MEETING REPORT

Clinical & Surgical Ophthalmology is pleased to present this Meeting Report of a presentation given by Dr. Joseph Blondeau (Saskatoon, Saskatchewan) at the 2012 Annual CSCRS Meeting in Vancouver, British Columbia on February 25, 2012. This Report has been made possible by an unrestricted medical education grant from Bausch & Lomb Canada.

North American Anti-Infectives Resistance Pattern Landscape: Implications for Ophthalmology

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Introduction

Dr. Blondeau began his presentation by stating that he would be providing an update on the current situation regarding antimicrobial resistance as it impacts ophthalmology. This is followed by his recommendations as to what ophthalmologists can do in order to deal with some of these issues that are impacting patient care.

Dr. Blondeau acknowledged that his antimicrobial research program has received substantial amounts of funding from numerous pharmaceutical companies. He stated that his purpose was not to endorse any particular products but rather, to present the data that he and others have generated. He noted that his diversity of funding dictated the need for him to be objective and hoped the audience would find that to be the case.

With regard to antimicrobial resistance, Dr. Blondeau displayed a diagram of a bacterial cell, identifying the key areas in which antimicrobial agents act. He stated that as a consequence of this knowledge, we also understand how resistance occurs. There are at least four major mechanisms summarized in Figure 1. In today's environment, a single bacterium may simultaneously possess all of these mechanisms, thereby conferring multi-drug resistant phenotypes. These are present both in bacteria associated with systemic infectious diseases and in ophthalmology.

In ophthalmology, particularly in eye infections, Gram-positive organisms predominate, regardless of conditions including keratitis, blepharitis, conjunctivitis or endophthalmitis. When broken down further, coagulase-negative staphylococci, *Staphylococcus aureus* predominate, and in conjunctivitis, *Streptococcus pneumonia* is a very prevalent pathogen. Even though Gram-negatives (i.e., *Pseudomonas aeruginosa* and others) do play an important role in some clinical presentations, eye infections are a predominantly Gram-positive arena.

Dr. Blondeau showed the results of a 2007 survey from the International Society for Infectious Diseases, in which community acquired methicillin-resistant *Staphylococcus aureus* ("CA-MRSA") was identifiable globally. The results of the survey confirmed in the USA and globally, CA-MRSA as a major infectious disease threat affecting human health in the world, and in North America. The survey noted other diseases, as well, such as HIV-AIDS, tuberculosis and malaria. This single resistant bacterium made the list of some of the most threatening infectious diseases affecting human health globally. This is a bug seen not only see in systemic infectious diseases; it appears to increase in frequency in ophthalmic infectious diseases, as well.

As far back as 1945, Dr. Alexander Fleming taught how antibiotics should be used. Dr. Blondeau pointed out that Dr. Fleming's recommendations were ignored. What he said in 1945 was, "Let's be careful. It's not difficult to make bugs resistant to penicillin in the lab when you expose them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the human body." He was saying that if you're going to expose an organism to an antibiotic, give enough of the drug in order to kill these bugs. Don't give inadequate amounts of antibiotic to tease these organisms because that just encourages them to become resistant. We now we have a global pandemic of resistance and multi-drug resistant pathogens are no longer uncommon. What Fleming predicted at that time has become a global reality.

Antibiotic Resistance in Ophthalmology

Dr. Blondeau cited a publication from the Bascom Eye Institute in Miami that looked at a ten-year review of consecutive conjunctival swabs which were positive for bacteria. He noted in particular the data showing that MRSA actually increased, from 4.4% to 42.9% over a

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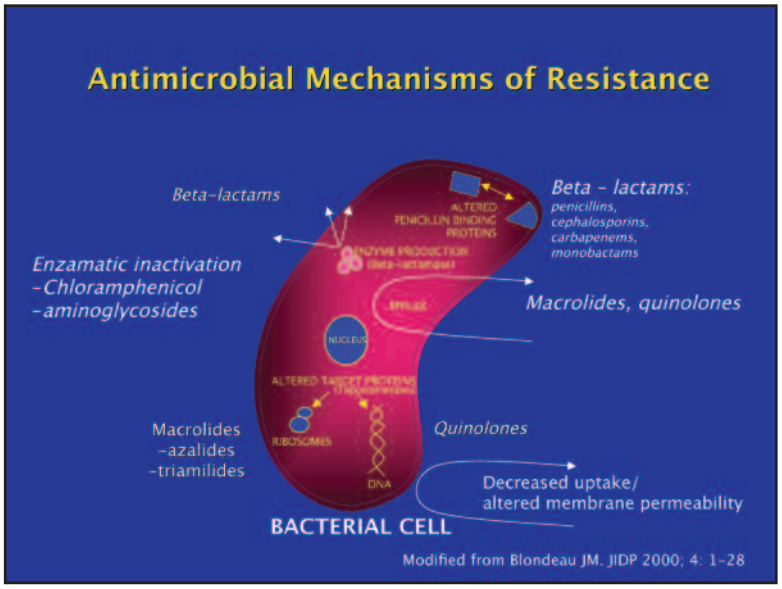

