ANTIBIOTIC RESISTANCE AND BIOFILM TOLERANCE: A COMBINED THREAT IN THE TREATMENT OF CHRONIC INFECTIONS

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INTRODUCTION

Bacteria were one of the first lifeforms to appear on earth >3.5 billion years ago, and have evolved as highly adaptable organisms capable of surviving in extreme and hostile environments. Their adaptability has been enabled by the ability of bacteria to produce exopolymeric substance (EPS) which provides protection from the outside world. Today, this self-produced protective matrix is known as biofilm. Similarly, antibiotic resistance has evolved naturally over millions of years as a survival strategy amongst bacterial species. However, it is only in the last 80 years or so since the availability of commercial antibiotics, that the true impact of antibiotic resistance has been realised. Since antibiotics, antibiotic resistance, and biofilm have evolved, it is reasonable to assume that inter-relationships exist. The following review addresses the importance of antibiotic resistance and biofilm tolerance in chronic infections, and the urgent need to develop combination therapies that will enable antimicrobial agents (antibiotics and antiseptics) to work most effectively in chronic infections.

ANTIBIOTICS AND RESISTANCE TODAY

The introduction of antibiotics into human medicine in the 1940s was one of the greatest medical advances. However, a post-antibiotic era is within sight due to excessive use of antibiotics in human and animal welfare, and antibiotic resistance is now a threat to human health. England's Chief Medical Officer predicts that common surgical procedures will become risky, and transplant medicine will become a thing of the past, marking 'the end of modern medicine'.[1] Furthermore a review of antimicrobial resistance commissioned by the UK Prime Minister in 2014 reported that by 2050 antibiotic resistance could account for an increase of up to 10 million deaths per year across the US and Europe, with associated costs in the region of the US$100 trillion worldwide.[2] Antibiotic stewardship programmes have been implemented worldwide to promote appropriate antibiotic prescribing practices to reduce the spread of antibiotic-resistant microorganisms.

The role of biofilm in antibiotic resistance

Biofilm plays an important role in the evolution of antibiotic resistance, in that it provides an environment that encourages horizontal spread of antibiotic resistant genes and virulence factors,[3] and conjugation between cells in biofilm is said to be 700-times more efficient than among planktonic bacterial cells.[4] Bacterial tolerance within biofilm is relatively unappreciated, while genetically-resistant bacteria living within biofilm creates additional physical/physiological tolerance to antimicrobial agents,[5] presenting a major threat to human health.

Chronic, non-healing wounds: a particularly challenging clinical paradigm

Biofilm is implicated in most bacterial infections in the human body,[6] and the clinical impact of this is evident in chronic, non- healing wounds (Fig 1), harbouring a complex, biofilm-predominant microflora,[7, 8] which is unresponsive to topical and systemic antimicrobial therapy. Biofilm-producing Enterococci from diabetic foot wounds showed a higher prevalence of resistance to erythromycin, tetracycline and ciprofloxacin than non- biofilm- producing isolates, [9] suggesting an inter-relationship between antibiotic-resistant bacteria and biofilm, which needs to be disrupted in order

