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The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern?

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Silver first gained regulatory approval for use as an antimicrobial agent in the early 20th century, but its usage diminished with the introduction of antibiotics in the 1940s. Recently, however, topical silver has gained popularity once again, principally in the management of open wounds. This has been largely due to the spread of methicillin-resistant *Staphylococcus* aureus and the resultant reduction in first-line antibiotic prescribing. The increase in the use of topical silver has raised issues concerning silver resistance, together with questions about the standardization of antimicrobial testing methods for silver. Issues related to silver product testing include a failure to establish standard procedures for determining MIC values, an absence of recognized breakpoints, a lack of conformity in the way different products release silver and variations in the effects of microbiological media on silver release and the measurement of inhibitory activity. The clinical incidence of silver resistance remains low, and emergence of resistance can be minimized if the level of silver ions released from products is high and the bactericidal activity rapid.

Keywords: silver, resistance, bacteria, healing, wounds

Introduction

The use of silver in wound management can be traced back to the 18th century, during which silver nitrate ($AgNO_3$) was used in the treatment of ulcers.[1] The antimicrobial activity of the silver ions was first identified in the 19th century, and colloidal silver was accepted by the US Food and Drug Administration (FDA) as being effective for wound management in the 1920s.[2,3] However, after the introduction of penicillin in the 1940s, antibiotics became the standard treatment for bacterial infections and the use of silver diminished.

Silver began to be used again for the management of burn patients in the 1960s, this time in the form of 0.5% $AgNO_3$ solution.[4,5] $AgNO_3$ was combined with a sulphonamide antibiotic in 1968 to produce silver sulfadiazine (SSD) cream, which created a broader spectrum silver-based antibacterial that continued to be prescribed mostly for the management of burns.[6,7] More recently, clinicians have turned to wound dressings that incorporate varying levels of silver, because the emergence and increase of antibiotic-resistant bacteria have resulted in clinical limitations in the prescription of antibiotics.[8]

At the same time, a greater variety of silver-based dressings have become available, such as Acticoat (Smith and Nephew) and Actisorb (Johnson and Johnson),[9] which offer wider therapeutic options. As well as infection management, these include the stimulation of healing in indolent wounds, prophylactic use for patients at risk of contracting a wound infection, and the management of critically colonized wounds.[10] In addition, silver-based vascular and urinary catheters have now entered clinical use.[9] As the use of silver and the number of available silver-based products increases, it is becoming more important to ensure that standard procedures are developed to measure the efficacy of each product. It is also essential to answer questions concerning mechanisms and clinical risk related to silver resistance.

Susceptibility standards for silver-based products

Since it was first established that the killing of pathogens ceased when the serum concentration of penicillin dropped below the MIC,[11] the determination of pharmacological indices has been pivotal in the comparison of antimicrobial agents and in the development of optimal dosing regimens. Hence, in the field of antibiotics, the assignment of MIC values and breakpoints is essential, particularly when considering the susceptibility of organisms to systemic and topical agents and the incidence of microbial resistance.[12,13] It is necessary to define these pharmacological parameters in order to predict antimicrobial efficacy in the treatment of infection.

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MIC values and categorical breakpoints (traditionally, susceptible, intermediate and resistant) are now defined by various professional organizations such as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the CLSI (formerly the NCCLS). However, breakpoints for silver ions have not been agreed,[14,15] and determination of MIC_{50} and MIC_{90} values, which are commonplace in the evaluation of the susceptibility of bacteria to antibiotics, have not been adopted by those studying silver-containing products.[16]

Where silver microbial susceptibility studies have been performed, the authors have assigned their own breakpoints to delineate susceptible and resistant strains. Most of these studies have produced different MIC data for $AgNO_3$, and this demonstrates the extent of variation that currently exists with regard to the pharmacological parameters of silver. For instance, results from the two studies that explored MIC values for *Staphylococcus aureus* (around 100 strains) range from 8 to 80 mg/L.[15,17] Similarly, the two largest studies examining silver ion MIC values for approximately 100 strains of *Pseudomonas aeruginosa* produced a range from 8 to 70 mg/L.[18,19]

Standardization of antimicrobial testing methods for silver

A further challenge exists because of a lack of standardization for silver ion antimicrobial testing methods. In part, this is due to complex solubility issues affecting the bioavailability of silver ions through formation of various silver-halide anionic complexes. This means that silver ion availability may be influenced by the sodium chloride content of the microbiological medium used for susceptibility testing.[14] Some researchers have used Mueller–Hinton media to test for silver susceptibility in a manner analogous to CLSI guidelines for antibiotics, but a variety of other media have also been adopted and the effects of these on final measurements with silver is not known.

To complicate matters further, most silver dressings make use of different delivery systems that release silver ions in a variety of concentrations. The question of whether one antimicrobial agent is necessarily representative of others is a recognized challenge for the standardization of antibiotic breakpoints.[20] However, the issue for modern silver-based dressings is far more complex. Thomas and McCubbin[21,22] tested 10 different silver-based dressings to compare their antimicrobial activity. Only four of these incorporated the same basic form of (ionic) silver as their active ingredient. The remainder used completely different types of silver. Also, none of the dressings incorporated silver within the same basic structure. All the dressings utilized different materials and silver ion release methods. The authors concluded that 'while total silver content is important, other factors also influence a dressing's ability to kill microorganisms. These include the distribution of silver within the dressing, its chemical and physical form and the dressing's affinity for moisture'.

