Non-antibiotic antimicrobial interventions and antimicrobial stewardship in wound care

Abstract: Control of wound infection today relies largely on antibiotics, but the continual emergence of antibiotic-resistant microorganisms threatens a return to the pre-antibiotic era when physicians used antiseptics to prevent and manage infection. Some of those antiseptics are still used today, and others have become available. A diverse variety of non-antibiotic antimicrobial interventions are found on modern formularies. Unlike the mode of action of antibiotics, which affect specific cellular target sites of pathogens, many non-antibiotic antimicrobials affect multiple cellular target sites in a non-specific way. Although this reduces the likelihood of selecting for resistant strains of microorganisms, some have emerged and cross-resistance between antibiotics and antiseptics has been detected. With the prospect of a post-antibiotic era looming, ways to maintain and extend our antimicrobial armamentarium must be found. In this narrative review, current and emerging non-antibiotic antimicrobial strategies will be considered and the need for antimicrobial stewardship in wound care will be explained.

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Caring for wounds has long involved antimicrobial treatments. Historically, topical remedies derived from local and natural sources were widely used; these included plant extracts, minerals, silver, grease, honey, wine and vinegar.[1,2] During the 19th century the development of the chemical industry provided antiseptics such as hypochlorite, iodine, phenol and hydrogen peroxide, [3] and ways to prevent the spread of infection were introduced—handwashing by Ignaz Semmelweis,[4] and decontamination of surgical equipment and environments (aseptic surgery) by Joseph Lister.[5] Since the late 19th century, when the role of microbial species in causing wound infection was established, a rationale for antimicrobial intervention has existed.

At the beginning of the 20th century Paul Ehrlich developed the concept of selective toxicity with 'magic bullets' designed to inhibit the pathogen rather than the host.[6] The discovery of antibiotics[7] later provided many generations of natural and semi-synthetic agents capable of rapidly inhibiting infectious agents by targeting specific intracellular sites or biosynthetic pathways not present in the host. Since the 1940s antibiotics have been used systemically for treating spreading and systemic infections of acute and chronic wounds. However, their widespread use and misuse in medicine and agriculture has allowed the emergence of microbial strains with resistance to one or more antibiotics.[8] Hence, efficacy has diminished and prospects for continued effective control of wound infection have lessened significantly. The lack of new antibiotics being developed is of particular concern.[9] Organisms implicated in wound infection were in the World Health Organization's (WHO) 2017 top five most urgent categories of pathogens for which the development of new antibiotics is urgently needed.[10]

Antimicrobial resistance (AMR) has now become a global crisis[11] which demands global action.[12] Demand for antibiotics increased by 40% between 2000 and 2010, which, together with international travel and migration, contributed to the spread of antibiotic-resistant pathogens.[9] By 2050, AMR is predicted to lead to 10 million annual deaths and economic losses of

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Table 1. Non-antibiotic antimicrobial agents used in wound care products

Antimicrobial agent	Formulation	Active component	Target site	Mode of action
Cadexomer iodine	Ointment/paste, powder, dressings	Iodine (I_2)	Bacterial DNA Bacterial membranes and cell walls	Oxidation of thiol groups, binding to DNA and reduction of fatty acids. Strong oxidising agent that destroys activity of cellular proteins and membrane function
Chlorhexidine (CHX) dihydrochloride CHX diacetate CHX digluconate	Solution, powder, dressings	CHX	Bacterial membranes Cell wall Cytoplasmic proteins	Denatures enzymes, causes loss of membrane potential and leads to leakage of cellular components and coagulation of cytosol
Dialkylcarbamoyl chloride (DACC)	Dressing	None	Bacteriostatic activity	Binds and inactivates bacteria
Gentian violet and methylene blue	Solution, dressings	Gentian violet Methylene blue	Not well defined Bacteriostatic activity	Redox potential altered to restrict bacterial growth
Honey	Medical grade honey, ointment, gel, dressings	Depends on floral origin: methylglyoxal, hydrogen peroxide, bee defensin-1, leptosperin	Bacterial cell cycle Bacterial cell envelope Bacterial DNA	Arrests cell division in staphylococci Disrupts cell walls of Gram-negative bacteria Binds to DNA to cause strand breakages Attenuates virulence
Polyhexamethyl-biguanide (PHMB)	Solution, dressings	PHMB	Bacterial membranes Bacterial DNA	Binds to phospholipids Condenses bacterial DNA and arrests cell division
Potassium permanganate ($KmnO_4$)	Solution	$KMnO_4$	Bacterial DNA Plasma membranes Intracellular enzymes	Oxidation of thiol groups
Povidone-iodine (PVP-I)	Solution, cream/ ointment, sprays, dressings	Iodine (I_2)	Bacterial DNA Bacterial membranes and cell walls	Oxidation of thiol groups, binding to DNA and reduction of fatty acids
Octenidine	Solution, gel, dressings	Octenidine dihydrochloride	Bacterial membranes	Disrupts membrane structure
ROS (enzyme alginogel and hydrogen peroxide)	Gel	Reactive oxygen species (ROS)	Bacterial DNA Bacterial membranes	Oxidation of thiol groups, react with lipids, proteins and DNA to increase cell permeability and cause breakage in DNA strands
Silver (salts, oxysalts, nanoparticles)	Solution, cream, dressings	Ionic silver (Ag^+, Ag^{++}, Ag^{+++})	Bacterial DNA Plasma membranes Intracellular enzymes	Binds to thiol groups and bases in DNA. Destroys membrane permeability and causes the release of potassium ions. Inhibits cell division and damages cell envelopes

$100 trillion.[9] The risks of AMR for wound care have been recognised,[13,14] especially the need to conserve the use of antibiotics.[15] However, because a diverse range of non-antibiotic antimicrobial interventions is used in managing wounds, it is imperative that clinical practices should minimise the possibility of selecting resistance to all of these therapies. With ageing populations, increased prevalence of diabetes,[16] rising costs of wound treatment[17] and diminishing prospects of developing new antibiotics,[9] novel approaches to optimising and conserving all antimicrobial interventions in wounds are indicated. The European Wound Management Association (EWMA) works actively to promote the concept of antimicrobial stewardship (AMS) in wound management. Here we aim to provide a narrative outlook on the potential challenges and opportunities of responsibly using non-antibiotic antimicrobial interventions in the future.

Conventional non-antibiotic antimicrobial agents used in wound care

A wide spectrum of non-antibiotic antimicrobial agents are used in managing wounds.[18] While some are antiseptic solutions employed in cleansing wounds, or decontaminating sites colonised by antibiotic resistance strains, many are incorporated into medical devices (Table 1). They include cadexomer iodine, chlorhexidine (CHX), gentian violet, honey, polyhexamethyl biguanide (PHMB), potassium permanganate, povidone-iodine (PVP-I), octenidine, silver, and agents that generate free radicals.