Fig. 1 Intracellular areas of antimicrobial resistance.

ten-year period, which was highly statistically significant. This does not necessarily mean that all these patients were infected with MRSA, but it certainly indicated that patients were colonized with MRSA. Practitioners know from other published literature that individuals get infected with organisms that colonize the periocular anatomy.

In Figure 2, looking specifically at bacterial conjunctivitis and MRSA isolates, a steady increase is observed from the mid-1990s to 2003, when it increased from approximately 5% to almost 30%. Therefore, there is absolutely no doubt that these organisms have increased in prevalence in ophthalmology, as they have in systemic infectious diseases.

At Dr. Blondeau's institution in Saskatoon, an examination of blood culture isolates from patients who were hospitalized and who had systemic infection, revealed that up to a third of *S. aureus* isolates were MRSA, so this is not a theoretical problem; it is a practical problem directly influencing appropriate antimicrobial therapy. As the frequency of multi-drug resistant organisms increase in ophthalmology, ophthalmologists will clearly be dealing with such problem pathogens more frequently in their clinical practices. A review by Penny Asbell that was published in the U.S. in 2008 clearly showed an increasing trend towards MRSA over time in ophthalmology.

The Mechanisms of Action of Quinolones

Dr. Blondeau emphasized that not all quinolones are the same. Older quinolones such as ciprofloxacin and levofloxacin, called third-generation agents in the ophthalmic literature, preferentially target the enzyme topoisomerase IV in Gram-positive organisms, and in Gram-negative organisms, these same quinolones target the enzyme, DNA gyrase.

When it comes to the fourth-generation agents, first with moxifloxacin and then gatifloxacin, and most recently besifloxacin, these compounds simultaneously target both of these enzymes in both Gram-positive and Gram-negative organisms. Moxifloxacin, gatifloxacin and besifloxacin (besifloxacin being the most recently approved) also have higher levels of intrinsic activity against Gram positive pathogens than do older quinolones as measurement by the minimum inhibitory concentration (MIC). The MIC measurement (performed in the laboratory) determines the minimum amount of drug required to inhibit the growth of the organism being tested. When the amount of drug required to inhibit bacterial growth exceeds a certain value, the organism is considered resistant.

Practically, an antibiotic targeting two intracellular targets would be less likely to select for resistant bacterial subpopulations than an antibiotic targeting one intracellular target. As such, the newer fluoroquinolone compounds would be expected to contribute to the selection of resistance with much less frequency than older compounds targeting one intracellular target. This has been proven and published both in the systemic infectious diseases literature, as well as in the ophthalmic literature.

Figure 3 demonstrates the current North American landscape. It includes data on ciprofloxacin, gatifloxacin, moxifloxacin and besifloxacin; these are MIC charts. If one looks at an organism that's considered to be susceptible to ciprofloxacin, it's susceptible to all of these quinolone compounds. The newest of the fluoroquinolones for *S aureus*, besifloxacin, has the lowest MIC values. For ciprofloxacin-resistant *S aureus*, the MICs are elevated for all of these compounds. Unfortunately, this is a class effect. When the data is segregated based on the individual compounds, the agent with the lowest MICs is besifloxacin based on this data set.

Regarding ciprofloxacin-susceptible *Staphylococcus epidermidis*, the same observation is seen: susceptible to all quinolones tested, with the lowest MICs for besifloxacin. For ciprofloxacin-resistant strains, MICs are elevated for all quinolones tested; however, the lowest MICs are seen with besifloxacin.

For *Streptococcus pneumoniae*, older quinolones are less potent (higher MIC values) than are the newer agents.

