BACTERIAL RESISTANCE ISSUES IN WOUND CARE AND WOUND DRESSINGS

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Summary

The dangers of bacterial colonization in wounds are well understood by caregivers – in fact the modern perspective on medical treatment dates back to Pasteur and the inception of aseptic techniques to prevent the infection of wounds. The challenge of resistant bacteria is that they do not respond as expected to the safety measures normally implemented to prevent bacterial infection. The mechanism of resistant bacteria is complex, and an educated caregiver is more able to implement preventative and protective measures to protect themselves and those around them and in their care. Within this presentation, we explore how antimicrobial agents function, and how this relates to the mechanisms through which bacteria are able to develop resistance to those agents. Quick-Med Technologies Inc. is a provider of wound care technologies, including NIMBUS® barrier dressing that has recently been cleared by FDA and is being commercialized with Derma Sciences as the Bioguard™ product line. This product was designed to kill bacteria by mechanisms that are not amenable to resistance development, as is confirmed experimentally.

Characterization of microbicides / biocides

Biocide = any substance that is specifically destructive to microbes and kills or retards the growth of microorganisms. There are a number of types of biocides, which vary with their level of target specificity, and applicability for various situations.

Antibiotics = natural or synthetic chemical substances that have the capacity, in dilute solution, to inhibit the growth of, or to kill microorganisms. Antibiotics are highly specialized in that they have specific cellular targets, and they require entry into a metabolically active cell in order to function. Because of their high degree of specificity, bacteria are most easily able to develop resistance to antibiotics.

Antiseptics = chemical agents used externally on living tissue to suppress bacterial growth. Antiseptics are applied to living tissue/skin to reduce the possibility of infection and/or sepsis – in a preventative fashion rather than as a treatment. There are many antiseptic types that work by different mechanisms. Some of the most common antiseptics include alcohols, quaternary ammonium compounds, chlorhexidines, biguanides, iodine, hydrogen peroxide, phenol compounds, and heavy metal compounds such as silver agents.

Disinfectants = antimicrobial used on non-living surfaces to destroy microorganisms. Disinfectants are non-selective agents and can be potentially harmful or toxic to living tissue at in-use concentrations.

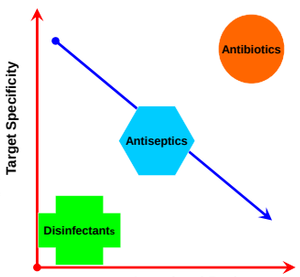


Figure 1. The relative likelihood of bacteria being able to develop resistance to an agent is related to the degree of target specificity of the mechanism of microbicidal action. Antibiotics are highly specific in both their targeting and mechanisms, and bacteria are most easily able to develop resistance. Disinfectants are non-specific and the dangers of bacteria developing resistance to disinfectants is very low.

Mechanisms by which bacteria develop resistance to microbicidal agents

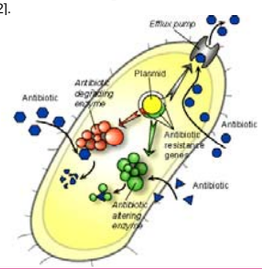
Intrinsic vs. acquired resistance. There are many instances where agents are known not to be effective against certain bacterial species – this is called natural or intrinsic resistance. Intrinsic resistances are well documented, and health care providers do not use certain agents to suppress the microbes against which they are not effective. Acquired resistance poses a risk in health-care and community settings because in this instance, bacterial species show decreased susceptibility to previously effective treatments. The term *resistant bacteria* describes the problem that it was expected the bacteria could be inhibited by some standard measure that has become ineffective.

Bacteria are able to acquire resistance to microbicidal agents by either *de novo* mutation, or through the acquisition of resistance genes from other bacteria. *De novo* mutation is also termed adaptation and is a vertical (hereditary) process: this represents the classically understood evolutionary process of environmental selection of individual bacteria that survive a challenge better, and thus become the progenitors of subsequent generations that carry forward this mutation that permits better survival. In contrast, the acquisition of resistance genes (these are transmitted as packets of gene sequences called *plasmids*) from other bacteria enable bacteria of the same generation to increase their resistance to an agent in a horizontal (non-hereditary) process [Poole, 2002].