Iodine has been used to treat wounds since the American Civil War,[3] but early preparations caused pain, irritation and marked staining of tissue.[19,20] Newer products such as cadexomer iodine and PVP-I were developed to overcome these limitations through the sustained delivery of low concentrations of iodine into the wound. Cadexomer iodine is composed of small spherical beads of hydrophilic starch containing 0.9% iodine; these absorb exudate in wounds and swell, allowing the slow release of iodine through pores in their surface. PVP-I is an iodophore comprised of tri-iodine bound as aggregates within polyvinylpyrrolidone (a synthetic polymer and surfactant). On dilution, aggregates slowly release elemental iodine. There are seven forms of iodine in aqueous solution, of which only three (hydrated iodine, hypoiodous acid and iodine cation) possess antimicrobial activity. Iodine binds avidly to thiol and sulphydryl groups in microbial proteins to cause irreversible defects in cellular structures.[19]

CHX is a chemically synthesised biguanide and PHMB is a cationic polymeric biguanide. Both have been used as an antiseptic scrub in the prevention of infection, as well as being used in wound dressings.[18,21] Biguanides are positively charged and bind to negatively charged phospholipids in cell membranes to disrupt integrity and allow leakage of essential components.[22] Octenidine hydrochloride is another cationic antiseptic used prophylactically and therapeutically in managing cutaneous lesions.[23,24]

Topical agents with a long history in wound care are potassium permanganate and gentian violet. Potassium permanganate has been used by dermatologists in treating exuding lesions,[25] and gentian violet (also known as crystal violet) is a triphenylmethane dye.[26]

Honey was used in treating wounds at least 4500 years ago. Modern wound care devices containing medical grade honey have been available since 1999.[27] The antimicrobial properties of honey are comprised of multiple components derived from bees and plants.[28] One of the antimicrobial mechanisms of honey is the action of glucose oxidase, which produces low levels of hydrogen peroxide that in turn give rise to free radicals or reactive oxygen species (ROS). A few other wound care products rely on enzyme action (such as glucose oxidase and lactoperoxidase) to generate ROS.[29–31]

The antimicrobial characteristics of silver have been known for more than 2000 years; in wounds silver nitrate was used during the 1800s.[32] In 1964 an ointment containing silver sulphadiazine (SSD) was introduced for burns patients to treat and prevent infection. There are a diverse range of wound care devices containing silver nitrate, SSD, silver chloride, silver acetate or nanocrystalline silver.[18] Differing concentrations of silver are associated with different types of dressing. Metallic silver is insoluble making it ineffective as an antimicrobial agent, so ionic silver (Ag+, Ag2+ or Ag3+) is required. This is achieved by ionic exchange with the chloride ions present in wound exudate so that silver ions are produced in either the wound bed or the wound dressing. Silver has been formulated into alginates, hydrogels, hydrocolloids and foams.[18] As with many of the agents above, silver interferes with many microbial processes by rapidly binding to thiol and disulphide groups in multiple cellular target sites (Table 1).

Unlike antibiotics, which inhibit infective agents by interacting on a specific microbial target site, non-antibiotic antimicrobial agents affect microbial functions in more a generalised (delocalised) manner by acting simultaneously on multiple target sites.[33] Most of these agents act as oxidising agents in binding to thiol groups of cysteine residues, leading to the disruption of stabilising disulphide links in proteins, which in turn results in loss of function in structural and metabolic proteins. Agents that bind to lipids, such as CHX and cationic detergents, impair membrane integrity, allowing leakage of cytoplasmic components and ingress of previously excluded substances. Many topical agents also bind to DNA and block DNA replication, gene expression and protein synthesis. These widespread intracellular perturbations (Table 1) confer a broad spectrum of inhibitory activity across the microbial cell and across microbial species which is less likely to lead to microbial resistance than antibiotics.

The activity of most non-antibiotic antimicrobial agents is influenced by their concentration, temperature, formulation, presence of organic matter and contact time.[34] The standardised suspension tests used to evaluate the antimicrobial efficacy of antimicrobial solutions *in vitro* are distinct from those used for antimicrobial dressings.[18,35] Because cytotoxicity has been associated with some of these agents, it has been suggested that their clinical potential (or biocompatibility) be assessed by comparing antibacterial activity with cytotoxicity *in vitro*.[36–38] Observations from early animal models warned against the cytotoxic effects of certain antiseptics, particularly undiluted hypochlorite solutions.[39,40] Such studies illustrate the importance of balancing antimicrobial activity with possible toxic effects *in vivo*. The irritant and allergenic properties of topical agents must also be considered.[20]

Antimicrobial wound dressings

The ability of an antimicrobial dressing to prevent the movement of pathogens into or out of a wound is important. Additionally, wound dressings are designed to provide the optimal conditions to facilitate wound healing. Materials used in dressings include alginate-hydrofibre, collagen, films, foams, amorphous gels, hydrocolloids, hydrogels and non-adherent contact layers. The relative performance characteristics and clinical applications of these components have been collated.[41] The ideal characteristics of an antimicrobial dressing suitable for treating chronic wounds include: broad spectrum antimicrobial activity, rapid bactericidal activity, reduction of malodour, activity in the presence of the proteins found in body fluids and wound exudate, residual or sustained activity on the skin (to avoid frequent application), localised skin absorption without systemic absorption, low cytotoxicity and low allergenicity, relatively ease of application to the wound, low potential to select for resistant microbial strain and ease of application to the wound.[42,43] Ideally, antimicrobial interventions must also satisfy patient and clinician expectations, maintain a moist wound healing environment, manage exudation, remove necrotic tissue, assist in wound bed preparation, and be conformable.[43,44]

Wound dressings containing antimicrobial agents are not intended for the elimination of a spreading infection which normally requires systemic antibiotic therapy, or for treating uninfected wounds.[45,46] However, they may be appropriate within a package of care for locally infected wounds.[47] Knowledge of the wound healing process, the differential characteristics of dressings, and how to assess patients' needs is essential if suitable selection choices are to be made.

Both the advantages and disadvantages associated with topical antimicrobial agents must be evaluated, and the rationale for using an antimicrobial dressing should be documented in the patient's notes.[43]

Additional non-antibiotic antimicrobial interventions used in wound care

In addition to non-antibiotic antimicrobial agents (also known as biocides) that are well established as medical devices in wound care, there are further topical interventions for wounds which have the potential to influence microbial populations and reduce the risks of infection.

Maggots

Insect larvae (maggots) have been used in wound care intermittently since the late 16th century and larvae of *Lucilia sericata* were reintroduced into modern medicine for chronic wound management in the 1990s. Their excretions/secretions contain a complex mixture of bioactive components that contribute to wound healing. Inhibitory activity is derived from antimicrobial peptides, such as lucifensins,[48,49] and lucimycin,[50] and ammonia.[51,52] Activity against staphylococci and β-haemolytic streptococci is greater than against Gram-negative bacteria[53,54] and there are even indications that *Pseudomonas aeruginosa* can defend themselves against the antimicrobial activity of maggots.[55] Maggot chymotrypsin disrupts staphylococcal biofilms[56] and combinations of maggot secretions/secretions together with antibiotics inhibit biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[57] A possible explanation is that insect nuclease digests extracellular DNA within the extracellular polymeric matrix of a biofilm, facilitating access of inhibitors to bacteria.[58,59] The inhibition on biofilm formation seems to be concentration dependant as lower concentration of the maggot excretions/secretions enhance the biofilm formation in an experimental set up.[60] In addition to antimicrobial activity, insect proteolytic enzymes assist wound healing in debridement,[61,62] as well as activation of fibroblast migration, angiogenesis and remodelling.[63]

