Antimicrobial agents have changed human and animal health systems by revolutionizing our weaponry in the war against infectious diseases, resulting in improved survivability for both humans and animals. However, this health triumph has been tempered by the subsequent realization that bacterial populations can quickly modify themselves to resist antimicrobial agents, propagate these resistance traits, and even share resistance genes with other bacteria within their environment. Recently, these abilities have seriously compromised the usefulness of antibiotics in the war against microbes and have created concerns over a future when antimicrobials may have limited usefulness in the management and control of microbial infections.

Resistance is a condition in which an antibiotic agent fails to harm a pathogen enough to manage or cure an infection or disease. The resistant bacteria have mutated and adapted to their changed environment by the normal evolutionary processes of living organisms. Bacteria, including human pathogens, have developed defense mechanisms for protection against human-produced poisons (ie, antibiotics). Bacteria can develop resistance to antibiotics by various mechanisms including the mutation of existing genes or the acquisition or sharing of genes from other strains or species.

The sharing of genes between bacteria by horizontal gene transfer occurs by many different mechanisms. Mobile genetic elements, including plasmids, phages, and transposons, mediate the exchange of resistance genes. In other circumstances, the presence of low levels of an antibiotic in the environment promotes gene transfer, perhaps ensuring that a whole microbial community develops protection against these killer agents.

The rapid development of antibiotic resistance has led to a continual need to develop new antibiotics, but in recent years, the pace of antibiotic development has slowed, creating concerns that some microbes may gain the upper hand when they cause infections.

**MECHANISMS OF RESISTANCE**

**Drug Degradation or Inhibition**

One mechanism by which bacteria have gained resistance against certain kinds of antibiotics is through the secretion of enzymes that degrade and inhibit the drugs’ bacteriostatic and bactericidal properties. Antibiotic resistance coevolved with biosynthesis as a means of bacterial self-immunity strategies for the production of toxic secondary metabolites in antibiotic-producing bacteria. This coevolution strategy
BESIFLOXACIN FOR THE TREATMENT OF BACTERIAL KERATITIS

BY BARRY A. SCHECHTER, MD

Bacterial keratitis is a serious and potentially sight-threatening condition, which can cause corneal scarring and opacification and, occasionally, perforation. Traditionally, treatment has involved frequent dosing by instillation and/or injection of compounded, fortified antibacterial solutions or a combination of commercially available topical antibacterial agents. Of late, fourth-generation fluoroquinolones have become the standard of care for eyes with small or peripheral corneal infiltrates, whereas fortified antibiotics remain the treatment of choice for more severe ulcers or when the risk of permanent vision loss is high.

Jai Parekh, MD, William Trattler, MD, and I recently published the results of a retrospective, postmarketing surveillance study of the safety of besifloxacin ophthalmic suspension 0.6% (Besivance; Bausch + Lomb) for the treatment of bacterial keratitis.1 Our goal was to assess the safety of this treatment approach. The research was conducted at 10 clinical centers across the United States. It included 142 patients who underwent treatment in one or both eyes with besifloxacin and, for perspective, 85 patients who instead received moxifloxacin ophthalmic solution 0.5% (Vigamox; Alcon), an older medication.

Primarily, we sought to identify the types and rates of adverse events reported during treatment with besifloxacin. We also looked at the development of corneal scarring and neovascularization, patients’ final visual acuities, how long they experienced pain before and after treatment, and measured bacterial eradication based on clinical appearances.

Our findings were consistent with those reported for larger, prospective, controlled studies of besifloxacin for the treatment of bacterial conjunctivitis.2-5 In our study, the difference in the frequency of adverse events was low and not significant between the besifloxacin and moxifloxacin groups, and both drugs were associated with high rates of bacterial eradication (> 90%).

Although our findings suggest that besifloxacin is efficacious and safe for the treatment of bacterial keratitis, a major limitation of our study is its retrospective nature. That being said, most clinically relevant adverse events would likely have been reported. A future prospective clinical trial is warranted to confirm our findings and to isolate the therapeutic contribution of besifloxacin, because patients in both treatment groups received additional antibiotics.


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phenicol in a process called *acetylation* and in Gram-negative and Gram-positive bacteria against aminoglycosides through phosphorylation, adenylation, and acetylation.¹

### Alteration of Bacterial Proteins

Bacterial proteins are common targets of antimicrobials. The alteration of bacterial proteins has become a widely used drug resistance mechanism for bacteria. This is one of the three major mechanisms of resistance, along with reduction of drug permeability to its target and drug modification.

