A supplement to clinician's brief

Skin and Ear Infections: Bacterial Resistance & Biofilm

AN EXPERT ROUNDTABLE ON A NOVEL APPROACH TO TREATMENT

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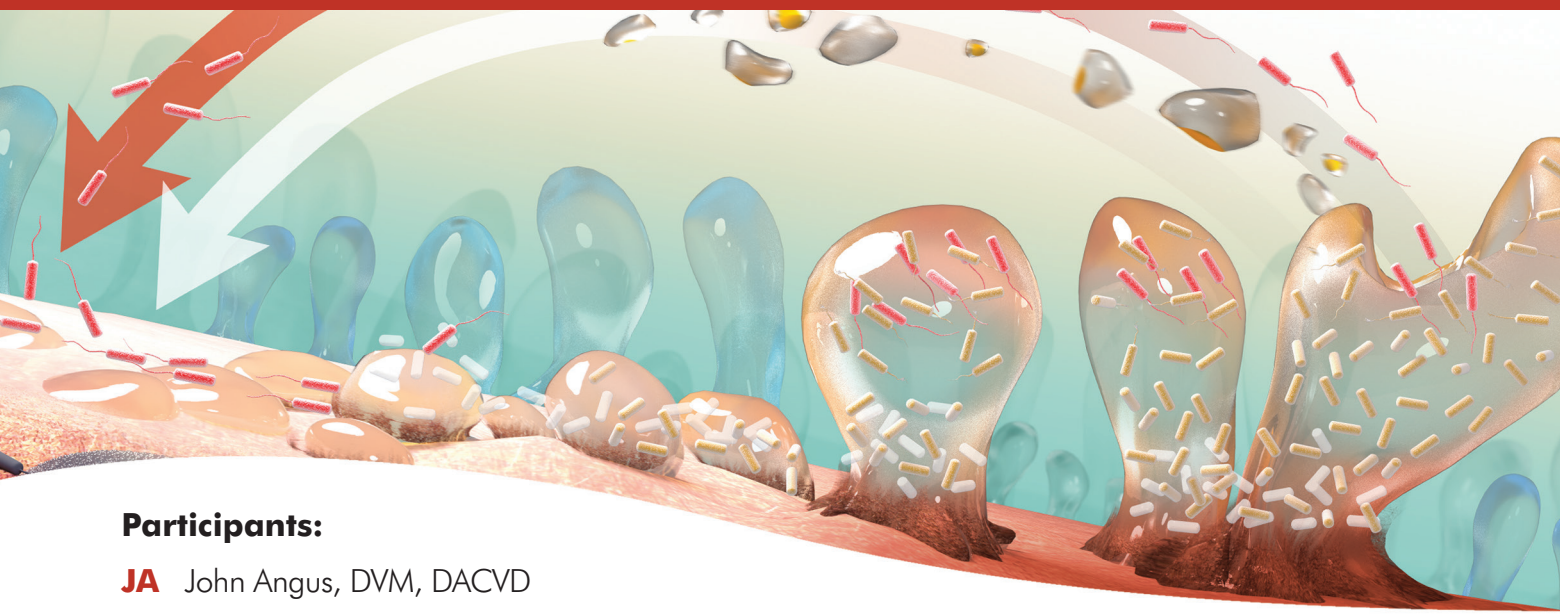

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What are the most common treatment regimens in veterinary practice when animals are affected by skin or ear infections?

DG = It is important to differentiate skin and ears as the approaches are different—as a rule, ear infections are initially treated topically and systemic antibiotic therapy is reserved for chronic and complicated cases, whereas skin infections are most often managed with systemic antibiotics with or without topical therapy. However there is a trend towards topical products because of the emerging increase of resistant antimicrobial organisms.

PM = Does it vary with the type of condition? And its severity? Such as superficial vs deep pyoderma?

DG = Yes—definitely. Usually for superficial pyoderma we treat for 21 days with systemic antibiotic and for deeper infections 6 to 8 weeks or more. It is important to treat for the proper length of time: two weeks past clinical resolution, but not all clients keep their follow-up appointments.

