the more acidic (pH 5.5) than alkaline (pH 8.5) environment for the SA but not the SCMC dressing.

For *S. aureus* at a pH of 5.5 and 7.0 both dressings showed similar activity. However, at a pH of 8.5 the SCMC dressing showed superior performance ($P < 0.05$) than the SA dressing. At pH 8.5 and 5.5 the mean CZOI were compared for each dressing and the zones were found to be significantly ($P < 0.05$) larger in the more acidic (pH 5.5) than alkaline (pH 8.5) environment for the SA dressing but not the SCMC dressing.

Overall, the mean CZOI for the Gram-positive isolates at a pH of 5.5 were very similar for both dressings with a mean CZOI of 5 mm for the SCMC dressing and 5.4 mm for the SA dressing. At pH 7.0 both dressings in general showed similar activity. However, at a pH of 8.5 a mean CZOI of 4.1 mm was found for the SCMC dressing and 2.6 mm for the SA dressing.

The effect of pH on the activity of silver (Gram-negative bacteria)

For *A. baumannii* it was found that changing pH from 8.5 to 5.5 increased antimicrobial performances for each wound dressing (Figure 2). This increase in activity was found to be significantly larger ($P < 0.05$) for the SA dressing only. At pH 5.5 antimicrobial performances was slightly higher for the SA compared to the SCMC dressing. However, at a pH of 8.5 the SCMC dressing showed overall better activity ($P < 0.05$) than the SA dressing but at a pH of 7.0 both dressings showed similar activity. The mean CZOI for each dressing at pH 8.5 compared to a pH of 5.5 were found to be significantly ($P < 0.05$) larger in the more acidic than alkaline conditions.

The effect of pH on the antimicrobial activity of silver dressings was also showed for the

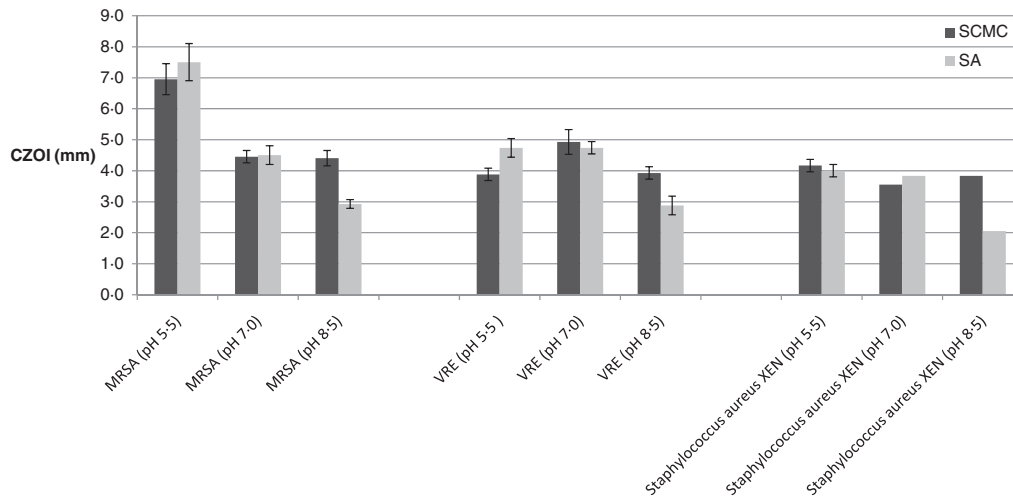


Figure 1. Mean corrected zone of inhibition (mm) of a silver carboxymethyl cellulose and a silver alginate dressing on antibiotic-resistant Gram-positive bacteria at pH 5.5, 7.0 and 8.5.