Negative pressure wound therapy (NPWT)

NPWT is an advanced technique intended to manage hard-to-heal chronic wounds. The therapeutic goals include the management of exudate, removal of slough, reduction of pain and wound odour, and prevention of infection by bacterial load reduction. Negative pressure is applied to the wound bed to remove wound exudate, debris and microbial cells away from the surface via a wound contact layer. Animal models have demonstrated that NPWT, combined with antiseptics, disrupt biofilms.[64] To date, systematic reviews for NPWT have provided only low grade clinical evidence to support efficacy in enhancing wound healing with or without simultaneous irrigation.[65–69] Nevertheless, NPWT is widely used throughout the world. There is conflicting evidence for the role of NPWT in reducing wound bioburden.[70–72]

Physical removal of microbial cells from wounds

Dressings in contact with wound surfaces bind microbial cells to varying degrees and thereby facilitate bacterial removal at dressing changes. In laboratory studies, it has been shown that dressings coated with a fatty acid derivative irreversibly bind a range of planktonic microbial cells,[73] and enhance binding of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms.[74] The addition of surfactants and/or chelators offers another way to disrupt aggregated microbial cells and biofilms.[75–79]

Emerging non-antibiotic antimicrobial interventions for wound care

Distinguishing between an emerging antimicrobial therapy and an established therapy is not easy because there are always procedures/devices in various stages of development and acceptance. A recently launched antimicrobial dressing, for example, is one made of carbon alone.[80] New antimicrobial technologies for wounds are emerging. Many are non-invasive and pain-free and some also positively influence wound healing. Three examples are described here. There is scarce clinical data from randomised control trials (RCTs) for evaluation by systematic review.

Cold plasma

Non-thermal atmospheric pressure plasma, also known as cold plasma, is partially ionised gas that has been developed for the treatment of cancer, skin conditions and wounds. As well as stimulating wound healing, by promoting the proliferation and migration of cells intimately involved in tissue repair and regeneration, cold plasma might also possess antimicrobial properties. These effects are due to several types of radiation that generate reactive oxygen and nitrogen species.[81,82] Evidence to support claims of the safety and antibacterial efficacy of this technology have been claimed.[83–85]

Phototherapy

Phototherapy is the use of light for therapeutic purposes. There are four approaches: photodynamic therapy (PDT), ultraviolet irradiation, blue light therapy (BLLT) and low-level laser therapy (LLLT). In PDT a photosensitive non-toxic dye is activated by light of a specific wavelength to generate ROS in the presence of oxygen. Although originally developed for treating tumours on or near the surface of the body, PDT has activity against a broad spectrum of microbial species and this has extended its application to dental disorders, acne and wounds. Potential in managing burns,[86,87] chronic wounds[88] and biofilms[89] has been proposed. Ultraviolet can be detrimental to human cells, but ultraviolet-C light has been shown to inhibit pathogens introduced into murine wounds without detected adverse effects.[90] By activating human porphyrins directly, blue light can elicit antimicrobial effects without the addition of a photosensitiser.[91–94] Of the phototherapy techniques available, LLLT has probably

Table 2. A summary of Cochrane reviews concerning non-antibiotic antimicrobial interventions for wounds that were published from 2013 to 2017

Intervention	Wound type (number of studies)	No participants	Observations	Conclusions
Antibiotics and antiseptics[114]	Venous leg ulcers (45)	4486	Some support for cadexomer iodine	Further good quality research required. Antibacterial preparations should only be used in cases of clinical infection not colonisation
Antibiotics and antiseptics[115]	Surgical wounds healing by secondary intention (11)	886	Evidence was limited by the size of the studies and the ways in which they were conducted and reported	No robust evidence on the effectiveness of any antiseptic/antibiotic/antibacterial preparation
Antiseptics[116]	Burns (56)	5807	Almost all trials had poorly reported methodology; most used silver sulfadiazine (SSD) as the comparator. Low certainty evidence that some antiseptics may increase healing compared with SSD. High certainty evidence that burns treated with honey heal more quickly than those given a range of other non-antibacterial treatments	It was often uncertain whether antiseptics were associated with any difference in healing, infections, or other outcomes. Low confidence that trials were free of risk, due to poor reporting
Topical antibiotics and antiseptics[117]	Pressure ulcers (12)	576	All studies had low numbers of participants; many had incomplete methodology. Quality of evidence ranged from moderate to very low	Relative effects of systemic and topical antimicrobial treatments on pressure ulcers are not clear. More, research of better quality is needed
Dressings and topical agents[118]	Pressure ulcers (39)	2127	Unclear whether one topical agent or dressing was better than another, or better that saline gauze. Certainty of the evidence was very low or low (due to risk of bias and imprecision)	Unable to determine which dressing or topical agents were most likely to heal pressure ulcers. More research of better quality is needed
Topical agents[119]	Fungating wounds (4)	164	Weak evidence for foam dressings containing silver to reduce malodour	More research needed
Topical antimicrobial agents[120]	Foot ulcers in people with diabetes (22)	2310	Not all trials reported important data (such as infection) making the reliability of results uncertain. Low certainty evidence from five trials suggested that use of some type of antimicrobial dressing may increase the number of ulcers healed in the medium-term when compared with non-antimicrobial dressing	The quality of the evidence was too low to allow certain deductions to be made about the benefits and harms of topical antimicrobial treatments for foot ulcer management in people with diabetes. More RCTs needed
Pre-operatives antiseptics[121]	Surgical wounds after clean surgery (13)	2623	Very little good evidence to discriminate between antiseptics	More research needed
Honey[122]	Minor wounds (3) Burns (11) Chronic (12) TOTAL (26)	3011	High-quality evidence that honey heals partial thickness burns 4–5 days earlier than comparator dressings. Evidence of low and very low quality was reported	The range of comparators and wound types precluded overall conclusions
Ozone[123]	Foot ulcers in diabetic people (3)	212	Limited and poor-quality evidence available; methodology was unclear	Unable to draw any firm conclusions
NPWT[65]	Pressure ulcers (4)	149	Trials small and poorly described	More, higher quality research needed
NPWT[66]	Leg ulcers (1)	60	Evidence from rigorous RCTs very limited	NPWT may reduce time-to-healing as part of a treatment that includes skin grafting
NPWT[67]	Surgical wounds healing by secondary intention (2)	69	No rigorous RCT evidence available	Potential effects of NWPT compared with alternatives remain unknown
Phototherapy[124]	Pressure ulcers (7)	403	All trials were at unclear risk of bias	Quality of evidence was very low due to unclear risk of bias and small number of trials
Phototherapy[95]	Foot ulcers in people with diabetes (8)	316	Studies that reported valid data for complete time-to-healing were not identified. Mostly single-centre studies. Sample size ranged from 14 to 84	Phototherapy compared with no phototherapy/placebo may increase the proportion of wounds completely healed; no evidence that phototherapy improves quality of life. Quality of evidence was low; large, well-designed RCTs needed

been the most extensively investigated in the clinical treatment of wounds to date. Like cold plasma therapy, adequate RCTs are required before widespread introduction into wound management. A recent systematic review of the clinical evidence concerning the use of phototherapy in treating foot ulcers in people with diabetes reported the inadequacy of evidence on healing outcomes, and there was judged to be insufficient evidence to make deductions about its impact in treating infection.[95]