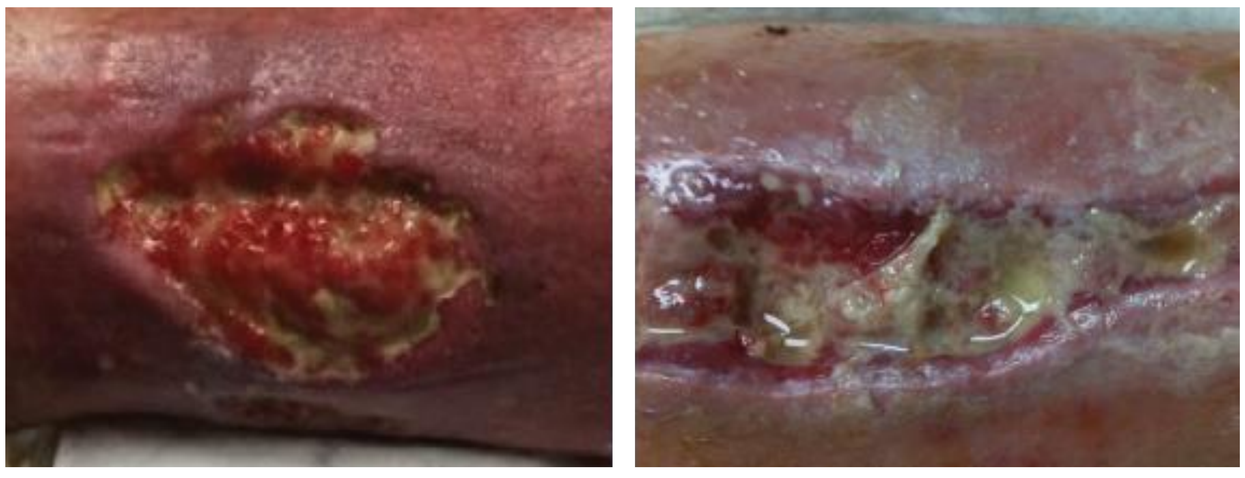

Figure 1. Examples of chronic, non-healing wounds characterised by likely presence of biofilm, antibiotic-resistant pathogens and tissue ischaemia

In addition to biofilm compromising antimicrobial effectiveness in chronic wounds, tissue ischemia can prevent antibiotics from reaching therapeutic concentrations at the wound site, which may encourage the development of antibiotic resistance (Fig 2).[10] This has been reported in several studies, particularly when peripheral arterial disease is involved.[11,12] 68% of patients with a chronic wound in primary care received at least one antibiotic course, compared to 29% of patients without a chronic wound[13]. Furthermore, it was reported that costs per patient with diabetic foot ulcers were four times greater with an infection, with costs largely attributed to antibiotics, hospitalization and amputations.[14]

The British Society of Antimicrobial Chemotherapy (BSAC) and the European Wound Management Association (EWMA) jointly published a paper, with the primary objective being to provide guidance on the appropriate use of systemic and topical antibiotics to ensure the most clinically effective therapy is implemented to manage infected wounds.[15] Bacteria often work in synergy as a pathogenic consortium, therefore, the use of narrow-spectrum antibiotics in polymicrobial wounds is unlikely to be successful.[16] In contrast, topical antiseptic agents, such as ionic silver and molecular iodine, exhibit a broader spectrum of activity and are less likely to induce bacterial resistance.[17] Topical antiseptics can be advantageous in that they have direct access to the superficial wound[18] (Fig 2), however, biofilm can act as a barrier to this and prevent the antimicrobial agent from contacting and killing the bacterial cells, making the biofilm bacteria tolerant. Therefore, alternative strategies such as transforming protected, tolerant biofilm bacteria into exposed, susceptible bacteria must be developed to enhance antimicrobial effectiveness.