WR = I also think that practitioners are more comfortable using topical therapy for maintenance and prevention of recurrent infections. We are becoming more aware of alternative options to systemic antibiotics especially when dealing with resistant bacterial infections.

DG = Most treatment regimens for pets with skin and ear infections revolve around the use of systemic and topical antimicrobial agents, often in combination with antipruritic and/or anti-inflammatory medications.

Products that also contain ingredients associated with improving epidermal barrier function have become popular in pruritic patients. As veterinarians work to be "good stewards" of antimicrobial use by decreasing the use of systemic antibiotics, topical therapy is becoming more important than ever. This is particularly important as we are facing more and more resistance to systemic antibiotics. The development of newer technologies is aiding the increased efficacy of topical products.

"There is a trend towards products because of the emerging increase of resistant antimicrobial organisms"
—Dunbar Gram, DVM, DACVD

What are the most common causes of failure associated with the classic or current approaches?

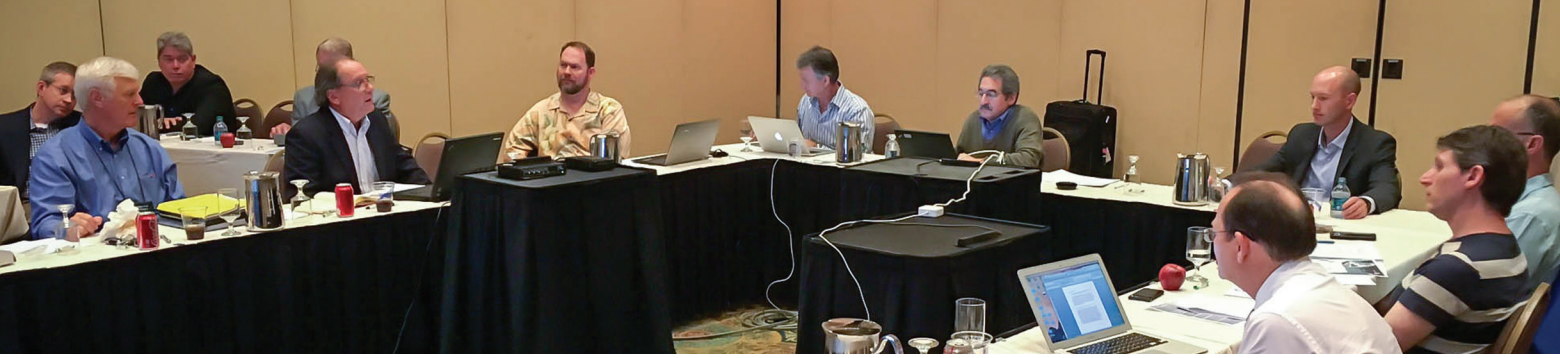
DG = The most common causes of failure are:

- Failure to address or diagnose the underlying cause
- Inadequate duration of treatment.

We increasingly see super-resistant bacterial infections in referral practices. Actually it is somewhat rare for me to see cases of bacterial ear or skin infections that are not associated with bacterial resistance.

WR = We still see "sensitive infections" … but we become more concerned as we are seeing increasing numbers of resistant infections and the worry is "when is it going to stop"?

DS = At University of Florida officially about 60% of the skin infections we culture are resistant to routinely used antibiotics!



"So my approach today is, when I am facing a superficial pyoderma case, to aggressively treat with topical therapy ALONE—but if you choose to use systemic antibiotics, always couple it with topical therapy."

—John Angus, DVM, DACVD

CG = Same for me. About 60% of the skin and ear infections are resistant!

But we should consider 2 groups:

- Dogs that are chronically recurrent, for which the issue is the underlying problem…
- Dogs that are chronically affected because they don't respond…

WR = Another cause of failure we should not neglect is client compliance!