Bacteriophage (phage) therapy

Bacteriophages are ubiquitously distributed viruses that act as obligate, intracellular parasites with high

specificity for their target-host, bacterial species. When they infect their specific host bacterium, virus replication is either immediate (for lytic phage) or later (for lysogenic or temperate phage).[96] The replication process is short (approximately 30 minutes); on completion of the viral replication cycle each bacterial host cell dies, lyses and releases many viral copies.[96] Each virus can then infect another bacterium, so bacterial numbers decline rapidly when a lytic bacteriophage encounters its specific host bacterium. The clinical potential of lytic phages in treating bacterial infection was realised soon after their discovery a century ago,[96–99] and enteric infections were successfully treated with bactericidal phages up to the 1940s.[96] This antibacterial approach gradually lost favour in Western medicine following the start of the antibiotic era. Conversely, in Georgia, where phage therapy was firmly established, it continued to be used extensively, as well as throughout the former Soviet Union and Eastern Europe.[99] With the increasing concern of antibiotic-resistant pathogens and healthcare associated infections (HCAIs), phage therapy has attracted renewed interest.[100]

Effective bacterial control relies on characterising an infecting bacterial species in order to select an appropriate viral strain for treatment. Therefore, modern wound care products containing a cocktail of bacteriophages against common wound pathogens are being developed.[101,102] It is also possible that phage endolysins (the enzymes that attack peptidoglycan) will become important in the control of wound infections by lysing bacterial cell walls.[103]

Despite the long clinical use of bacteriophages, publications in Georgian, Russian or Polish remain largely unrecognised by wider communities. Modern indications of the potential of phage therapy for treating wounds comes mostly from animal models.[104–109] Clinical control of *Pseudomonas aeruginosa* in burns has been reported[110] and the sporadic use of phages, prepared in-house for burns patients infected with antibiotic-resistant bacteria, began in a Belgium hospital in 2007.[101,111] Safety of bacteriophage therapy in venous leg ulcers (VLUs) has been demonstrated in a phase I trial[112] and control of staphylococci in diabetic toe ulcers has been recently reported.[113] This technology offers hope of controlling pathogens with AMR.

Clinical efficacy of non-antibiotic antimicrobial interventions for wounds

Despite the long-term use of some non-antibiotic antimicrobial interventions (particularly antiseptics), Cochrane reviews indicate that there is weak clinical evidence of efficacy (Table 2).[65–67,95,114–124] Furthermore, an evidence summary issued by NICE in 2016 stated that:

> *'Systematic reviews and meta-analyses identified little good quality evidence from randomised controlled trials (RCTs) to support the use of advanced or antimicrobial dressings (such as iodine, honey or silver dressings) for chronic wounds'.*[125]

These deductions arise from limitations in RCTS due to the size of patient cohorts (underpowered studies) and methodology (such as definition of outcomes, biased randomisation processes, poor surveillance, low compliance, and inadequate follow-up). Many studies have focused on wound healing as the primary outcome, rather than antimicrobial effect. Even though infection may delay wound healing, time-to-heal does not necessarily reflect the antimicrobial efficacy of an intervention. Future studies might consider monitoring indicators of infection, levels of malodour and the presence of specific pathogens. Nevertheless, from these systematic reviews, deductions about the benefits of topical agents are possible. For example, more patients with venous ulcers healed when treated with cadexomer iodine compared with standard care[114] and weak evidence showed that foam dressings containing silver were effective in reducing malodour in malignant wounds.[119]

A systematic review of the effects of antiseptics on burns analysed 56 trials in three ways: antiseptics versus topical antibiotics, antiseptics versus alternative antiseptics, and antiseptics versus non-antibacterial comparators.[116] Overall the quality of evidence was poor.[116] Most studies used silver sulfadiazine (SSD) as the comparator and there was low certainty of evidence that some antiseptics (silver-based antiseptics and sodium hypochlorite) increased average healing times, and moderate certainty evidence for honey. A possible reduction in healing time was found for burns treated with PVP-I compared with CHX. Burns treated with honey healed more quickly (high certainty evidence) and were more likely to heal (moderate certainty evidence) than those treated with non-antibacterial treatments, but some of the comparators were unconventional ones. There was moderate certainty evidence that wounds treated with nanocrystalline silver probably had shorter time-to-heal than those treated with Vaseline gauze or other non-antimicrobial treatments. However, it was uncertain whether infection rates in burns treated with either silver-based antiseptics or honey differed in comparison to other non-antimicrobial therapies.[116]

For the newer interventions (such as NPWT, phototherapy and ozone)[65–67,95,123,124] the small number and size of clinical trials was a limitation. Generally, better designed studies with improved reporting are needed to generate higher level evidence for all antimicrobial wound therapies.

Studies into the effects of antimicrobial interventions on microbial communities in wounds have traditionally relied on routine cultural methodologies. Yet molecular techniques allow the detection of a significantly broader range of microbial species[126–128] and accurate estimations of numbers. Bacterial load in DFUs was shown to be underestimated by factors ranging between 100 and 1,000,000 using cultural methods when compared to molecular technique.[128] A recent

investigation into the effects of cadexomer iodine *in vivo* on the microbial burden of chronic non-healing DFUs complicated by biofilm used a combination of molecular and microscopic techniques together with zymography. During a six-month period, 17 patients were enrolled and the presence of biofilm was confirmed by scanning electron microscopy (SEM) and or fluorescence in situ hybridisation (FISH). DNA sequencing, and real-time quantitative PCR (qPCR) was used to determine the microbial diversity and load, and gel zymography was used to monitor levels of wound proteases before and after treatment. Significant reductions in microbial load, which correlated with decreases in proteases, were found following treatment with cadexomer iodine.[129] This study illustrates an innovative approach to evaluating the clinical efficacy of an antimicrobial intervention.

Antibiotic resistance

The history of antibiotic discovery and the evolution of antibiotic resistance are well documented.[130] Antibiotics are largely derived from microbial species isolated from the soil, many of which carry genes that confer resistance to their own antimicrobial products. Strains recovered from ecological niches, such as cave sediments or permafrost that have been isolated from human existence since ancient times, possess antibiotic-resistance determinants that pre-date the era of the clinical use of antibiotics.[131,132]

Definitions of resistance may be confusing.[133] Laboratory testing of the susceptibility of clinical isolates to antibiotics informs clinical practice and those reported to be sensitive are likely to succumb to appropriate therapeutic regimens. Those with levels of susceptibility likely to result in therapeutic failure are termed resistant. Species which have not demonstrated susceptibility to an antimicrobial agent (either antibiotic or non-antibiotic) are considered to exhibit innate (or intrinsic) resistance.[133–135] In the latter case, intrinsic resistance is considered to occur naturally, being independent of antimicrobial exposure and not caused by horizontal gene transfer. Impermeability of cell envelopes, sporulation, lack of suitable drug targets or the activity of multidrug efflux pumps are examples of adaptations that may account for the phenomenon.[133–135] The genes that code for these attributes are usually located on the bacterial chromosome,[33] but the mechanisms controlling intrinsic resistance are not entirely understood.[136]

Species with newly decreased susceptibility due to a permanent genetic change (mutation) are regarded as having acquired antibiotic resistance.[130,133] This can arise either following a spontaneous mutagenic event in a relevant gene, or by acquiring an appropriate resistance gene on a mobile genetic element (such as integrons, transposons or plasmids) from a neighbouring resistant strain. Movement of genetic elements between bacteria is achieved by transformation, transduction and conjugation. Novel resistance mechanisms, genes and mobile genetic vectors continue to be described, but essentially five types of adaptations confer antibiotic resistance:[137]

- Possession of an enzyme (such as β-lactamase) that degrades an antibiotic
- Reduced permeability of the cell envelope to prevent ingress of an antibiotic
- Structural changes in the microbial target site that prevent binding of the antibiotic
- Acquisition of alternative enzymes/pathways to obviate the original target site
- Acquisition of efflux pumps to remove antibiotic from the cytoplasm within the target cell.