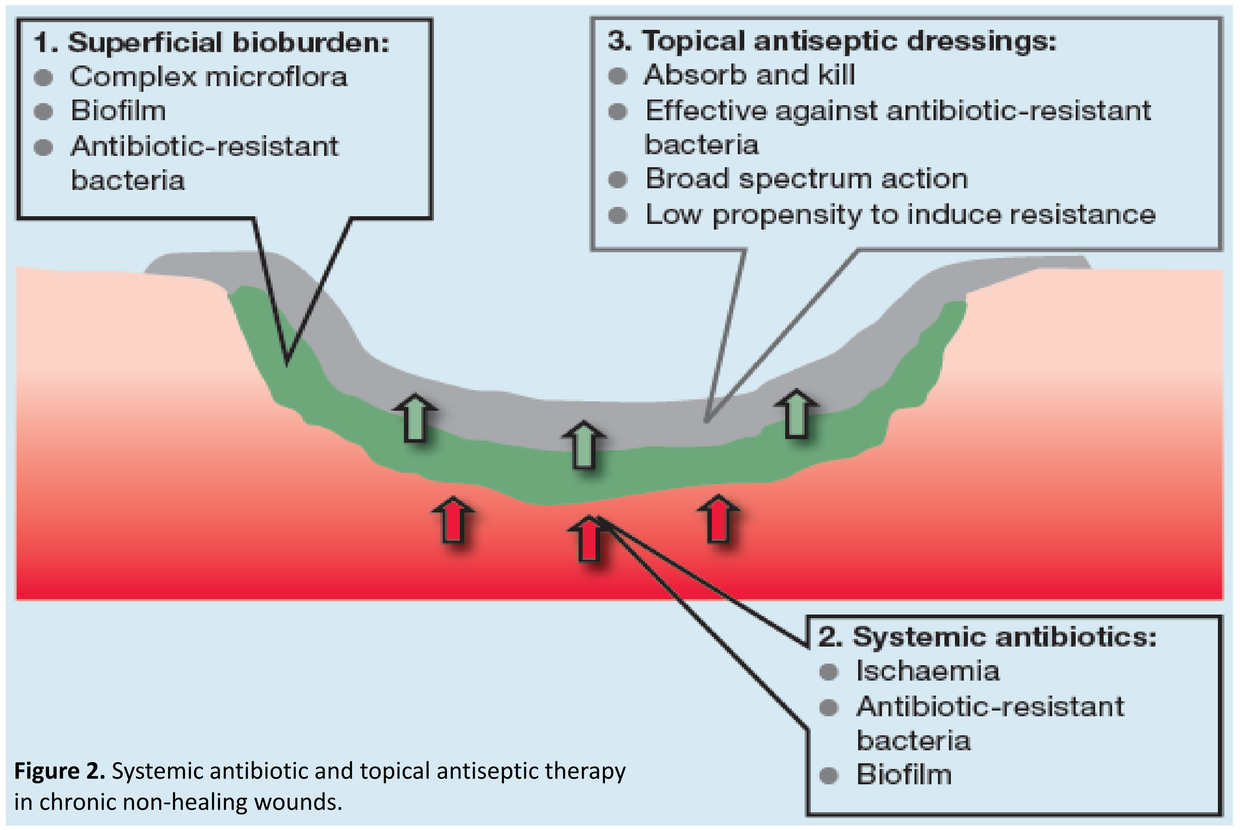

Figure 2. Systemic antibiotic and topical antiseptic therapy in chronic non-healing wounds.

An alternative antimicrobial strategy

New approaches are required to combat biofilm-associated infections. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines to improve treatment of biofilm infections, advocate antibiofilm strategies in combination with antimicrobial agents which could provide several benefits, such as increasing bacterial susceptibility and reducing the propensity for antibiotic resistance.[19] An antibiofilm wound gel was found to facilitate wound healing more effectively when used with topical antibiotics.[20] The antimicrobial efficacy of a silver-containing wound dressing was found to be enhanced by an enzyme capable of dispersing poly-N-acetyl glucosamine, a component of biofilm.[21] In 2014, following extensive research to identify safe chemical technologies that work most efficiently with ionic silver, a wound dressing was introduced that was designed to more effectively combat wound bioburden, improve antimicrobial effectiveness, and reduce the need for systemic antibiotics. The effects of this wound dressing have been shown *in vitro* and *in vivo* to have superior efficacy compared to the same device without the technology and ionic silver.[22, 23] This dressing, now available as AQUACEL® Ag+ Extra™ (in the US as AQUACEL® Ag Advantage™), has also been shown to improve healing in bioburden-impaired wounds, that were previously unresponsive to antimicrobial agents.[24,25]

SUMMARY

- Bacteria were among the first, and will most likely be the last surviving organisms on earth due to their evolutionary adaptability to produce and overcome antimicrobial molecules, and tolerate extreme environmental conditions.
- In recent years, we have discovered that biofilm tolerance and antibiotic resistance are linked, impacting, amongst others, the management of chronic and infected wounds.
- This knowledge has encouraged us to think differently about how to develop and implement new antimicrobial strategies to combat antimicrobial resistance and tolerance.
- Combinations of antibiofilm and antimicrobial agents look promising in the management of chronic infections, and may contribute to the success of future antibiotic stewardship.