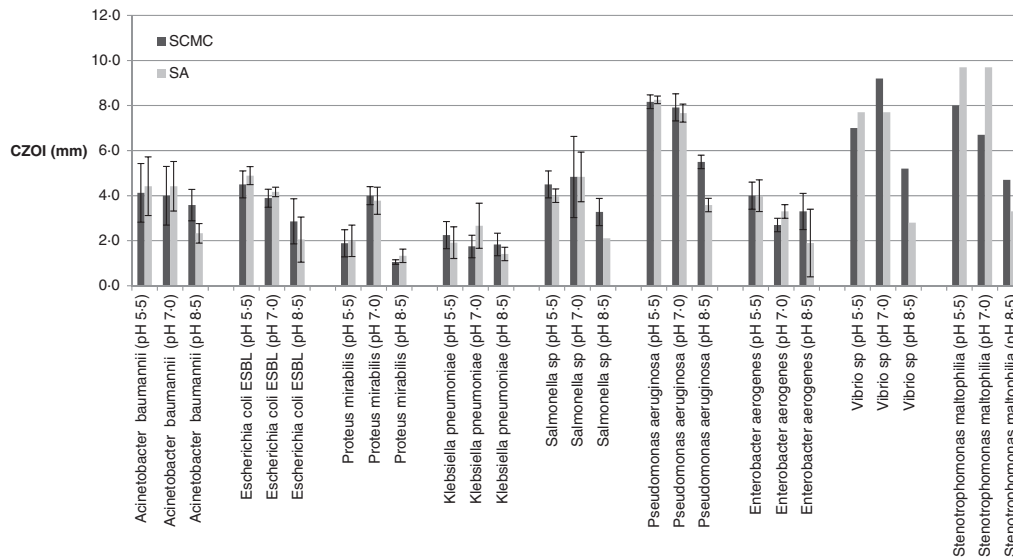


Figure 2. Mean corrected zone of inhibition (mm) of a silver carboxymethyl cellulose and a silver alginate dressing on antibiotic-resistant Gram-negative bacteria at pH 5.5, 7.0 and 8.5.

ESBL producing *E. coli* isolates. Both dressings showed similar activity at both a pH of 5.5 and 7.0. However, at a pH of 8.5 a larger mean CZOI for the SCMC was observed. Changing pH from an alkaline (pH 8.5) to an acidic (pH 5.5) environment significantly increased ($P < 0.05$) the efficacy of each silver containing wound dressing against *E. coli*.

P. mirabilis were most susceptible to the silver dressings at a pH of 7.0. Increasing acidity significantly increased ($P < 0.05$) the mean CZOI for the SCMC dressing only.

For *K. pneumoniae* both dressings showed similar activity over the different pH ranges.

Both silver containing dressings showed a very similar performance on *P. aeruginosa* at pH 5.5 and 7.0. However, the SCMC dressing was superior to the activity of the SA dressing at a pH of 8.5. Increasing acidity increased the mean CZOI for each dressing.

The performance of the SCMC and the SA dressings against *E. aerogenes* was enhanced at a pH of 5.5 compared to a pH of 8.5. Both dressing showed similar activity at all pH values studied.

For *Vibrio* sp the dressings showed very similar activities but at pH 8.5 the SCMC dressing showed enhanced activity compared with the

Key Points

- our aim in this article was to evaluate the antimicrobial efficacy of two silver containing wound dressings, a SA and a SCMC dressing, at three different pH values, 5.5, 7.0 and 8.5, on antibiotic-resistant bacteria isolated from burn patients
- the study found that pH affected the activity of silver containing wound dressings on antibiotic-resistant bacteria
- in particular, it was found on the whole that decreasing pH (increasing acidity), from 8.5 to 5.5, enhanced the activity of each silver dressing
- the susceptibility of bacteria to silver was genera, species and strain specific at the different pH values
- as wounds are known to reside in differing pH ranges it seems clinically relevant to monitor pH as part of a wound management strategy to ensure that maximum antimicrobial performance can be achieved in at risk or infected wounds
- further studies are on going to substantiate the findings reported in this study

SA dressing. Changing pH from 8.5 to 5.5 increased antimicrobial performances for each wound dressing.