In bacteria five types of transporter have been described:

- The major facilitator superfamily (MFS)
- Multidrug and toxic efflux (MATE)
- Resistance-nodulation-division (RND)
- Small multidrug resistance (SMR)
- Adenosine triphosphate (ATP) binding cassette (ABC).[138]

Efflux pumps are transport proteins that actively export potentially toxic substances from within cells. Some carry a specific molecule, but many export a variety of different classes of substances including antibiotics. Efflux pumps are thought to explain intrinsic resistance of many Gram-negative bacteria.[138]

Multidrug resistance (MDR) occurs in strains which have acquired resistance to more than one class of antibiotic from different mechanisms.[133] Species with MDR in all but one or two classes of antibiotic are extensively drug resistant (XDR), and species without susceptibility are said to be pan-resistant (PDR).[139] Gram-negative rods with extended spectrum β-lactamases (ESBLs) are a concern.[140] MDR and PDR infections in wounds caused by MRSA, vancomycin-resistant *Staphylococcus aureus* (VRSA), *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and ESBLs are a concern for practitioners caring for wounds. The high density of microbial population sizes, their relatively short generation times and contact with antibiotics increase opportunities for the emergence of resistance strains.

Resistance to non-antibiotic antimicrobial agents

As with antibiotics, since the 1950s there have been reports of resistance to non-antibiotic antimicrobial agents pertinent to wound care, namely quaternary ammonium compounds (benzalkonium chloride and cetrimide), CHX, silver, PVP-I, sodium hypochlorite, hydrogen peroxide and gentian violet.[21,33,133] Mechanisms of resistance to these agents are not entirely elucidated, but those investigated to date mirror those mechanisms associated with antibiotic resistance:

- Enzymic degradation converts active silver ions to inactive metallic silver, while catalase and superoxide dismutase inactivate free radicals generated from hydrogen peroxide[141]
- Reduced permeability in Gram-negative bacteria following changes in outer membrane components (such as lipopolysaccharide, proteins, fatty acids and

phospholipids) have been implicated in decreased susceptibility to quaternary ammonium compounds and CHX[133]
- Structural modifications in a target site (enoyl reductase) resulted in resistance to triclosan[142]
- Changes in biosynthetic pathways may explain resistance to quaternary ammonium compounds and triclosan[133]
- Efflux pumps exporting biocides from microbial cells have been reported.[133,137] Resistance to quaternary ammonium compounds, CHX, cetrimide, benzalkonium chloride, biguanides, triclosan and silver has been linked to efflux pumps.[33,133,143]

Acquisition of resistance to benzalkonium chloride and quaternary ammonium compounds in staphylococci has been associated with plasmids,[143,144] whereas genes coding for efflux pumps in many Gram-negative bacteria are chromosomal, with some associated with potentially mobile integrons.[143] Reports of resistance to silver have been accumulating since the 1970s.[145–150] Resistant organisms include MRSA, *Klebsiella pneumoniae* and *Enterobacter cloacae*, isolated from DFUs, chronic leg ulcers or burns patients.

Following an outbreak of resistance to silver nitrate on a burns ward in Massachusetts General Hospital which led to several fatalities, resistance to silver nitrate, mercuric chloride, chloramphenicol, ampicillin, tetracycline, streptomycin, and suphonamides was detected in *Salmonella typhimurium*. The resistance was transferrable between *Salmonella typhimurium* and *Escherichia coli* in mating experiments (i.e. by conjugation).[151] Subsequently, a plasmid carrying the resistance genes was isolated and characterised. In the silver resistance gene cluster, nine genes in three transcription units were recognised. Expression of the encoded genetic information was for a periplasmic silver-specific binding protein and two effluc pumps (one was an ABC pump and the other a RND efflux pump).[152]

The same genes were identified on further plasmids and similar silver resistance genes were found in enteric bacteria.[145] Silver-resistant mutants of *Escherichia coli* displayed active efflux of silver ions, as well as decreased uptake of silver ions due to deficient outer membrane proteins.[153] Exposure of *Escherichia coli* to sub-lethal concentrations of silver nitrate for six days *in vitro* resulted in two point mutations that conferred silver resistance. One caused loss of function in an outer membrane porin associated with silver uptake, the other caused increased activity of a RND efflux pump by derepression (or activation). Thus, endogenous resistance (spontaneous mutation) led to decreased import and increased of export silver.[154] Additionally, exogenous silver resistance involved activation of another RND efflux pump and expression of a periplasmic silver-sequestration protein. The genes coding for these products were located on a plasmid that had been previously acquired by the bacterium.[154] Thus, silver resistance in *Escherichia coli* was conferred by mutation as well as gene acquisition. In another study, rapid evolution of resistance to silver nanoparticles in *Escherichia coli* in the laboratory illustrated ease of selection of resistant strains.[155]

Although there are standardised laboratory tests for determining antibiotic susceptibility and antiseptic efficacy, methods of detecting resistance to non-antibiotic antimicrobials in clinical isolates are less well developed.[133] In particular, a lack of consensus on methods to test for silver sensitivity was noted by Muller and Merrett in 2014.[156] In previously published studies, detecting *sil* genes had been the basis for identifying silver resistance, and the absence of these genes was interpreted as evidence of silver susceptibility and low prevalence of silver resistance.[145–155] However, a highly significant positive correlation between production of pyocyanin by clinical strains of *Pseudomonas aeruginosa* and resistance to silver was discovered.[156] Pyocyanin is an extracelluar redox-active pigment produced constitutively by *Pseudomonas aeruginosa*. It conferred intrinsic resistance to ionic silver by reducing it to metallic silver outside the cell, so silver ions did not accumulate within this bacterium, and there was no necessity to acquire the genes coding for silver resistance in order to be protected against silver toxicity.[156] The absence of genes coding for silver resistance in *Pseudomonas aeruginosa*, therefore, cannot be inferred as susceptibility to silver. It is probable that insusceptibility to silver in *Pseudomonas aeruginosa* has been underestimated, and it raises questions about the validity of testing methods. In terms of using non-antibiotic antimicrobial agents clinically, the priority is to determine whether the pathogens responsible for a wound infection are susceptible to a prospective therapy rather than to determine the mechanism of insusceptibility (intrinsic or acquired resistance).