We can send home the proper treatment and tell clients what to do but then is it always getting done properly at home? So any therapies that promote better compliance would help increase the response to treatment. Many of our oral and topical treatment regimens that cut dosing to once a day are moving in the right direction…

AUDIENCE: Do you see a trend and increase in resistant bacterial infections?

WR = In our practice in Tustin, California, in 2012 49/190 cases or 26% were methicillin resistant *Staphylococcus pseudintermedius* (MRSP). In the same location, 2 years later, we repeated the study, and found 139/233 cases or 60% were MRSP. So this is alarming as we doubled the number of MRSP in a 2-year period!

JA = As veterinarians, we know that the risk factor for developing resistance to antibiotics is directly correlated with the usage of systemic antibiotics. The logic would be to avoid such usage to prevent this increased resistance phenomenon.

So my approach today is, when I am facing a superficial pyoderma case, to aggressively treat with topical therapy ALONE—but if you choose to use systemic antibiotics, always couple it with topical therapy. By using such an approach, you can now look back at the cause of failure being either

- Failure to diagnose underlying disease
- Failure to treat for sufficient amount of time rather than NON-responsive treatment due to resistance to the antibiotics.

PM = So what are your criteria to establish "failure" to a treatment for pyoderma?

DG = We look at clinical signs—not only cytology and culture results—so the clinical examination and the client reports are important. My definition of success is the resolution of clinical signs within the expected period. For superficial pyoderma that period is 3 weeks. Once clinical signs (inflammation, papules, scales, flakes, etc...not the hair growth, which takes much longer…) are no longer present, we would continue the treatment for at least 2 additional weeks.

WR = It really varies from case to case. You can often determine failure faster. So for example: if a dog is on antibiotics for 3 to 7 days and there is development of new lesions in the presence of proper antimicrobial treatment— that is a sign of failure!

CG = I have a similar approach to Wayne. I tell my clients "if you see any development of new lesions during the course of the treatment, stop the antibiotics and come in for a culture after 2 to 3 days."

What is (are) the definition(s) of "biofilm"?

DS = Biofilm has different definitions. The most commonly accepted is an aggregation of different populations of bacteria. These bacteria live together and create a "community." I like to think of biofilm as a metropolis with different species and types of bacteria all living together and all sharing tools to fight whatever aggression is potentially outside. So they secrete material to protect themselves such as polysaccharides; the slimy material that we see. When the metropolis is getting too big, they migrate and split. Paradoxically, the inflammation (the body) helps the metropolis to grow.

WR = When I think of biofilm, I think of it in a localized site in or on the body, as opposed to it spreading all over the body. It may also be a very important component that can contribute to bacterial "resistance." A perfect localized example is a biofilm that may occur in ears and contribute to resistant cases of otitis externa or media.

CG = The original definition of biofilm included a group of bacteria adhering to a solid surface—such as a rock in a stream, in the manufacturing industry on some equipment such as solid equipment in paper mills. Biofilms allow these groups of bacteria to persist in environments where there is movement of fluid over solid surfaces. Recently biofilms have been recognized in medicine, particularly with solid devices such as implants and catheters. … Then it was discovered that biofilms also occurred in tissues, where there are no attachments to solid surfaces. In tissues we are dealing with aggregates that are smaller than those found on solid surfaces or implants.

To prove the presence of a biofilm—which is not clinically easy—my suggestion is to identify the presence of aggregates and polysaccharides.

SW = My understanding is that the more different organisms are involved in a set biofilm, the more it becomes difficult to get rid of it…

CG = Some bacteria have the ability to produce a biofilm and some don't. It was shown 40% of canine otic *Pseudomonas* isolates and 30% of the canine otitis Staph strains are able to produce biofilm.[1,2] A study on canine *Malassezia* showed that 95% can produce a biofilm.[3]

Malassezia pachydermatis from dogs can produce a biofilm, which goes back to Dunbar's question earlier about resistance and failure of treatment with *Malassezia*…

PM = Clinically, how does a practitioner recognize that he is dealing with a biofilm?

