

The Horizon: What's New in Antimicrobial Drug Development

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In the past decade, multiple-antibiotic-resistant enterococci and pneumococci have appeared in the clinical setting, spread intercontinentally, and increased in incidence in the hospital and community environments. Development of resistant phenotypes in these organisms has rendered facile treatment of diseases caused by both groups of organisms problematic in the clinical setting and has had the unfortunate consequence of dramatically increasing the cost of effective therapy. Additionally, serious infection caused by methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* has been a long-standing serious nosocomial problem where vancomycin has emerged as the antibiotic of choice and often is the only useful antibiotic in treating these organisms. Vancomycin resistance in the enterococci and recent cases of low level vancomycin-resistance in human *S. aureus* isolates has raised the specter of antibiotic-untreatable staphylococcal infections.

The appearance of serious gram-positive infections accompanied by new resistance mechanisms occurred at a time when the antimicrobial business in the pharmaceutical industry was at a low ebb in terms of delivering new antimicrobial agents. That state of affairs, while alarming and well documented in the scientific and popular literature, stemmed from the industry entering into a dramatic period of change with regard to how new antimicrobial agents are discovered.

The forces for change in the paradigm of antimicrobial discovery follow and derive from the great success of the antibiotic business from the 1950's to present. During that period, several antibiotic classes with distinct mechanisms of action were discovered and introduced to the market. Often intense synthetic chemistry efforts introduced successive waves of improved compounds in each class as exemplified by the penicillins and cephalosporins. