Challenging silver: a comparison of *in vitro* testing methods



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There is no current British Standard for testing the antimicrobial efficacy of dressings. Several other methods have been devised by microbiologists to demonstrate efficacy of the antimicrobial agent. In this study, three methods – transmission, translocation and challenge testing – were used to assess the antimicrobial efficacy of seven silver dressings. Four dressings were tested alongside their non-silver counterpart and all were tested with gauze as a control. The performance of the antimicrobial within the dressing varied depending upon the method used, the organism and the control chosen to calculate the log reduction in the challenge test. Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* showed greater susceptibility to silver than Gram-positive *Staphylococcus aureus* and MRSA. The yeast *Candida albicans* showed a varied response.

odern wound dressings are very sophisticated and offer benefits of physical protection, infection prevention, promotion of autolytic debridement, odour reduction, exudate absorption, pain reduction and maintaining a moist environment at the wound surface. Dressings vary from films, foams, fibrous products, beads, hydrogels or hydrocolloids and there are more than 50 different classes of dressing available for the wound care market in the UK (Wound Care Handbook, 2017). Increasingly, antimicrobial agents are incorporated into dressings to prevent and treat wound infection, and to reduce bioburden in chronic wounds. Topical silver is a popular antimicrobial of choice because of efficacy, low toxicity and few cases of resistance (Cutting et al, 2009). Costs are variable and the UK spend on silver dressings is estimated to be over £23 million per annum (Chambers et al, 2007). Cost is outweighed by benefits as they can reduce the length of morbidity and save drastically on nursing time while having to dress an infected wound (Jemec et al, 2014).

Recently, the use of silver dressings has been questioned, as the costs continue to rise without sufficient evidence on their efficacy. Two Cochrane reviews concluded that there was insufficient robust evidence to establish whether silver could prevent infection or treat infected wounds (Vermeulen et al, 2007; Storm-Versloot et al, 2010). However, an international consensus review on the use of silver dressings on wounds by an expert panel of practitioners in 2012 concluded that silver dressings should continue to be used in practice (International Consensus, 2012). Therefore, until there are sufficient robust data through clinical trials, the practitioner must continue to make decisions based on evidence provided by the dressing manufacturer, through clinical evaluation and *in vitro* studies.

In vitro studies used to assess the antimicrobial effect of dressings are varied and limited as model systems do not always assess the needs of the user. In 2014, a draft British Standard was proposed to assess the antimicrobial efficacy of wound dressings. This considers the log reduction of numbers of specific organisms over a 24-hour period in very defined parameters and would allow this method to be portable between laboratories (British Standards Institution, 2014).

In this study, three methods were used to challenge the *in vitro* efficacy of silver in a variety of wound dressings with five organisms, four bacteria frequently found in wounds and one yeast, *Candida (C) albicans*. Previously published *in vitro* tests to assess wound dressings were utilised, including the transmission, translocation and challenge tests (Thomas and McCubbin, 2003; Gallant-Behm et al, 2005; Strohal et al, 2005; Edwards-Jones, 2006). All these methods have been used previously and are frequently reproduced to compare antimicrobial efficacy irrespective

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Materials and methods Wound dressings

Twelve dressings were used in this study, seven containing silver and five without silver. There were four paired dressings (silver dressing with a non-silver counterpart) and three silver dressings without a non-silver counterpart. A gauze dressing was used as a control dressing throughout the study [Table 1].

All dressings were purchased from a local pharmacy and were those most freely available and accessible.

Organisms and culture

Five organisms were used: *Staphylococcus (S) aureus* ATCC 6538, *Escherichia (E) coli* ATCC 8739, *Pseudomonas (P) aeruginosa* ATCC 9027, MRSA 16 (phage typed) and *C albicans* ATCC 10231.

Tryptone soya agar (TSA) plates (CM0131 type, Oxoid, UK) were used to maintain and to grow the bacterial species, and Sabouraud (SAB) dextrose agar plates (CM0041, Oxoid) used to maintain and grow the yeast.

Preparation of the inoculum

An overnight broth culture (ONBC) was prepared by adding two or three colonies from a fresh pure culture of the microorganism into tryptone soya (TS) broth (CM0129, Oxoid) for the four bacterial species and Sabouraud broth (CM0147) for *C albicans* and incubating it overnight (approximately 18 hours) at 37°C in static conditions. These were further diluted to 5% (v/v) with TS and SAB broth for *C albicans* and then incubated for 25 minutes at 35°C in an orbital shaking incubator rotating at 150 rpm.

Transmission test

The transmission test examines the effect of silver preventing transmission of microorganisms via a lateral wicking effect.

An island of agar was produced in each plate by removing two areas of agar – one central part and one to the left of one area of the plate [Figure 1]. Effectively, a channel was created between the two halves of the agar with a ditch to the left. The surface of the island of agar was inoculated with 0.1 ml of the organism being investigated using a sterile swab. Sterile strips of the test dressing approximately 10mm × 50mm were aseptically placed from the inoculated side of the island, across the central channel to the uninoculated side of the plate, acting as a bridge between the two halves of the plate.

| Table 1: Dressings used in this study. | | | |
|---|---|--|--|
| Dressings containing silver | Dressings used as controls containing no silver | | |
| A: foam dressing containing silver sulphadiazine | B: foam dressing counterpart — no silver sulphadiazine | | |
| C: microfiber dressing containing 1.2% ionic silver | D: microfiber dressing counterpart – no ionic silver | | |
| E: foam dressing with silver in the silicone layer | F: foam dressing counterpart — no silver in the silicone layer) | | |
| G: colloid dressing impregnated with silver | H: colloid dressing counterpart — not impregnated with silver | | |
| l: nanocrystalline silver dressing | | | |
| J: activated carbon dressing impregnated with metallic silver | | | |
| K: silver-coated nylon fibre dressing) | | | |
| | L: gauze dressing — no silver | | |

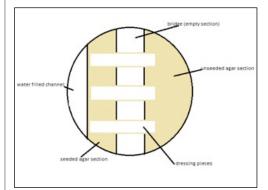


Figure 1. Diagram of the final view of the test.

Sterile distilled water was then added to the reservoir ditch on the left of the inoculated side of the plate, filling to the brim of the ditch but not overflowing onto the agar surface. These plates were incubated for 24 hours at 35°C in a moistened environment. They were examined for transmission of the organism to the uninoculated side of the plate. If this was obvious, the plates were removed. If not, further sterile distilled water was added to the reservoir ditch and plates were incubated for a further 24 hours and then re-examined. All dressings were assessed in triplicate, i.e. three plates with three strips of dressing each were prepared, giving nine replicate results.

A gauze dressing containing no antimicrobial was used as the universal control dressing for

all samples, however, where available, the same dressing without silver was also used.

Translocation test

This assesses the effect of silver preventing translocation of microorganisms via a vertical wicking effect and release of silver from the dressing inhibiting growth underneath.

Columbia blood agar plates (Columbia agar base, CM0331, Oxoid) plus 5% (v/v) sterile defibrinated horse blood (TCS Biosciences, Botolph, UK) were used for the bacterial strains and SAB dextrose agar (Oxoid) plates for Calbicans. A sterile test dressing (4cm²) was aseptically placed onto the centre of a pre-inoculated plate of the relevant organism. The plates had been previously inoculated by adding 0.1 ml of 10⁻² dilution of an ONBC (equating to 105-106 cells) in a circular fashion using a rotary plater (RPO157, Mast Laboratories, UK). Triplicate plates were prepared for each organism and for each time point being tested with each dressing (i.e. 1, 2, 4, 24, 48 and 72 hours).

Plates were then incubated at 35°C in a moist environment to prevent the dressing from drying out.