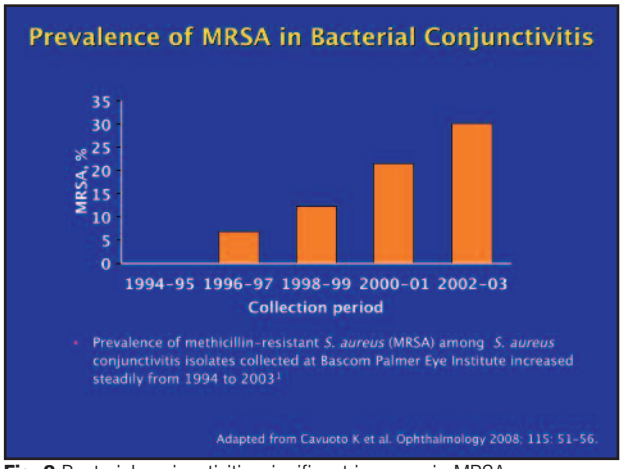

Fig. 2 Bacterial conjunctivitis: significant increase in MRSA.

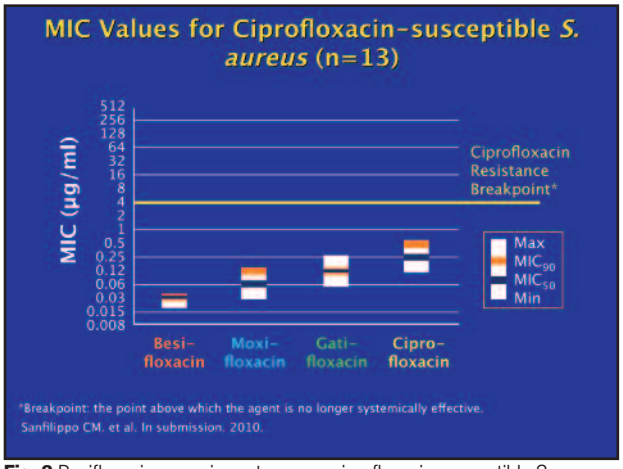

Fig. 3 Besifloxacin: superior potency vs. ciprofloxacin susceptible S. aureus.

Agents such as gatifloxacin, moxifloxacin and besifloxacin are more potent (lower MIC values). For the data set summarized, the lower MICs tend to favor besifloxacin but are also low for the other two agents.

For *Hemophilus influenzae*, all quinolones have high levels of activity and differentiating between compounds would be difficult- either in a test tube or clinically.

Dr. Blondeau cited the ARMOR study, which contains the most recent and comprehensive data for North America. He and his colleagues are in the process of adding a number of Canadian sites in order to generate some Canadian-specific data that would be relevant for ophthalmology. They are hoping to have this up and running later this year. The data examines ophthalmic isolates collected from across the U.S. It illustrates to date that, taking into account the resistance issues, vancomycin and besifloxacin are approximately equivalent, with an MIC_{90} value of 1, as compared to two other quinolones, moxifloxacin and ciprofloxacin – or tobramycin and azithromycin – where the MIC values were substantially higher. Dr. Blondeau commented that the reason why azithromycin continues to be a factor in ophthalmology continues to be a surprise to him. Clearly, at the time this study was done, 39% of the isolates were methicillin-resistant, and 38% were also quinolone resistant, which is an important observation.

For coagulase-negative *Staphylococcus*, besifloxacin was equivalent to vancomycin in terms of MICs. For the other compounds tested, the MICs were substantially higher, including moxifloxacin and ciprofloxacin, as well as for tobramycin and azithromycin. Once again, 53% were methicillin-resistant and 43% were quinolone resistant. In terms of the Gram-positive pathogens associated with eye infections, a substantial number of these are methicillin-resistant, regardless of whether they're coagulase-negative *Staphylococcus* or *S. aureus*. In Dr. Blondeau's study of MRSA isolates, over half of the isolates were quinolone-resistant, which is consistent with the data shown in the ARMOR study.

Dr. Blondeau posed the question, "Is there any hope in the midst of all this?" Figure 4 depicts one of Dr. Blondeau's first piece of work which was presented at ARVO in 2005. The investigators examined a number of organisms, including *S aureus*, both methicillin-susceptible and resistant, *Streptococcus pneumoniae*, *E. coli*, *Pseudomonas*, *Hemophilus* spp.- both beta-lactamase-positive and negative strains. Organisms were tested against gatifloxacin alone and in combination with benzalkonium chloride (BAK), which is a constituent of Zymar® (Allergan, Markham, ON) and Zymaxid® (Allergan, Irvine, CA) formulations. It is present at a concentration of 50 µg/mL in the Zymar and Zymaxid formulations. Besifloxacin, the newest compound to be introduced onto the market, contains benzalkonium chloride (BAK) as well, but at a concentration of 100 µg/mL – double the concentrations present in the Zymar and Zymaxid formulations. What the investigators were able to show – and this has captured a lot of attention around the world – is that the activity of a quinolone in combination with BAK offers something that neither the quinolone alone, nor BAK alone, offers against some of these organisms including multi-drug resistant Gram-positive organisms such as MRSA. This is represented by data that shows extremely low MICs (i.e. 0.004 µg/mL) when gatifloxacin is tested in combination with BAK.