JA = To me the best way to suspect such a cause (other than the factors described earlier) is when a culture comes back positive for a specific antibiotic and the animal is not responding despite proper compliance. Then the biofilm should be included in the differential diagnosis as a cause of failure. It is not a true resistance but more of a "shield factor"…

Are biofilms a reality in dogs and cats?

"There is no reason to believe that biofilms are not a reality in dogs and cats and may help to explain the resistance seen to some antimicrobial agents."

Wayne Rosenkrantz, DVM, DACVD

PM = Is the biofilm a recent complicating factor?

WR = Biofilms have likely existed for a long period of time and have been reported in human medicine for many years. Biofilms and their role in many types of human infections have been extensively evaluated and, besides dental plaque, biofilms may form in the lungs of patients with cystic fibrosis, within wounds, on catheters or surgical implants, and within the middle ear.

CG = I think that dental plaque has been well described in animals and is recognized as a common biofilm problem in dogs and cats.

SW = I was at the ECVD (European College of Veterinary Dermatology) last week in Poland and there was a paper about the ability of *Staphylococcus pseudintermedius* to form biofilms in healthy dogs. They cultured *S pseudintermedius* from 36 healthy dogs by swabbing the abdomen and the interdigital space.

Out of these 36 animals they grew *S pseudintermedius* in 7 from the abdomen and 13 from the interdigital spaces. These bacteria were able to produce biofilms based on growth on agar plates in 100% of samples from the abdomen (7 out of 7) and in 85% of those from the interdigital spaces (11 out of 13).

"So biofilms are present and probably more often than we think. … We simply don't document it …"

Stephen White, DVM, DACVD

CG = One of the reasons biofilms are not well documented in skin and ear disease may be because they often don't grow in culture. In human otitis media for example, the cultures are routinely sterile and the discovery of biofilms was associated with the use of new techniques such as confocal laser scanning microscopy and fluorescence in situ hybridization (FISH) studies or electron microscopy.

Q What are the most common manifestations of biofilm in clinical practice?

CG = The clinician should consider biofilms as a potential problem in any chronic skin or ear infection that persists while the patient is receiving what is normally a routine effective therapy. Other clinical features that raise suspicion for a biofilm are a slimy purulent exudate and foul odor. A recurrent infection that occurs at the exact same site soon after treatments are stopped also makes me concerned about biofilm.

PM = What are the criteria you use to determine the presence of a biofilm in veterinary dermatology?

WR = I would commonly consider biofilm formation if a number of the following criteria are met:

1. Infecting bacteria are adherent to a substrate or are surface associated.
2. Direct examination of infected tissue shows bacteria living in cell clusters, or microcolonies or clumping of bacteria, encased in an extracellular matrix.
3. The infection is generally confined to a particular site. It is a localized lesion. … Although spreading may occur, it is a secondary phenomenon.
4. The infection is often difficult to eradicate with antibiotics despite the fact that the responsible organisms are susceptible to killing in the planktonic state.
5. It is often a chronic issue.
6. A foul odor is also often present.
7. The lesions include a "slimy appearance."

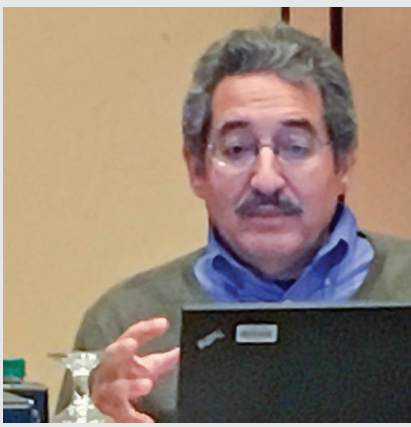
CG = We don't always see the slimy look…

For me what I like to document is the presence of bacterial aggregates on cytology, though I do not know how often you will find them…

PM = How do you define an aggregate?

CG = A biofilm aggregate is a 3 dimensional cluster of bacteria that is not derived from a single specific organism; it is not a colony. These are often different sized rods or mixed rod and cocci. If I can identify more than 1 morphology of organism, then it is likely the cluster is not a colony. For the cluster to be an aggregate it has to also have some mass.

In human otitis media the biofilm aggregates are usually 4–40 microns in size.[4] At 40× to 100× magnification the depth of field is about 1 to 0.2 microns. Therefore when I see a cluster of bacteria I can focus up and down on the cluster. If different bacteria in the cluster go in and out of focus as I focus up at several different fields, then I know that cluster has mass and is over 1–2 microns in size.



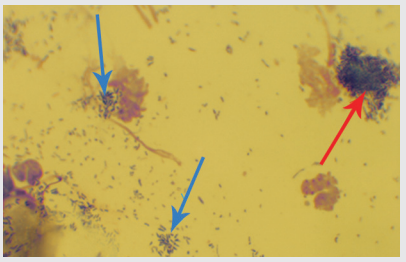


Figure 1. Cytology of ear exudate showing degenerate swollen nuclei of neutrophils with blue arrows pointing at two clusters of bacteria. These clusters do appear to have different morphologic types and therefore are not colonies. Also note how the bacterial cells are mainly in focus. The cluster indicated by the red arrow is larger, has multiple morphology, and also contains both bacteria that are not in focus and others that are. ©Craig Griffin

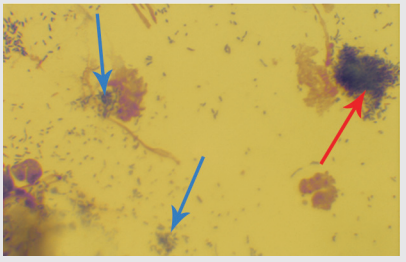


Figure 2. Same field as Figure 1 at different focal length. Note how the two clusters of bacteria that are identified by the blue arrows are now out of focus. The aggregate that is identified by the red arrow now has different bacteria in focus and others that had been in focus out of focus. You can also see the pinkish gray material around some of the margins of the aggregate. ©Craig Griffin

How would you approach a patient with complications by biofilm formation in both skin and ear infections?

"I think there is sort of an intuitive feeling that the more I can wash things out, the better it will be."

Stephen White, DVM, DACVD

SW = For the ears I envision the biofilm as the thick and sticky stuff you find at the bottom of the pot after cooking pasta! If you just use water, it does not wash out as rapidly as if you use soap or some other detergent. … I think this is even more so for the body… and for local lesions such as intertriginous areas, washing with something that has a degree of surfactant or something that would be able to strip away the exudate as well as something that could kill off the organisms…that would be ideal. … In terms of ingredients, on the skin, I would use a product that contains chlorhexidine and miconazole at least to kill bacteria and yeast and just the mechanics of it would also help to get rid of the biofilm. … In the ears, I would use a combination of a cleanser and a treatment. The cleanser would ideally be a product that would strip off or break off the biofilm. The treatment would be a product that kills the organisms, recognizing that these days several cleansers also have anti-microbial activities…

WR = I agree entirely with Stephen. Again thinking of these biofilms as a well protected environment for bacteria with polysaccharide coatings and envelopes—is there a way to disrupt biofilm formation?

In the ear, physically cleaning, flushing, and removal of purulent debris, but then also following with the addition of various disinfectants that would penetrate and break down the biofilm.

CG = This is where some new molecules and a mix of new technologies such as MicroSilver could play a role.

There are some recent products currently available that contain MicroSilver that seem to act specifically on biofilm prevention, and potential elimination of existing biofilms. What is the mechanism of action of micronized silver?

WR = Silver has been around for many years and used as an antiseptic and potential antibacterial. It is used in dressings, in prosthetic devices and implants, even in food packaging, textiles, cosmetics, dental products, and others. In Hong Kong, subways are sprayed with silver molecules to minimize the incidence of transmittal infections.

There are different types of silver molecules that primarily vary by the size of silver particles. Currently there are silver salts, nanoparticles, and most recently, MicroSilver.

"MicroSilver has no systemic absorption so this is a big benefit over the nanoparticle formulations."