After the incubation period, dressings were aseptically removed to an uninoculated plate and were deposited in the same alignment as the original plate. This allowed the numbers of microorganism remaining on the bottom of the dressing to be examined. After two minutes (to allow an impression of the dressing to be formed on the new plate), the dressing was removed and flipped over onto another uninoculated plate. This was left for two minutes. Examination of the top surface of the dressing for translocation of the organism was thus facilitated.

The original plates were sub-cultured in the area from where the dressing had been removed onto either TSA or SAB agar plates and then both the subculture and the original plates were incubated for a further 24 hours to determine whether the organism on the original plate under the dressing was killed (bactericidal) or inhibited (bacteriostatic).

Gauze dressing was used as a universal control dressing for the system. All tests were performed in triplicate.

Challenge test

This assesses the effect of silver on log reduction of organism numbers and time to kill. The test included samples assessed at 2, 4 and 24 hours. There was limited nutrient source included in this system and desiccation of the organism was considered.

Test dressings (4 cm²) were placed aseptically into sterile, empty Petri-dishes. To prevent drying out of the dressing, a filter paper disc (sterile) was added to each plate and dampened by adding 0.5 ml sterile distilled water. Dressings containing silver were also dampened by aseptically adding 1 ml sterile distilled water directly to facilitate activation of the silver.

Then 0.2 ml of the inoculum of the organism under test (approximately 10^5 cfu/ml) was added aseptically to the dressing using an autopipette and sterile tips, dampening as much of the dressing area as possible. After inoculation, the dishes were incubated for 2, 4 or 24 hours at 35° C in a moist chamber.

After incubation, the dressings were aseptically removed to 10 ml sterile peptone waters (0.1% v/v) to neutralise the effect of silver by dilution and vortex-mixed vigorously for 30 seconds. Serial dilutions were then performed using 9 ml sterile peptone waters (0.1%). Then 50 μ l of each of the resulting dilutions was inoculated onto either TSA or SAB plates in a spiral fashion using a WASP 2 model (Don Whitley, Shipley, Yorkshire, UK). All tests were set up in triplicate.

The resultant plates were then incubated aerobically for 24 or 48 hours and colonies counted using an Acolyte counting machine (Don Whitley).

In this test, the effect of the dressing was investigated by examining reduction in the number of organisms/ml at 24 hours compared to the original inoculum, the gauze dressing control and where available, the dressing control. The time taken to effect a 3 log reduction compared to the gauze control was determined at 2, 4 or 24 hours and if no organisms were detected in the sample the dressing was placed in 10 ml TS broth or SAB liquid medium to detect very low levels of residual organisms below the limit of detection — a sterility test.

Results

Transmission test

There was transmission of all organisms in the control dressing and the non-silver-containing dressings (dressings B, D, E and L) within 24 hours. Therefore, any inhibition of transmission was effected by the silver in the dressing. The inhibition of transmission was very varied depending upon the dressing, the time period and the organism under test. Generally, more organisms were inhibited at 48 hours compared

| Table 2. Transmission of S aureus ATCC 6538 and MRSA 16. | | | | |
|--|--------------------|----------------------------|----------------------------|--|
| Dressing | Organism (control) | Transmission (24 hours) | Transmission (48 hours) | |
| Dressing A | +++ | | | |
| Dressing C | +++ | | | |
| Dressing E | +++ | | | |
| Dressing G | +++ | | +++ | |
| Dressing I | +++ | | | |
| Dressing J | +++ | +++ * | +++ | |
| Dressing K | +++ | +++ | +++ | |
| Dressing B, D, F, H, L | +++ | +++ | +++ | |
| | | | | |

Key: – no transmission, + transmission. *MRSA 16 transmitted in all replicates at 24 hours in dressing J.

| Table 3. Transmission of E. coli ATCC 8739 and P. aeruginosa ATCC 9027. | | | | |
|---|--------------------|----------------------------|----------------------------|--|
| Dressing | Organism (control) | Transmission (24 hours) | Transmission (48 hours) | |
| Dressing A | +++ | | | |
| Dressing C | +++ | | | |
| Dressing E | +++ | | | |
| Dressing G | +++ | | | |
| Dressing I | +++ | | | |
| Dressing J | +++ | +++ | +++ | |
| Dressing K | +++ | | | |
| Dressing B, D, F, H, L | +++ | +++ | +++ | |
| Key: – no transmission, + transmission. | | | | |

| Table 4. Transmission of C. albicans ATCC 10231. | | | | |
|--|---|--|--|--|
| Organism (control) | Transmission (24 hours) | Transmission (48 hours) | | |
| +++ | | | | |
| +++ | | | | |
| +++ | | | | |
| +++ | | +* | | |
| +++ | | | | |
| +++ | | +++ | | |
| +++ | | +++ | | |
| +++ | +++ | +++ | | |
| | Organism (control) ++++ ++++ ++++ ++++ ++++ ++++ ++++ + | Organism (control) Transmission (24 hours) ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ ++++ | | |

to 24 hours, with the Gram-negative organisms being more susceptible than the Gram-positive organisms. The yeast was very variable, depending upon the dressing.

There was no transmission of any of the five organisms in the test system using Dressing A, C, E and I, showing these dressings inhibited lateral wicking of the five organisms tested. Dressing G prevented transmission of *S* aureus, MRSA and *C* albicans at 24 hours, but this did occur at 48 hours. Transmission of *P* aeruginosa and *E* coli was inhibited at both time points.

Dressing J prevented transmission of *S aureus* and *C albicans* at 24 hours, but not MRSA, but allowed transmission at 48 hours. *P aeruginosa* and *E coli* were transmitted at 24 hours.

Dressing K prevented transmission of *P aeruginosa* and *E coli* at 24 and 48 hours. Transmission of *C albicans* was prevented at 24 hours but not 48 hours. Transmission of *S aureus* and MRSA was not prevented at any time point. The data is shown in *Tables 2–4*.

Translocation test

Dressings B, D, F, H and L did not inhibit translocation of microorganisms through the dressing and this happened very quickly (less than 1 hour). The seven dressings containing silver varied, depending upon the time period and the organism under test.

Two dressings prevented translocation of all organisms under test at all time periods, demonstrating the silver was activated quickly and inhibited the organisms, while one dressing did not have any effect at any time periods; this was thought to be due to the structure of the dressing. Others varied depending upon the organism and time.

Dressing A prevented translocation of MRSA, *S aureus* and *C albicans* at all time periods, however translocation of *P aeruginosa* and *E coli* occurred between 4 and 24 hours.

Dressing C prevented translocation of *P aeruginosa* and *E coli* at all time periods but not *S aureus*, MRSA (translocation occurring within 1hour) or *C albicans* (translocation occurred between 48 and 72 hours).

Dressing E and I prevented translocation of all organisms at all time periods up to 72 hours.

Dressings G did not prevent translocation of any of the microorganisms tested.

Dressing J did not prevent translocation of any bacteria tested but delayed translocation of *C albicans* until 4–24 hours.

Dressing K delayed translocation of *S aureus* and MRSA until 2–4 hours and *P aeruginosa, E coli* and *C albicans* until 4–24 hours.

Effect underneath the dressing

Following removal of the dressings on the original culture plate, the area underneath the dressing was inspected for growth. If there was no growth, then the original plate was incubated for a further 24 hours without the dressing to determine of the organisms would grow once

| | S aureus MRSA 16 E coli P aeruginosa C albican | | | | | |
|------------|--|------------|------------|------------|------------|--|
| | ATCC 6538 | WINDA TO | ATCC 8739 | ATCC 9027 | ATCC 10231 | |
| Dressing A | 4–24 hours | 4–24 hours | 0–2 hours | 0–2 hours | 4–24 hours | |
| Dressing C | DNA (2.81) | 4–24 hours | 2–4 hours | 0–2 hours | 4–24 hours | |
| Dressing E | 4–24 hours | 4–24 hours | 0–2 hours | 0–2 hours | 0–2 hours | |
| Dressing G | 4–24 hours | 4–24 hours | 0–2 hours | 0–2 hours | DNA | |
| Dressing I | 0–2 hours | 0–2 hours | 0–2 hours | 0–2 hours | 0–2 hours | |
| Dressing J | DNA(2.70) | DNA (2.16) | 4–24 hours | 4–24 hours | DNA (2.77) | |
| Dressing K | 4-24 hours | 4-24 hours | 4-24 hours | 2–4 hours | 4–24 hours | |

number in parenthesis is the log reduction actieved.

the dressing had been removed. All dressings except dressing I allowed growth underneath the dressing, indicating that this dressing leached sufficient levels of silver from the dressing to kill the microorganisms, whereas all other dressings either did not release silver from the dressing or that the levels were not high enough to kill the organism under test.

Challenge test

This assay determined the reduction of organism numbers over time including the time taken to demonstrate a 3 log reduction compared to the gauze control within a 24-hour period. Dressing I was the most effective dressing at reducing all organism numbers by greater than 3 log compared to the gauze control and this occurred within a two hour period. Dressing E showed a greater than 3 log reduction of all organisms within a 24-hour period compared to the gauze control, with a greater than 3 log reduction of *E coli*, *P aeruginosa* and *C albicans* within 2 hours.

Other dressings showed a varying reduction of organism numbers and time to kill within a 24-hour period compared to the gauze control, with *E coli* and *P aeruginosa* showing greater susceptibility to silver than the *S aureus*, MRSA and *C albicans*. The time frame taken to effect a 3 log reduction compared to the gauze control dressing is shown for each dressing in *Table 5*.

The log reduction of organism numbers at 24 hours was calculated using three possible controls: the gauze control dressing, the dressing control and the starting inoculum. These data are shown in *Table 6*.

Discussion

Incorporation of antimicrobial agents into wound dressings has made their application

more standardised compared to 20 years ago when many were supplied for use as a cream or lotion without any control of release or delivery. Most dressings are classed as medical devices and manufacturers have to show the efficacy of the dressing through *in vivo* and *in vitro* studies. Not all in vitro methods are appropriate for all classes of antimicrobial dressings and this study demonstrates that activity of antimicrobial dressings can vary depending of the method used.

The transmission test described by Thomas and McCubbin (2003) is a simple laboratory method used to study the lateral wicking effect of the dressing. In addition, the ability to inhibit microorganisms within the dressing by an antimicrobial agent can be demonstrated as the water should activate the silver and also give the microorganisms a means of laterally moving along the dressing. Three dressings allowed transmission of some of the organisms at within 24 hours indicating (but not proving) that either the silver was not activated sufficiently before the organisms transmitted or there was insufficient silver to inhibit that particular organism.

The minimal inhibitory concentration (MIC) of silver does vary for organism type – Gram-negative organisms such as *E coli* and *P aeruginosa* are more susceptible than Gram-positive organisms such as *S aureus* and MRSA; and fungi (such as *C albicans*) have variable MICs (Hamilton-Miller et al, 1993). Beacuse MICs of silver vary for different groups of microorganisms, it is important to test a range of organisms whenever possible – preferably standard organisms that can be compared between laboratories.

The translocation test was used to determine if the dressing could prevent translocation of the organism through the dressing from the surface of a seeded agar plate (to model the surface of the wound) to the outer surface of the dressing (Strohal et al, 2005; Edwards-Jones, 2006). The plates were incubated in a moist incubator and the culture plates were moist, but there is no 'active exudate' in the model system as would be seen in a moist wound. This could be a problem as activation of silver in the dressing is essential to inhibit microorganisms.

The method was easy to perform and care had to be taken when handling the dressing to ensure there was no cross contamination from the seeded plates. The control dressing and the non-silver counterpart dressings all facilitated rapid movement of the organisms (occurring within 1 hour), so any delay caused was assumed to be due to the presence of the silver. There appeared to be sufficient moisture in the test system to activate the silver because four dressings (A,C, E and I) prevented translocation of *E coli* and *P aeruginosa* as early as 1 hour and maintained activity until the end of the test period at 72 hours. Dressing G had small holes within the dressing, so the test method was inappropriate for this dressing as the organisms could access the surface without passing through the dressing.