Figure 2. Biocide resistance mechanisms: antibiotic agents. Bacteria have shown a number of different mechanisms to increase resistance to biocides. Image from Science Quarterly, 07-08

Antibiotic agents typically target specific metabolic processes. Defense mechanisms include alterations in the metabolic pathway to circumvent antibiotic activity, or the production of enzymes that degrade or alter the antibiotic to render it ineffective.

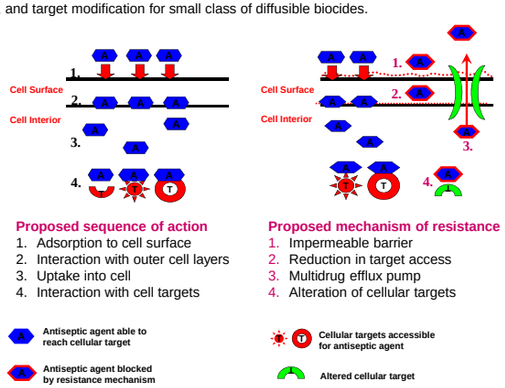
A resistance mechanism that is common for small molecules is the efflux pump: a mechanism by which the bacteria pumps the biocidal agent out of the cell through the use of a transport protein. This enables the bacteria to withstand much higher concentrations of the agent (either antibiotic or antiseptic).



Mechanism of resistance generation to antiseptic agents

The mechanism of bacterial resistance depends on the mode of action of the antimicrobial agent. While bacteria do not develop resistance to **antiseptic agents** as readily as to antibiotics, many of the same defense mechanisms apply. The reasons for the difference in the response of bacteria to antiseptics are still poorly understood, but the chemical composition of the outer cellular layers is considered to be the primary factor for intrinsic resistance mechanisms. Acquired resistance mechanisms include stress response (adaptations to limit uptake of antimicrobial agent), the presence of efflux pumps, and target modification for small class of diffusible biocides.

Figure 3. Mode of action and proposed mechanism of resistance generation to antiseptic agents. The mode of action of most antiseptics is to approach the cell wall, transfer into the cell interior and act on a cellular target, as illustrated by the sequence at left. Bacteria develop resistance by implementing the mechanisms illustrated on the right, including arresting the biocidal agent at the surface by presenting a barrier that is difficult to traverse, altering the cellular targets that are being acted on, and/or by using transport proteins to eject the invading agent from the cell in a efflux pump mechanism.



The action of antiseptic biocides illustrated in **Figure 3** is attributed to high affinity binding to the negatively charged bacterial walls and membranes, and is directed against a wide target spectrum. This broad-action class of agents has significantly less potential for resistance development because resistance has to be expressed against a variety of structurally diverse targets.

Antiseptic agents

There are a wide variety of antiseptic agents that have been utilized as topical antimicrobials, and as active ingredients for antimicrobial wound dressings. Noted below are some small-molecule antiseptic agents.

Silver agents represent the bulk of the market for the US. Silver based antimicrobials exist in various chemistries, including as metallic silver (Silvercel™ - J&J, Silverlon™ - Argentum), Silver Chloride (AquaCel Ag™ - Convatec, Silvasorb™ - Medline), and nanocrystalline silver (Acticoat™ – Smith & Nephew). Despite the diversity of silver chemistries, all silver dressings rely fundamentally on the release of silver ions or particulates, and the accumulation of silver ions within the cells. Within the cells, silver ions poison respiratory enzymes, denature proteins and destroy cell viability by interfering with DNA replication. [Percival, 2005]

Other antiseptic agents worth noting are iodine, which is utilized both as povidone iodine, and more recently as cadexamer iodine (Iodoflex™ - Smith & Nephew.) Iodine also functions by means of protein denaturation and nucleic acid breakdown, as well as damaging the plasma membrane – again acting on the interior of the cell.

Cationic agents

Cationic antiseptics include a range of agents that can be chemically distinguished through the number of charge sites resident on the molecule (or molecular repeat units for polymeric agents). Monocationic quaternary ammonium compounds (QACs) are best known through the common antiseptic Benzalkonium Chloride (one commercial product based on this chemistry is Bactine™ (Bayer)). Bisbiguanides have two cationic phospholipid binding sites: Chlorhexidine is an example of this chemistry. Polyhexamethylene Biguanide (PHMB) is a small polymeric biguanide that is used in contact lens solutions and is the active antimicrobial in Kerlix AMD™ (Covidien).