Wayne Rosenkrantz, DVM, DACVD

In terms of toxicity and in particular regarding concerns related to systemic effects of silver, this is seen mainly with nanoparticle silver. This is due to the ability of these smaller particles to potentially be absorbed systemically. Besides the size of the silver molecule, the shape of the silver particles also plays a role in the mechanism of action. The bulky shape of the micronized silver, for example, increases the release of Ag+ ions and it is these ions that have the antimicrobial properties we are interested in. It also gives a high concentration at the skin surface and no significant penetration, so no systemic reactions. It may enter the superficial infundibulum of the hair follicle and provide some antimicrobial effects in the outer hair follicle.

The ions also inactivate intracellular enzymes, which further damages the production of protein by the nucleic acids of these bacteria, inhibiting the reproduction of these organisms. There are many reports supporting the benefits of silver preventing biofilm

formation. As far as its ability to disrupt existing biofilms there are reports suggesting this but more research is needed in this area.

"The proposed mechanism of action of the MicroSilver (Ag+) ions against bacteria is related to its ability to inhibit the transmembrane transport of protein. This results in lysis of the bacterial cell wall."

Wayne Rosenkrantz, DVM, DACVD

PM = Can you expand about micronized silver and eradication of biofilm?

WR = We know that the MicroSilver (Ag+) will prevent bacterial adhesion and, if you recall, adhesion is an important component of the biofilm formation.

There is also some indication that Ag+ will destabilize the binding sites of bacteria to proteins.

Most recently VetBiotek sponsored a research project documenting that topical products containing various concentrations of MicroSilver (Ag+) were effective at eradicating biofilm formation in an established *in vitro* model for *Staphylococcus intermedius* and *Pseudomonas aeruginosa*.

This research was conducted by an independent laboratory that utilized an established model for biofilm studies. This study will be presented at the World Dermatology Veterinary Congress next May in Bordeaux, France as it was accepted in the supporting original studies session. We are excited about using Ag+ products as we have several clinical cases showing significant improvement with MicroSilver. These are cases that were not responding to multiple treatment regimens.

We also know Dr. Alan Mundell has been a precursor and he is using MicroSilver for some time with some great results in dogs with chronic resistant ear infections.

CG = What was the lowest concentration of MicroSilver that was effective in that study?

WR = It was 0.05% MicroSilver, a relatively low concentration as compared to the treatment products that are now released, which contain 0.1%.

JA = My experience with the MicroSilver is amazing. It is a radical leap forward in ear therapy. I've treated about 5 total nightmare otitis cases with weekly flush and me applying the micronized silver in clinic.

On cytology I see the silver particles still present on the swab one week later. Owners are clamoring to be able to take it home.

Cosmetically—no odor. Looks like gray paint in the canal…

Q MicroSilver has been used for one decade in various human products. What are the benefits from such an ingredient?

KR = Most silvers used initially were particles in some media—including adding silver suspensions to some beverages. No benefits from these have been scientifically documented…but the vast majority are colloidal/nanosilver suspensions or silver salts or other chemical silver compounds. MicroSilver is highly porous and micronized…from 100% pure silver. The key benefit is that these particles are about 10 microns in size…so well above the definition of nanoparticles (100 nm). … And the MicroSilver will NOT penetrate skin or mucosal tissue and will not be detrimental to the resident skin flora. There are numerous studies showing that micronized Ag does not penetrate the skin. Another interesting feature of MicroSilver is that using pure metallic silver particles, you have a reservoir of

"My experience with the MicroSilver is amazing. It is a radical leap forward in ear therapy."

John Angus, DVM, DACVD

silver to continuously generate silver ions as they are depleted and this results in very long-lasting antimicrobial activity when compared to silver salts and other silver products. … It is quite different with silver salts and other silver compounds where ions are released in high quantities (sometimes too high), with an immediate peak and then an acute drop. … With micronized Ag, there is a continuous effect, and the porous sponge-like morphology of the silver particles physically clings well to skin and hair follicles…

The micronized silver is also not water soluble so in presence of water or blood or other fluids it will NOT be readily absorbed into the skin nor be as readily removed and washed away in comparison to water soluble compounds.