Previous *in vitro* studies using this method showed that MRSA was inhibited by Acticoat[™] (Smith and Nephew) and the barrier effect was also demonstrated *in vivo* (Strohal et al, 2005; Edwards-Jones, 2006).

The translocation test is a simple method and a good indicator of the vertical

movement of microorganisms through the dressing. It can also demonstrate whether or not the antimicrobial agent is released from the dressing as it can inhibit or kill the microorganisms underneath the dressing. In this study, only dressing I killed all the microorganisms under the dressing, implying that silver was released from the dressing.

The challenge test is frequently undertaken to test the efficacy of dressings. This is a modification of the AATCC 100 method for testing antimicrobial textiles (AATCC, 2018). The US Food and Drug Administration often requires this test to be undertaken by dressing manufacturers when registering their product as a medical device, but also requires a 4 log reduction compared to a control to be demonstrated over the wear period of the

| Table 6. Log red | uction observed at 24hr | s compared to differe | ent controls used. | | | |
|--------------------|---|---|--|---|---|--|
| Dressing | Log reduction at 24 hours (test vs gauze control dressing) | Log reduction at 24 hours (test vs own control dressing) | Log reduction at 24 hours (test vs original inoculum) | Log reduction at 24 hours (test vs gauze control dressing) | Log reduction at 24 hours (test vs own control dressing) | Log reduction at 24 hours (test vs original inoculum) |
| S aureus ATCC 6538 | | | | MRSA 16 | | |
| Dressing A | 5.65 | 3.36 | 2.43 | 5.55 | 3.88 | 2.95 |
| Dressing C | 2.81 | -0.53 | 0.41 | 4.37 | 1.53 | 1.77 |
| Dressing E | 8.44 | 5.67 | 5.22 | 7.86 | 5.84 | 5.26 |
| Dressing G | 5.89 | 3.00 | 2.67 | 6.24 | 4.03 | 3.64 |
| Dressing I | 8.44 | NA | 5.22 | 7.86 | NA | 5.26 |
| Dressing J | 2.70 | NA | -0.52 | 2.16 | NA | -0.44 |
| Dressing K | 4.81 | NA | 1.59 | 4.57 | NA | 1.97 |
| | E coli A | TCC 8739 | | F | aeruginosa ATCC 90 | 27 |
| Dressing A | 8.64 | 6.56 | 5.26 | 8.62 | 6.74 | 5.38 |
| Dressing C | 8.64 | 6.03 | 5.26 | 8.62 | 6.60 | 5.38 |
| Dressing E | 8.64 | 6.47 | 5.26 | 8.62 | 5.97 | 5.38 |
| Dressing G | 8.64 | 6.76 | 5.26 | 8.62 | 6.78 | 5.38 |
| Dressing I | 8.64 | NA | 5.26 | 8.62 | NA | 5.38 |
| Dressing J | 8.64 | NA | 5.26 | 8.62 | NA | 5.38 |
| Dressing K | 4.19 | NA | 0.81 | 8.62 | NA | 5.38 |
| | C albicans | ATCC 10231 | | | | |
| Dressing A | 4.48 | 2.89 | 1.73 | _ | | |
| Dressing C | 3.86 | 1.47 | 1.11 | | | |
| Dressing E | 7.75 | 4.84 | 5.00 | | | |
| Dressing G | 5.25 | 2.80 | 2.50 | | | |
| Dressing I | 7.75 | NA | 5.00 | | | |
| Dressing J | 2.77 | NA | 0.02 | | | |
| Dressing K | 3.36 | NA | 0.61 | 1 | | |
| NA=no dressing | control available. | | | | | |

dressing. Only a 3 log reduction is required in other countries, including the UK.