References

1. McDonough J. Chief medic warns of the post-antibiotic apocalypse. CEO Magazine, October 13, 2017.
2. O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. HM Government, 2014.
3. Flemming et al. Biofilms as an emergent form of bacterial life. Nat Rev Microbiol 2016; 11: 563-75.
4. Savage et al. *Staphylococcus aureus* biofilms promote horizontal transfer of antibiotic resistance. Antimicrob Agents Chemother 2013; 57: 1968-70.
5. Stewart PS. Antimicrobial tolerance in biofilms. Microbiol Spectr 2015; 3: 1-30.
6. National Institutes of Health. Research on Microbial Biofilms Guidance Report, 2002.
7. Hurlow et al. Clinical investigation of biofilm in non-healing wounds by high resolution microscopy techniques. J Wound Care 2016; 25 Suppl 9: S11-S22.
8. Malone et al. The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data. J Wound Care 2017; 26: 20-5.
9. Shettigar et al. Severity of drug resistance and co-existence of *Enterococcus faecalis* in diabetic foot ulcer infections. Folia Microbiol (Praha) 2018; 63: 115-22.
10. Pereira et al. Microbiota of chronic diabetic wounds: ecology, impact and potential for innovative treatment strategies. Frontiers Microbiol 2017; 8: 1791.
11. Uçkay et al. Diabetic foot infections: what have we learned in the last 30 years? Int J Infect Dis 2015; 40: 81-91.
12. Vella et al. Factors affecting penetration of ciprofloxacin in lower extremity ischemic tissues. Int J Low Extrem Wounds 2016; 15:
13. Howell-Jones et al. Antibiotic prescribing for chronic skin wounds in primary care. Wound Repair Regen 2006; 14: 387-93.
14. Prompers et al. Resource utilisation and costs associated with the treatment of diabetic foot ulcers. Prospective data from the Eurodiale Study. Diabetologia 2008; 51: 1826-34.
15. Lipsky et al. Antimicrobial stewardship in wound care: a position paper from the British Society for Antimicrobial Therapy and the European Wound Management Association. J Antimicrob Chemother 2016; 71: 3026-35.
16. Bowler et al. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001; 14: 244-69 .
17. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev 1999; 12: 147-79.
18. Bowler et al. Multidrug-resistant organisms, wounds and topical antimicrobial protection. Int Wound J 2012; 9: 387-96.
19. Høiby et al. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clin Microbiol Infect 2015; 21 Sup1: S1-25.
20. Wolcott R. Disrupting the biofilm matrix improves wound healing outcomes. J Wound Care 2015; 24: 366–71.
21. Gawande et al. Antimicrobial efficacy of Dispersin B wound spray used in combination with a silver wound dressing. Microbiol Insights 2014; 7: S13914.
22. Bowler PG, Parsons D. Combatting wound biofilm and recalcitrance with a novel anti-biofilm Hydrofiber wound dressing. Wound Medicine 2016; 14: 6-11.
23. Seth et al. Impact of a novel, antimicrobial dressing on *in vivo*, *Pseudomonas* aeruginosa wound biofilm: quantitative comparative analysis using a rabbit ear model. Wound Repair Regen 2014; 22: 712-9.
24. Metcalf et al. A real-life clinical evaluation of a next generation antimicrobial wound dressing in the United Kingdom and Ireland. J Wound Care 2016; 25: 132-8.
25. Metcalf et al. Safety and effectiveness of a new antimicrobial wound dressing designed to manage exudate, infection and biofilm. Int Wound J 2017; 14: 203-13.