Resistance by the general mechanism of drug target modification can be brought about by a remarkable variety of means, which have been exploited by different clinically important bacteria. The modification mechanism often results in an alteration of the original drug target structure, so that the drug binds poorly or not at all. This change in the structure can be brought about by naturally occurring spontaneous mutations in the gene or genes encoding the drug target. These mutations result in modification of single or limited sequences of amino acids in the target protein, often in the region of a known putative drug binding site.¹¹

Examples of this mechanism include quinolone resistance due to alterations in target enzymes DNA gyrase and topoisomerase IV involved in DNA synthesis, rifampicin resistance due to alterations in the β-subunit of the target RNA polymerase involved in RNA synthesis, and low-level penicillin resistance in *Streptococcus pneumoniae* due to alterations in the transpeptidases (PBPs) involved in cell-wall synthesis.¹³

More extensive modifications of drug targets often require other genetic mechanisms. In the case of high-level penicillin resistance of *S pneumoniae*, more extensive modifications of the target transpeptidases involved in cell-wall synthesis are possible because of this organism’s ability to exchange DNA segments with related bacterial species, some of which have transpeptidases that bind penicillin poorly, allowing the generation of mosaic transpeptidases with extensively modified regions of these target enzymes in *S pneumoniae*.¹¹

In other cases, such as glycopeptide resistance in enterococci and macrolide resistance in many bacteria, the target structures to which these drugs bind, specifically the cell wall in glycopeptides and the bacterial ribosome in macrolides, are exogenously modified by enzymes encoded by DNA acquired on mobile genetic elements, such as plasmids and transposons, which can be transferred between bacteria. In other cases, such as tetracycline resistance in many bacteria and plasmid-encoded quinolone resistance due to Qnr proteins in enteric Gram-negative bacteria, the drug targets are protected from drug action but not modified by the resistance-determining proteins.⁶

### Altered Metabolic Pathway

Another novel variation of the altered-target mechanism is overexpression of unmodified drug target binding sites in such a way that binding of drug to these extra sites limits access of the drug to a subset of critical target binding sites. This is thought to be the cause of low-level glycopeptide resistance in staphylococci.

Finally, in a number of cases, such as resistance to methicillin and other β-lactams in staphylococci, resistance to mupirocin in staphylococci, and resistance to trimethoprim in many species, bacteria have acquired genes. Sometimes on mobile genetic elements, these acquired genes encode alternative or bypass drug-resistant target enzymes. This enzyme then provides the functions that would otherwise have been inhibited by the drug, allowing growth in the presence of the antimicrobial.¹³

Some resistant bacteria evade antimicrobials by reprogramming or camouflaging critical target sites to avoid recognition. Therefore, regardless of the presence of an intact and active antimicrobial compound, no binding or inhibition takes place. This type of evasion has been observed in staphylococci against methicillin and other β-lactams, specifically through changes or acquisition of different PBPs that do not sufficiently bind β-lactams to inhibit cell-wall synthesis. Enterococci are able to resist vancomycin by the alteration in cell-wall precursor components to decrease binding of vancomycin. *Mycobacterium* spp. effectively use this type of mechanism for resistance against streptomycin through modification of ribosomal proteins or rRNA, against the rifamycins through mutations in RNA polymerase, and against the quinolones through mutations in DNA gyrase.¹

Thus, the creativity of nature in developing resistance mechanisms under selective pressure has been capable of meeting the many challenges posed by the development of new antimicrobial drugs.

### Drug Penetration Pathways

Most antibiotics target intracellular processes, and activity is achieved by penetration of the molecule into the bacteria. The outer membrane of Gram-negative bacteria provides a naturally occurring shield that becomes an additional barrier to molecular penetration of antibiotics.

For these bacterial entities, there are essentially two pathways that allow penetration through the outer mem-

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“Another novel variation of the altered-target mechanism is overexpression of unmodified drug target binding sites in such a way that binding of drug to these extra sites limits access of the drug to a subset of critical target binding sites.”
The existence of drug-resistant strains in a large number of bacterial species due to subtle lipid or protein modifications in the composition of the outer membrane highlights the importance of the outer membrane barrier in antibiotic sensitivity.13

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