The CA-MRSA strain, by definition, is a strain that occurs in patients who are considered to be hospital-naïve, meaning that they have no association with, nor any history of having been in, a health care facility for any period of time. They tend to infect otherwise healthy, younger people

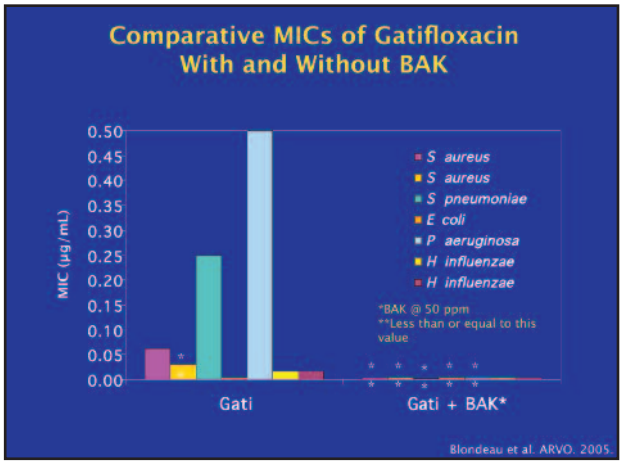

Fig. 4 Gatifloxacin: comparative MICS with/without BAK.

in the community and have been associated with extremely aggressive infections, in some instances resulting in mortality in patients with systemic infections.

In the mid-2000s, there were two papers relating the presence of these MRSA strains to ophthalmology. These studies are slightly dated, but at the time, the significance of some of these organisms in ophthalmology was somewhat unclear. Table I shows a study that looked at over 540 cases of external eye infections where *S. aureus* was the pathogen. They identified only 3% as MRSA. Dr. Blondeau stated that he believes that one could make the argument that in today's environment, practitioners are going to start seeing more reports indicating that these numbers are now higher. At the time, these authors concluded that CA-MRSA was an infrequent cause of external eye infections.

In the same year, a second study examined a CA-MRSA clone that exists in Canada and North America called the USA-300 clone. This clone has been shown to be associated with aggressive infections in the eye and periorbital region and demonstrated an ability to invade ocular tissue, again in hospital-naïve patients. These authors rightfully concluded that MRSA infections in ophthalmology were likely to increase. Dr. Blondeau commented that data coming out of the U.S., and certainly out of Miami, indicates the incidence of drug resistant organisms associated with ophthalmology has risen dramatically and needs to inform our thinking about drug selection and use in ophthalmology.

One paper Dr. Blondeau and his colleagues published was a comparative study investigating MRSA. It contains data that examined the MICs for moxifloxacin alone with values ranging from 4 µg/mL to 16 µg/mL for quinolone non-susceptible strains. When the researchers tested these same organisms with gatifloxacin in the presence of BAK, the combined MIC value of the quinolone plus BAK were exceedingly low, almost unmeasurable (<0.008 µg/mL). During the review process, the editors insisted that when the paper was published, these experiments be repeated to see whether or not the same phenomenon would recur if BAK was added to moxifloxacin and. in fact, the exact same phenomenon occurred. This was interpreted to mean that a quinolone, in combination with BAK, offers something that neither the quinolone alone, nor BAK alone, provides. Other researchers have also shown that BAK has its own intrinsic antimicrobial activity. It behaves like an antibiotic, and is active by itself against MRSA.

Dr. Blondeau presented what MRSA looks like on a chromogenic agar plate: a specimen is inoculated; if after 18 to 24 hours of incubation, colonies show a denim-blue colour, there is a 96% probability that this is MRSA. A quick confirmatory test is then done. In their laboratory, these organisms are identified and reported quickly.

Why Resistance Occurs

Dr. Blondeau stated that perhaps one of the most exciting things that has worked on, and which he felt was relevant to this discussion, is the concept of trying to understand why resistance occurs. He stated that he and his colleagues have researched why it happens and how the dosing of a drug may actually contribute to the selection of resistance. They have repeatedly published that both in human and veterinary medicine, it is possible to use a drug in such a way that when a substantial amount of drug exposure is given, reducing the likelihood that one selects for resistance in the presence of these drugs can occur. This concentration is called the Mutant Prevention Concentration (MPC). The question is, does it have any relevance for some of the drugs that practitioners use in ophthalmology? The answer, in his opinion, is clearly, yes.