Mechanisms of silver resistance

To date, there are less than 20 published reports of silver resistance in bacteria and only a few of these include data that help clarify resistance mechanisms.[23] McHugh *et al.*[24] described the first instance when a silver-resistant strain of *Salmonella typhimurium* emerged in a hospital burns unit. It was reported that a silver-resistant determinant occurred on a conjugally transferable plasmid, which also encoded resistance to mercuric chloride, ampicillin, chloramphenicol, tetracycline, streptomycin and sulphonamides. Subsequently, this plasmid was named pMG101 and defined as a 180 kb plasmid belonging to the IncH1 incompatibility class. The region of pMG101 that confers inducible silver resistance has been sequenced and the function of the gene products deduced. The silver-resistantce determinant contains seven genes and two open-reading frames of unknown function.[14,25]

Early clinical studies identified silver resistance in other members of the Enterobacteriaceae, but the resistance phenotype was unstable in the absence of silver selection pressure.[26,27] This could reflect reversion of chromosomal mutations conferring silver resistance, especially if they imposed fitness costs, or possibly loss of plasmids encoding resistance. Unfortunately, no further genetic or biochemical studies were undertaken on the isolates described by these groups.

Bridges *et al.*[28] isolated silver-resistant *P. aeruginosa* from burn patients. A loss of resistance on subculture suggests that resistance may have been plasmid-mediated. Deshpande and Chopade[29] discovered a 54 kb plasmid (pUPI199) encoding resistance to silver nitrate in an environmental isolate of *Acinetobacter baumannii* that was transferable to *Escherichia coli* by conjugation. The plasmid did not encode resistance to other metal ions or antibiotics. The mechanism of silver resistance may have involved intracellular detoxification of silver ions by pUPI199.

Li *et al.*[30] concluded that silver resistance in *E. coli* may depend on a mechanism that works in combination with porin loss. They described the characterization of silver-resistant mutants of *E. coli* selected by stepwise exposure of sensitive stains to $AgNO_3$ or SSD and reported that enhanced efflux of silver ions was detected in silver-resistant mutants. This suggests that activation of an endogenous silver efflux system together with porin mutations provides the basis for silver resistance. Subsequent work by Gupta *et al.*[31] proposed that candidates for the endogenous chromosomal genes could be genomic homologues of the *silA* and *silP* genes known to have a role in the efflux of silver ions mediated by plasmid pMG101. An ATP-dependent copper efflux protein that also mediates removal of silver ions was identified as the major component conferring tolerance to silver in *Candida albicans*.[32]

High-level, single-step, target-based mutation to silver resistance is unlikely because of the multifaceted mode of action of the silver ion. This hypothesis is supported by a study that examined the frequency of spontaneous mutation to silver-containing compounds.[33] The authors were unable to recover silver-resistant mutants of *S. aureus* (i.e. frequency $<10^{-9}$) when SSD was used as the selecting agent. This contrasts with the situation for mutational resistance in bacteria arising in single antibiotic targets where spontaneous mutation frequencies of about 10^{-8} are usually recorded.[16]

Silver-based products and the clinical reality of resistance

There is no doubt that bacterial resistance to silver can occur.[9,14] However, whether resistance is a threat in the clinical

environment is unclear. The lack of standardized methods to determine bacterial susceptibility to silver, and the absence of recognized breakpoints, certainly complicate interpretation of silver susceptibility and resistance data. If an attempt to define breakpoints for silver was made, the wide variation in product delivery systems and silver formulations would present unique challenges above and beyond those normally encountered when setting breakpoints for antibiotics.

Silver-based dressings release different amounts of silver ions in different ways via different materials. There is no standard methodology currently available that will ensure a uniform release and concentration of silver ions from different products on which to base MIC values and breakpoints. When the effectiveness of different silver-based dressings against a range of bacteria has been determined, a wide range of data has been generated.[21,22,34] Despite these difficulties, there have been fewer than 20 documented reports of bacterial resistance to silver since 1975.

The increased use of silver dressings has occurred because alternatives are required to replace antibiotics in the management of infected wounds.[10,21,22,34,35] Until more clarity is available concerning MIC levels and breakpoints relating to silver, clinicians should take the common sense measure of using silver dressings that are effective against both Gram-positive and Gram-negative bacteria, with a proven high degree of silver ion release and rapid bactericidal activity.[34–36] Some of the higher silver release formulations produce ionic silver concentrations that reach 70–100 parts per million when measured in ionic water and kill relevant bacteria within 30 min.[36–38] They also provide a continuous supply of new ionized silver molecules.[37,38] There is also evidence to support their efficacy in the clinical environment.[39]

Dressings that release low levels of silver ions are likely to be more dangerous in terms of selection for resistance, especially if the silver ion concentration is sublethal. Faster acting dressings will inevitably present less risk because organisms are more likely to be killed, thereby eliminating possibilities for enrichment of the resistant population through growth and division, especially in the context of mutational development of resistance.

Conclusions

There is a need for silver MIC levels and breakpoints to be developed and standardized. However, even though silver resistance has been documented, current evidence suggests the clinical threat is low. The two most likely mechanisms of silver resistance are plasmid acquisition and gene mutation to decrease silver ion uptake or promote efflux. There is no direct evidence that silver resistance mechanisms confer cross-resistance to antibiotics. However, genetic linkage of silver resistance genes and antibiotic resistance genes has been reported in the context of plasmid-mediated silver resistance.

Some silver-based dressings appear to provide an effective alternative to antibiotics in the management of wound infection. However, dressings that release low levels of silver ions are likely to be more problematic in terms of selection for resistance, especially if the silver concentration is sublethal. In order to minimize the risk of silver resistance, clinicians should choose dressings that release high levels of silver ions and that demonstrate rapid bactericidal activity.

Transparency declarations

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