Concern about the lack of standardised methods to determine resistance to CHX has also been expressed.[157] Resistance to CHX has been found in *Pseudomonas*, *Acinetobacter*, *Klebsiella oxytoca*, MRSA and *Staphylococcus epidermidis* recovered from burns and surgical wounds.[158–161] The implications of reduced susceptibility of staphylococci to CHX have been highlighted.[157] Furthermore, nosocomial outbreaks attributed to contaminated solutions of antiseptics or disinfectants have occurred and some of these have led to infected wounds.[162] These outbreaks were not caused by contaminated wound dressings. They were due to antiseptic solutions being contaminated during manufacture, by dilution with tap water before use or by storage of diluted solutions in unsterile vessels. These manufacturing issues have been overcome. These events, however, illustrate the metabolic diversity of some bacteria.

The prevalence of genes coding for non-antibiotic antimicrobial resistance in wound isolates has raised some concern,[150,158,160] as has the ease of the selection of silver resistance after three weeks of clinical treatment.[149] Increased surveillance of silver resistance and tighter control of silver usage have been advocated.[154,163]

Cross-resistance to antibiotics and antiseptics

The discovery of strains with both antibiotic and antiseptic-resistance has also raised alarm.[21,133] Frequently co- or cross-resistance is mediated by the possession of multidrug efflux pumps capable of exporting both antibiotics and antiseptics.[143] CHX resistance has been linked to resistance to mupirocin in *Staphylococcus aureus*,[164] to vancomycin resistance in *Enterococcus faecium*,[165] to colistin resistance in *Klebsiella pneumoniae*[166] and to the presence of β-lactamases in *Acinetobacter baumannii*.[167]

The diversity of silver resistance genes located on plasmids carrying antibiotic resistance genes has been described.[168] Silver resistance, linked to ESBL resistance in *Escherichia coli*, has caused alarm in Sweden where silver resistance was observed in human isolates but not isolates recovered from wild birds. Since levels of silver are low in the environment, it was postulated that human exposure to silver promoted the prevalence of these XDR-resistant strains.[169]

Surveillance programmes monitor the prevalence and distribution of antibiotic resistance in many countries, but susceptibility to non-antibiotic antimicrobials is not determined. In order to determine the scale of the threat of resistance to these agents, there is a need to develop rapid tests to detect resistance to non-antibiotic antimicrobial agents and to implement an international surveillance study. Tests for screening clinical isolates would also be valuable in supporting clinical decisions on topical therapies.

Tolerance to antimicrobial agents

Antibiotic sensitivity is communicated from the microbiology laboratory to the clinician so that antibiotic treatment can be adjusted to fit the antimicrobial strain and its resistance pattern. However, antibiotic susceptibility test methods usually use suspensions of strains isolated from clinical specimens (otherwise known as planktonic cells). This artificial environment does not accurately mimic the natural environment, where microbes normally adopt the biofilm mode of growth.[170] Biofilm formation induces antimicrobial tolerance with cells becoming up to 1000 times less susceptible to antimicrobial agents.[170–172] Tolerance can encompass a wide range of unrelated antimicrobial agents.[173] Biofilms comprised of mixed species are ubiquitously found in nature.[170] Permanent changes in biofilm members, such as mutations or gene acquisitions that confer resistance, will be retained by those cells on leaving the biofilm. However, phenotypic changes that confer the ability of biofilm members to tolerate high concentrations of inhibitors are due to transient physiological and biochemical adaptations which will be lost when cells leave the biofilm.[170–173]

Although not completely explained, tolerance is influenced by a number of different factors. The composition of biofilm matrix varies because its distinct constituent species contribute to its composition.[174] It is comprised of polysaccharides,[175] proteins, lipids, extracellular DNA and small amounts of RNA. Movement of quaternary ammonium compounds, biguanides, halogens and hydrogen peroxide through biofilm matrix can be retarded by adsorption to matrix components or chemical quenching.[176,177] Sometimes biofilm matrix may contain enzymes that inactivate an antimicrobial.[178] Variations in the distribution and supply of nutrients and oxygen within biofilms, influences the development of different phenotypes such that limitations lead to slower growth rates and increased antimicrobial recalcitrance.[179–181] Tolerance to antimicrobial agents is subject to change over time, due to changes in the environment and changes in gene expression controlled by intercellular communication (quorum sensing) that affect the status of the biofilm.[182,183] Since failure to heal was associated with the presence of biofilm in wounds,[184,185] interest in finding effective antibiofilm therapies has developed.

The tolerance of biofilm bacteria is, to some extent, indicated in the concept of minimal biofilm inhibitory concentration (MBIC),[186] as an equivalent of minimal inhibitory concentration (MIC) which is used in determination of antimicrobial susceptibility. The MBIC is determined in a standardised set-up but its validity in a clinical setting is not determined.

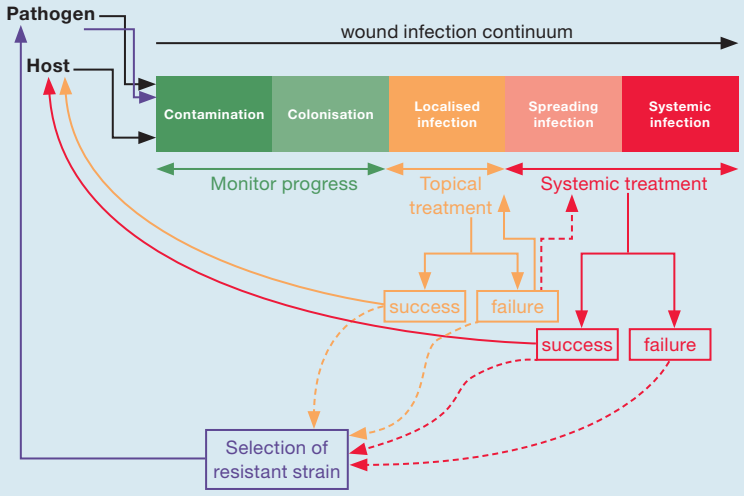
Resistance and tolerance cause failure in antimicrobial treatment but although the former will be genetically transferred to the next generation, the latter may not. The micro-environment of the wound could be influenced by debridement, increased compression therapy and perhaps even dressing changes. The concept of 'The Window of Opportunity' is based on this.[187] Four distinct experimental models demonstrated increasing antibiotic susceptibility with time-dependent biofilm maturity. Thus, sharp debridement followed by topical antimicrobial therapy is a plausible strategy for the management of biofilms in wounds.[187]

Using existing antimicrobial agents effectively in clinical practice

The presence of microorganisms in wounds is not necessarily a matter for concern, because wounds do not have to be sterile to heal. What is important is detecting, at the earliest opportunity, when an infection is present, and whether it is deteriorating or resolving, so that appropriate intervention can be initiated, changed or concluded.[15]

The wound is a challenging environment for microbial cells due to the variety of host strategies designed to remove foreign cells. Microbial survival depends on the expression of virulence mechanisms, such as adherence, invasiveness, toxigenicity and the ability to overcome host immune responses.[188] Increased bacterial numbers favour the expression of virulence genes controlled by quorum sensing and complex host-pathogen interactions dictate whether an infection results or not.

Fig 1. The antimicrobial resistance selection pathway in wounds



The location of a microbial species in any natural situation is non-random. It is influenced by the chemical, physical and biological requirements of that organism; temperature, oxygen, the presence of essential nutrients and growth factors (like iron or vitamins) contribute to the factors that influence microbial distribution patterns.[189] In chronic wounds biofilms of *Staphylococcus aureus* tend to remain near the surface, whereas *Pseudomonas aeruginosa* biofilms occur deeper within wound tissue.[190–192] Numbers of bacterial also vary throughout the wound.[191] Hence, the method of sampling influences what is detected in clinical specimens. A biopsy, for example, is recommended for wounds suspected of having a biofilm.[193] It is likely that the uneven spatial patterns of microorganisms within wounds also affect the efficacy of certain antimicrobial interventions. To date, processing of wound samples has relied on cultivation techniques, which are biased towards identifying organisms that grow in the laboratory. Molecular methods, which allow the characterisation of culture-independent microorganisms, are able to provide detailed information on microbial load and diversity,[126–129] and are becoming increasingly important in understanding the role of microorganisms in health and disease.[194–196]

A necrotic wound bed may facilitate bacterial colonisation and the growth of anaerobes. Fundamental wound management aims to restore vascular supply (arterial and venous), decrease excessive interstitial fluid (oedema), remove necrotic tissue and reduce repetitive mechanical tissue damage that leads to the development of pressure ulcers and DFUs.[197] Failure to address all of these factors may favour an environment that supports a high bioburden.

Antibiotics have provided a safe and effective means of preventing and treating infections for 70 years but continued emergence of antibiotic resistance threatens their future efficacy. Non-antibiotic antimicrobial strategies are likely to become much more important in wound care, even though they may pose an additional but unquantifiable risk of selecting for resistant strains. Events that select antimicrobial resistant strains in modern wound care must not be overlooked (Fig 1). Essentially, the use of any antimicrobial agent, whether it be in clinical treatment or in cleaning healthcare facilities, provides chances for strains resistant to that agent to flourish, while sensitive strains do not. Hence, it is essential that all antimicrobial interventions are used appropriately. That means choosing the right agent in the right dose at the right time for the right duration. This is the basis of antimicrobial stewardship.

Effective control of wound infection depends on early diagnosis, rapid identification of causative agents and determination of antimicrobial susceptibility.[15] The development of reliable point of care tests are urgently required to manage antimicrobial resistance in wound pathogens. Suitable tests do not yet exist, but one competition to reward successful innovation was initiated in 2014.[198]

Selection of appropriate antimicrobial intervention depends on the availability of resources locally, practitioners' knowledge and experience, and patients' preferences.[15] In clinics where non-antibiotic antimicrobials are non-prescription devices that can be initiated by any member of the wound care team, it is not uncommon to encounter antimicrobial treatments on wounds without appropriate basic treatment. Antimicrobial interventions must be precipitated by a comprehensive evaluation of the basic wound care in order to favour healing and limit necrotic tissue. Factors that should be considered in selecting a wound dressing have been summarised as: the stage of healing, amount of exudates, infection, odour, ease of removal, irritation of dressing adhesive, adsorption, frequency of dressing changes, pain caused at dressing changes, protection of surrounding skin, and patient preferences.[125]

Factors contributing to antimicrobial misuse by practitioners treating wounds are diagnostic uncertainty, clinical ignorance, clinician fear *'of failing to treat properly, or of having a bad outcome'* and patient demands.[15] Whereas advice on treating wound infection with antibiotics is readily available,[15,44,45] advice on topical non-antibiotic antimicrobial interventions is less prescriptive.[44,45,199,200] With the passage of time, additions to the range of antimicrobial therapies designed for wound care, changes in formulations of existing products and staff turnover create a continual demand for education. A survey of competencies pertinent to specialised wound care nurses in six European countries showed that a wide range of personnel involved in managing wounds were found to have experienced inconsistent educational opportunities.[201] Significant variations in nurses' knowledge of basic wound management were recognised in several studies.[202–205] A survey of

136 nurses at three different levels (advanced clinics, home care and general hospital care) identified shortfalls in the evidence base that underpins wound care and in links between objective evidence and clinical practice;[205] differences in theoretical knowledge were not associated with length of service.[205] Two studies have reported ritualistic practice.[204,205] The need for structured education for pre- and post-registration nurses, and for better clinical evidence, was emphasised in one study,[205] and improvements in dressing selection following education have been demonstrated.[206]

Wound care in a post-antibiotic era

With the limited evidence of clinical efficacy for antimicrobial interventions outlined above, the possibility of inconsistencies in dressing selection, and continuing emergence of antimicrobial resistance, control of wound infection in a post-antibiotic era seems rather bleak. Using existing resources in a responsible manner is paramount. Additional measures are also needed.

Preventing infection

In the pre-antibiotic era tetanus and gas gangrene were frequent causes of wound infection which caused high rates of morbidity and mortality following surgical procedures.[207] Up until the 1950s wound care relied on antiseptics and 'good hygiene' to prevent infection. Infection control is still an important function today, and it may become more critical in the future. Emphasis on handwashing, aseptic non-touch technique (ANTT), effective environmental cleaning and patient placement will increase. Preventing wound infection by immunisation may become routine. Some progress has been made in this area with animal studies,[208–210] but human studies are limited.[211] The prime candidates for vaccine development are MRSA,[208,209,211] *Pseudomonas aeruginosa*[210] and *Candida*.[211]

Another approach to preventing wound infection concerns colonisation resistance using natural flora, pre- or probiotic bacteria to replace pathogens. This concept was initially developed to treat gut disorders, but it may have wider effects and has been suggested for treating acne, atopic dermatitis and wounds.[212] Much of the data published to date concerns the effect of *Lactobacillus plantarum* on burns,[213,214] chronic leg ulcers[215] and DFUs.[216] *Staphylococcus lugdunensis* was shown to produce a novel antibiotic called lugdunin. It is bactericidal against a broad range of human pathogens, active in animal models and not prone to elicit resistance in *Staphylococcus aureus*. It reduced nasal carriage rate of *Staphylococcus aureus* in humans, and therefore has potential in treating infected wounds.[217]

Treating infection with novel agents

Although the research and development process is long and expensive, natural products are on the horizon for wound care. One antimicrobial agent previously used — vinegar — promises to regain a position on modern formularies. Its clinical use declined during the last 100 years. The ability of acetic acid (both as an acidic solution and as sodium diacetate) to inhibit planktonic bacteria, and eradicate biofilms alone[218,219] and in combination with selected antibiotics, has been investigated *in vitro*.[218] Instillation of acetic acid into chronic wounds together with NPWT has also been described.[218]

All organisms possess innate immune defence mechanisms that use antimicrobial peptides (AMPs). Typically, AMPs are a relatively heterogeneous group of small, cationic molecules with broad spectrum antimicrobial activity. Their mode of action is not uniform, but many insert themselves into microbial membranes by electrostatic attraction to negatively charged phospholipids leading to the formation of pores which results in membrane disruption.[220] In addition to antimicrobial activity (including biofilms), AMPs offer therapeutic potential as mediators of wound healing.[221,222] Examples are:

- Lactoferrin, β-defensin, and cathelicidins (of human origin)[223–225]
- Pexiganan and temporins (from frogs)[226,227]
- β-defensin, cecropins and lucifensins (from insects)[28,228,229]
- Snake toxin and tylotoin (from reptiles)[230,231]
- Bacteriocins and lantibiotics (from bacteria).[232,233]

The role of efflux pumps in intrinsic resistance and MDR makes the development of efflux pump inhibitors an important future control strategy.[137,234] One approach is to search for potential inhibitors by virtual screening using computer models, followed by laboratory evaluation of identified candidate compounds.[235] Natural products, such as flavonoids, seem to offer promise as efflux pump inhibitors.[236,237]

Quorum sensing inhibitors have also emerged as an innovative means to control biofilms and infections caused by pathogens with antimicrobial resistance. Quorum sensing is an intercellular communication mechanism that regulates the expression of microbial genes by chemical signals. It is involved in the control of virulence, biofilm formation, sporulation and motility.[188] Although synthetic quorum sensing inhibitors have been identified, phytochemicals, such as flavonoids, flavones, polyphenols and essential oils, have also emerged as quorum sensing inhibitors.[238–240] Many traditional herbal or medicinal plants are being screened for these molecules and the list of inhibitors is likely to increase with time. One of the first plant-derived quorum sensing inhibitors to be investigated was garlic. The mechanism of quorum sensing inhibition induced by ajoene (an extract of garlic) was recently elucidated in *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[241] Thus, garlic extract is a potential therapy for wound infection. Honey also inhibits bacterial quorum sensing *in vitro*.[242,243]

Extraction of aromatic plants yields complex mixtures containing essential oils (EO).[244,245] Many

EOs, particularly terpenes and terpenoids, possess broad spectrum antimicrobial activity and some also act as quorum sensing inhibitors.[246] They have already been used extensively in foods and cosmetics,[244,245] and their future in controlling multidrug resistant bacteria is recognised.[247] Tea tree oil (TTO) has attracted most attention in dermatology,[248] although the evidence to support the use of TTO in wound healing is limited.[249,250] Low solubility of EOs has hampered laboratory investigation, but the mode of action of TTO is linked to penetration of bacterial and fungal membranes leading to cytoplasmic loss and destabilisation of internal organelles.[251] Because low solubility of EOs also affects bioavailability, encapsulation into lipid nanoparticles, liposomes or polymers allows prolonged delivery and improved stability.[252] A formulation of marigold oil has been developed for a wound dressing.[253] Further to essential oils, other phytochemicals are being evaluated for future wound care.[248,254–256]

With the low level of investment in searching for new antibiotics at present, future remedies may be rediscovered by re-examining discarded therapies. Revaluating existing drugs with a view to re-purposing them is one strategy being considered with antibiotics.[257–259]

Combination therapy

Another strategy for coping with antimicrobial resistance is to employ combination therapy. Long before antibiotic-resistance became a global problem, the idea of using two agents simultaneously to treat bacterial infections was conceived.[260] Important benefits that have been attributed to combination therapy are the ability to combat[261] and to prevent acquired antibiotic resistance.[262] Some demonstrate synergistic action in that the antimicrobial activity of the combination is greater than the sum of the components within the mixture. Many combinations have already been suggested (Table 3).[76,107,263–275] Synergistic combinations involving antibiotics suggest that the usefulness of conventional antibiotics may be prolonged when used simultaneously with non-antibiotic antimicrobial agents.[270,271,276,277]

Conclusion

The thought of returning to a pre-antibiotic era is frightening and remote. Advances in the development of innovative antimicrobial interventions will surely take us into a post-antibiotic era. The WHO has recommended that the rational use of medicines requires:

> *'that patients receive medications appropriate to their clinical needs, in doses that meet their own individual requirements, for an adequate period of time, and at the lowest cost to them and their community'.*[278]

The latest advice on the use of antibiotics from WHO is to update the essential medicine list into three categories: ACCESS, WATCH and RESERVE; while some antibiotics would be readily available, some would be restricted to a limited number of infections and others would be used only as a last resort.[279]

Table 3. Examples of combination therapies for wound care

Antimicrobial agent		Combined with	Citation
Antiseptic	Acetic acid	NPWT	263
	Octenidine	NPWT	264
	Povidone-iodine	NPWT	265
Essential oil	Myrtle oils	Antibiotics	266
	Eucalyptus oils	Antibiotics	267
	Cinnamon bark oil	Antibiotics	268
Honey	Manuka honey	NPWT	269
	Manuka honey	Antibiotics	270
	Manuka honey	Antibiotics	271
	Heather honey	Lactic acid bacilli	272
	Portuguese honey	Phage	273
Silver	Silver sulfadiazine	Surfactant	274
	Ionic silver	Surfactant and chelator	76
	Ionic silver	Tea tree oil	275
Bacteriophage		Linezolid	107

Microbial evolution will dictate a constant search for new antimicrobial agents. In the immediate future, the continued emergence of antibiotic resistant strains will place greater reliance on non-antibiotic antimicrobials. Antimicrobial stewardship is the coordinated action required to select, use and monitor appropriate antimicrobial agents optimally in order to slow the emergence of resistant strains and to preserve their future effectiveness. In wound care, the need to institute antimicrobial stewardship to safeguard antibiotics has been discussed.[15] Antimicrobial stewardship in relation to the use of non-antibiotic antimicrobial agents (other than silver[150,154,163] or CHX[14,157,280]), however, has received scant attention.

This review demonstrates the substantial range of conventional non-antibiotic antimicrobials available for treating wounds, as well as those emerging, being developed and under investigation. It is imperative that misuse of these resources be avoided to safeguard their effectiveness in wound care. Unfortunately the clinical evidence to support the use of conventional antimicrobial agents in wound healing is weak (Table 2), and evidence of antimicrobial efficacy *in vivo* is sparse. Hence the evidence base necessary to inform good practice is deficient.[205] Limitations in the knowledge of wound care practitioners have been identified.[201–206] The prevalence of resistance to non-antibiotic antimicrobial agents is unknown, a consensus on suitable testing methods is not reached, and routine susceptibility testing and surveillance are not yet possible.

In wound management it is imperative that all antimicrobial interventions are used wisely. In order to implement AMS there is much work to do. Objective data on the clinical efficacy of non-antibiotic antimicrobial agents must be obtained and evaluated. Quality standards must be identified and robust antimicrobial guidelines developed. Methods to ➤

Reflective questions

- How is continuing emergence of antimicrobial resistance likely to impact on wound management?
- Should resistance to every antimicrobial agent used in treating wounds be monitored?
- What is the importance of resistance to non-antibiotic agents to wound care?

evaluate non-antibiotic susceptibility must be developed and surveillance programmes introduced. Readily accessible educational resources must be developed for all personnel involved in wound care and updating of knowledge encouraged. Prescribing practice should be routinely monitored and evaluated, with feedback provided to prescribers. Audit, review and effective communication should include health professionals across all settings, as well as patients. The process will difficult yet it cannot be ignored. **JWC**

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