Dr. Blondeau cited a landmark paper his group published. In 2009, in *Ocular Pharmacology* and *Therapeutics*, testing staphylococci, his research revealed the amount of drug required to prevent resistance with gatifloxacin alone was >4 µg/mL. BAK, alone, required at least 6 to 10 µg/mL for these particular strains. He stated, however, that when gatifloxacin and BAK were combined, the drug concentration was so low (0.004 µg/mL) it could scarcely be measured. When these two drugs were used in combination, the likelihood that resistance would occur was so infinitesimally small that one would argue that the use of these formulations containing BAK had a substantial reduced likelihood to select for resistance. Dr. Blondeau has followed this up with studies with besifloxacin, with similar observations.

A short while ago, in other measurements, Dr. Blondeau and his group demonstrated that with gatifloxacin combined with BAK, more pathogens are killed than with gatifloxacin or BAK alone. In addition,

Table I External ocular infections due to methicillin-resistant Staphylococcus aureus (MRSA)

- 548 external eye infections caused by *Staphylococcus aureus*
- 17 (3%) were MRSA
- All MRSA isolates – chloramphenicol "S"
- All isolates from patients over 50 years of age – ofloxacin "R"
- All patients had:
  - underlying history of ocular surface disease
  - malignancy
  - debilitating medical illness

Conclusion: MRSA – infrequent cause of external ocular infections

Adapted from: Shanmuganathan et al. Eye 2005; 19: 284-291.

when he and his colleagues compared Zymar or gatifloxacin plus BAK to the moxifloxacin or Vigamox formulation, they saw that they achieved better killing against MRSA in the formulation that contained BAK, versus that which did not contain BAK. This data has not yet been published (it has been presented at ASCRS 2009), but it's been written for publication and it is expected to be published later in 2012.

The Role of Besifloxacin

Dr. Blondeau referred to comparative kill studies, investigating besifloxacin compared to gatifloxacin or moxifloxacin against *S aureus* strains either susceptible of resistant to methicillin or susceptible or resistant to quinolones. In all instances investigated, faster and more complete killing was achieved with besifloxacin when compared to these other compounds against the strains investigated. With *S epidermidis*, the same observation was seen, with besifloxacin having a faster and more complete rate of kill than gatifloxacin or moxifloxacin.

Dr. Blondeau presented new data from his own laboratory, which had not previously been made public. It shows killing of *S epidermidis, Pseudomonas aeuruginosa, Haemophilus influenzae,* and MRSA. With these kill experiments following the first five to ten minutes after drug exposure, anywhere from 50% to 90% kill, (approaching 100% against some of these strains) in the presence of besifloxacin with BAK. This experiment contained BAK at 20 μg/mL. Why would one use 20 μg/mL of BAK, given that the besifloxacin ophthalmic formulation contains 100 μg/mL? The reason is that this is a more clinically-relevant concentration, based on how much drug is delivered to the eye with drops. One can look at absolute concentrations, or consider a more clinically-relevant concentration. Dr. Blondeau mentioned that he has 70 or 80 graphs from this set of experiments, where his team used 5, 10, 15, and 20 μg/mL of BAK, with concentrations of besifloxacin.

In 1946, Fleming wrote *"… the greatest possibility of evil in medication is the use of too small doses so that instead of clearing up infection the microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out, which can be passed to other individuals and from them to others, until they reach someone who gets septicemia or pneumonia which penicillin cannot save."*

The current area of concern is patients harboring drug-resistant organisms and acting as a reservoir for dissemination to other patients. Ultimately, they get into a patient where drug therapy is just not going to be satisfactory; unfortunately, Dr. Blondeau stated, this is seen with increasing frequency in systemic infectious disease.

Conclusion

Dr. Blondeau concluded his presentation by stating that BAK has intrinsic activity against Gram-positive strains, including MRSA; it is active against some Gram-negatives as well, but to a lesser degree. Certainly, its effect appears to be most beneficial when it is used at the same time as a quinolone, although the nature of this interaction is still unknown. The published literature indicates that fourth-generation agents are better, statistically, in preventing endophthalmitis. Both the besifloxacin and gatifloxacin formulations are formulated with BAK, and besifloxacin now shows rapid bactericidal activity. This has been demonstrated in Dr. Blondeau's laboratory, as well as in other laboratory settings. This combination of a quinolone with BAK points to a strong argument that these formulations do have a substantially reduced propensity to select for resistance. Whether or not they'll correct for all of the resistance that currently exists in ophthalmology is a separate question, a very intriguing question, but a separate one. ❑