Main features and benefits of micronized silver:

1. Long lasting antimicrobial effect
2. No known resistance to MicroSilver
3. Broad spectrum (gram +/gram – multiresistant bacteria, yeast, etc)
4. Particle size = large size—so no absorption
5. It remains on the skin, and it will not cause any detrimental harm to the "good flora."

Q Can biofilm be prevented?

SW = The best way is to get rid of the underlying cause of pyoderma. Once you have accomplished this first step, treat with sufficient doses of antibiotics, and for a proper duration, using topical therapy to wash away and break the biofilm OR prevent the biofilm from occurring. But it has to be a combination of all these actions.

The whole idea is, for example: You have a dog that comes in and is atopic—rubbing its face, licking its feet—and you don't find much in terms of organisms or just a few. If you have a shampoo with Ag+, you can now tell your client "if you use this product on a routine basis, not only will you be able to take care of any of the infection that takes place now, but you have a good chance to reduce the further incidence of any bacterial infection in relation to atopic dermatitis…"

WR = It would appear that the MicroSilver has benefits as a preventative agent for biofilm formation. In addition to shampoo therapy, other delivery systems (ie, mousse, gels, lotions, or sprays) may be more effective for localized disease.

AUDIENCE = What triggers the release of the Ag+ ions? Is it the acidity?

KR = Primarily moisture. So water, but any moisture on the particles, including blood, will trigger the release of Ag+ ions. Remember that the persistence is somewhat associated with the mechanical wash-out effect so the form of Ag+ delivery will affect the remanent on the skin. For example, a shampoo would remain less than a mousse or a wipe containing MicroSilver.

This will depend on how much you massage and get the Ag particles onto the skin, as they do cling well to the skin and hair and even after rinsing some Ag will remain. The Ag concentration will also play a role.

"So I see the benefits of MicroSilver spray & mousse over shampoos and also wipes for skin folds and localized lesions."
Dunbar Gram, DVM, DACVD

DG = So I see the benefits of MicroSilver spray and mousse over shampoos and also wipes for skin folds and localized lesions.

JA = For your information, regarding the residual of Ag+, when I used the otic product containing MicroSilver, after a single application, I found Ag+ particles on cytology after 1 week.

Q What are the potential future developments associated with the management of biofilms?

"What I would like to see in terms of ideal product when dealing with biofilms is one that:
- **Dissolves polysaccharides**
- **Kills bacteria."**

Domenico Santoro, DVM, PhD, DACVD

DS = So molecules like acetylcysteine, EDTA, or MicroSilver are able to disrupt the "shield" and open the door to antimicrobial products.

Some of these molecules such as MicroSilver also have an antimicrobial effect.

CG = That is one of the keys. It is not only how you kill the organisms BUT how you prevent resistance from developing. Multi-modal approaches are needed. An organism must have a genetic mutation that works to become resistant. If an organism needs not one but 2 or 3 genetic mutations, it becomes more difficult for that organism to become resistant.

So having, for example, miconazole, chlorhexidine, and MicroSilver should be more effective at preventing resistance.

JA = Most of our patients are atopic dogs and these are predisposed to overgrowth by *Staphylococcus intermedius*. A study in Japan on atopic dermatitis in people showed the correlation between secondary infections and the reduction of natural ceramides in the skin.

So it is not only the combination of the ingredients to kill bacteria that matters, but also the addition of ceramide.[3] This will contribute to a positive reaction in patients with pyoderma, as we know they have altered skin barrier function.

DS = Future developments should aim to destroy the biofilm. To do so, one option would be the inhibition of the cationic polysaccharide intercellular and capsular adhesion molecules that keep the bacteria populations together. In addition, such molecules prevent the attachment of antimicrobial peptides to bacterial surfaces or the activity of commonly used antibiotics. Once this "coating armor" is dissolved, the use of regular antimicrobials (topical and/or systemic) would be much more effective. ■

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