For efficacy against *S. maltophilia* the SA dressing was found, in this study, to be higher ($P < 0.05$) compared to the SCMC dressing at both a pH of 5.5 and 7.0. At a pH of 8.5, however, the SCMC dressing outperformed ($P < 0.05$) the SA dressing. Overall, the activity of both dressings was enhanced at a pH of 5.5 compared to a pH of 8.5. This enhanced activity was found to be significant ($P < 0.05$).

Within this study the mean CZOI for the Gram-negative isolates at a pH of 5.5 were similar for both dressings with a mean CZOI of 4.9 mm for the SCMC dressing compared to a slightly higher value of 5.2 for the SA dressing. At pH 7.0 the mean CZOI was 5.0 mm for the SCMC dressing but slightly higher at 5.4 mm for the SA dressing. At a pH of 8.5 a mean CZOI of 3.5 mm was observed for the SCMC dressing and 2.3 mm for the SA dressing.

DISCUSSION

Topical antimicrobials, such as silver, incorporated into wound dressings are used widely for the management of infected wounds colonised with a polymicrobial community of micro-organisms (25). The isolated bacteria found colonising the wound sometimes exhibit resistance to antibiotics following routine or directed sensitivity testing. Such a finding represents a concern to both wound healing and management (2,26,27). Hence, it is therefore very important that wound dressings containing silver show efficacy on the more problematic antibiotic-resistant bacteria (26). Furthermore, for antimicrobial wound dressings to have therapeutic value in the prevention and management of wound infections they need to retain their antimicrobial activity in fluctuating physiological and biochemical conditions (28). This is considered very important as non healing and infected wounds reside within an environment, which is in a state of dynamic biological flux. Consequently, chaotic conditions will inevitably affect the activity and performance of antimicrobials, particularly ionic silver. This is due to the high affinity of ionic silver to many chemical entities including proteins and polysaccharides (19,28). A factor known to fluctuate in a non healing, 'at risk' or infected wound is pH.

Fluctuations in pH are considered as an explanation as to why some topical treatment approaches often fail in infected wounds (15,29). Consequently, it is important to determine if pH has an effect on the antimicrobial efficacy of silver as this will affect the bioavailability of the active component, ionic silver (Ag^+) in a wound environment. Hence, our aim in this study was to evaluate the antimicrobial efficacy of two silver containing wound dressings, a SA and a SCMC dressing, at three different pH values, 5.5, 7.0 and 8.5, on antibiotic-resistant bacteria isolated from burn patients.

Interestingly, the study found that pH affected the activity of silver containing wound dressings on antibiotic-resistant bacteria. In particular, it was found on the whole that decreasing pH (increasing acidity), from 8.5 to 5.5, enhanced the activity of each silver dressing. This effect was showed for both Gram-positive and -negative isolates. Generally, the SA dressing appeared to exhibit a slightly superior antimicrobial performance than the SCMC dressing at pH 5.5. However, at a pH of 8.5 the activity of the SCMC dressing appeared to outperform the activity of the SA dressing on many of the isolates. Furthermore, the susceptibility of bacteria to silver was genera, species and strain specific at the different pH values.

The overall findings generated in this article substantiate conclusions found in other studies that suggest pH has a role to play in antimicrobial activity and performance of silver (23).

Alginate wound dressings are composed of alginic acid. This alginic acid by its very nature may assist in 'pushing' a wound environment to one which is slightly more acidic. The benefit of this would be exhibited in an alginate wound dressing containing silver as the acidic nature of the dressing will help to enhance the performance and bioavailability of ionic silver both within the dressing and at the wound/dressing interface. As wounds are known to reside in differing pH ranges it seems clinically relevant to monitor pH as part of a wound management strategy to ensure that maximum antimicrobial performance can be achieved in at risk or infected wounds. By ensuring maximum antimicrobial performance of a wound dressing improvements in positive clinical outcomes could be achieved (30) particularly when antibiotic-resistant bacteria

are involved. Further studies are on going to substantiate the findings reported in this study.

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