Quaternary ammonium compounds ('quats' or polyquats in the case of polymeric structures) have a fundamentally different mechanism of antimicrobial activity than small – molecule antiseptic agents such as silver or iodine, that require entry into the cell in order to exert antimicrobial activity. Quats bind rapidly to the cellular envelope and displace otherwise stable calcium ions to chemically destabilize the cell wall structures. Cationic biocides cause the membrane to fragment, leading to generalized cellular leakage.

Gilbert and Moore describe the mechanism of cell wall disruption induced by polymeric cationic biocides in excellent detail as shown in **Figure 4.** They specifically describe the activity of PHMB – which is a polymer of ~ 2,000 daltons molecular weight. The physical size of a polymer chain precludes entry into the cell for larger polymeric biocides, confirming that their antimicrobial efficacy is due solely to the cell wall disruption mechanism. This is critical in regard to bacterial resistance, because all known acquired resistance mechanisms operate against internalized agents.

Polycationic biocides: NIMBUS®

Quick-Med Technologies Inc. has designed the **NIMBUS®** antimicrobial polymeric treatments, which utilize a long chain (molecular weight >100,000 daltons) polyquaternary agent that is permanently bound to a solid substrate. Both the large size of the polymeric agent and the physical attachment to a surface preclude entry into cells, while the high charge density provided by hundreds of quaternary repeat units ensures high biocidal activity.

Figure 4 (right). Theory: action of polymeric cationic biocidal agent. Normal bacterial membranes (panel **a**) are stabilized by Ca+2 ions binding anionic charged phospholipids. NIMBUS® quat-polymer rapidly displaces Ca+2 (panel **b**) leading to loss of fluidity (panel **c**) and eventual phase separation of different lipids. Domains in the membrane then undergo a transition to additional smaller micelles. Image adapted from Gilbert and Moore, 2005.

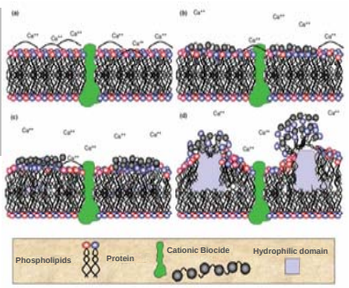
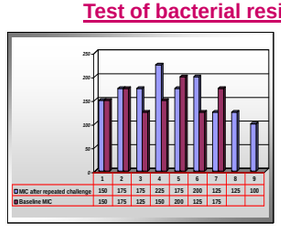
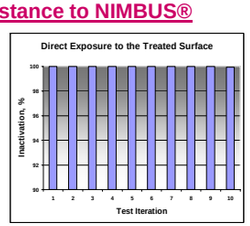


Figure 5 (left). SEM imaging of *E. coli* on untreated gauze wound dressing (top) and on NIMBUS treated wound dressing (bottom). NIMBUS does not require entry into the cell in order to exert antimicrobial activity but destabilizes the cell wall structures, inducing cellular collapse, as experimentally demonstrated by the high resolution SEM images in **Figure 5**. The chemistry of the cell wall is relatively immutable, so the generation of resistance to this mechanism is extremely unlikely.

Test of bacterial resistance to NIMBUS®



MIC of polycationic NIMBUS® compound for *E. coli* after repeated exposure to treated surface



Efficacy of NIMBUS® treated surface against step-wise trained *E. coli* cultures

Figure 6 (above). Determination of microbicidal activity of NIMBUS surface and testing of bacterial resistance. We evaluated changes in bacterial susceptibility to NIMBUS biocide after step-by-step adaptation training of *E. coli* culture to the active surface of the NIMBUS dressing. Sequential assessment of the minimum inhibitory concentration (MIC) was used as an additional confirmation experiment. The selection vector was created by exposing serial passages of bacteria to the NIMBUS treated surfaces. Three or more isolated survivor colonies were selected and propagated into new inoculum. Exposure to the treated substrate was repeated for ten passages. The results of these experiments demonstrated that *E. coli* did not become resistant to NIMBUS after a prolonged and repeated exposure.

Conclusion

Bacteria fail to develop resistance to **NIMBUS®** over the course of many successive generations. The NIMBUS antimicrobial surface destroys bacteria by causing irreversible damage to bacterial membranes rather than by targeting a specific intracellular target, and therefore carries a low risk of resistance development. The design of this technology purposely minimizes opportunities for bacteria to generate resistance, thus permitting safe and effective prophylactic application.

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