In this study, a modification of the challenge test was used to determine the time taken to effect a 3 log reduction or greater in a model system (Gallant-Behm et al, 2005). Reduction in organism numbers is dependent on a number of factors - the starting concentration of the organism, the phase of growth, the concentration and availability of the antimicrobial agent and the suspension medium. In this study, the initial organism numbers were approximately 10⁵⁻⁶ cfu/ml (which are the numbers associated with an infection), the microorganisms were in logarithmic phase (which means they were actively growing), the concentration and availability of the antimicrobial agent (silver) were determined by the dressing manufacturer and the suspension fluid was 0.1% peptone water, which would maintain organism viability but limit the growth.

All silver-containing dressings caused a log reduction in organism numbers, but this was dependent upon which control was used to determine the reduction. There was a greater log reduction observed when the gauze control was used to compare with the test dressing. This was because the gauze control did not inhibit growth and the 0.1% peptone water allowed an increase of the original inoculum from ~10⁵ cfu/ ml to ~10⁹ cfu/ml (3 log) after 24 hours. However, when log reductions were calculated compared to the original inoculum or the dressing control (dressing counterparts) the log reductions were greatly reduced [*Table 6*].

All the silver dressings inhibited growth of the microorganisms but some did not appear to kill them. The greater the log reduction compared to the original inoculum or the dressing control indicates that the organisms are actually being killed within the dressing rather than growth being inhibited and organism numbers remaining similar or the same.

Hamilton Miller in 1993 showed that a concentration of 16–64 µg/ml of silver sulphadiazine was required to kill over 409 strains of bacteria, including multi-drug resistant strains (Hamilton-Miller et al, 1993). The MIC of *E coli* and *P aeruginosa* was lower than that of *S aureus* and MRSA, corresponding to results seen in this study where generally the Gram-negative organisms were inhibited more quickly than the Gram-positive organisms.

E coli and *P aeruginosa* (Gram-negative) were inhibited more quickly (within 2 hours) compared to MRSA, *S aureus* and *C albicans*, which took between 4 and 24 hours to achieve a similar log reduction [Table 5]. This indicated (but did not prove) that the levels of available silver within the dressings varied between dressings.

The silver within dressings does vary considerably, with manufacturers using silver salts, metallic silver or nanocrystalline. The levels of silver in a dressing will also vary depending upon how quickly the silver ions are dissociated from the silver salts (many silver salts have differing dissociation constants) and with what ions they re-associate with as the exudate passes through the dressing. In this system, the fluid component did contain 0.1% peptone, which contains variable salts, but was not a substitute wound fluid. This could have had an effect on the data obtained and must be considered in future work.

The proposed British standard is an attempt to standardise the *in vitro* methods for testing wound dressings and proposes a classification system: microbicidal or microbiostatic for antimicrobial dressings (British Standards Institution, 2014). It is based on a challenge test with a single sample time (24 hours) and comparing the test dressing with its own counterpart control or a non-antimicrobial dressing from the same dressing group. At least a 3 log reduction compared to the dressing control would be classified as microbicidal, while less than 3 log reduction but prevention of growth compared to the original inoculum would be classified as microbiostatic.

Although the proposed method is still not adopted and does not cater for other attributes of the dressing (transmission or translocation), it does introduce standardisation for the challenge test and allow for an antimicrobial dressing classification that would be suitable for all antimicrobial agents, not just silver. The practitioner could choose a microbicidal dressing to reduce bioburden and to treat a topical wound infection and a microbiostatic dressing could control bioburden and prevent the wound from deteriorating. Any new antimicrobial dressings could be added to the classification system and easily compared to others.

Conclusion

The different methods used in this small study successfully demonstrated that the silver in the test dressings was effective, but the results were very varied, depending upon the organism, the method and the controls used for comparison (especially in the challenge test).

Future classification of antimicrobial activity of microbicidal or microbiostatic in a dressing

using a challenge test may help the clinician to compare dressings for their antimicrobial effectiveness and choose the appropriate antimicrobial dressing for the needs of the individual patient.

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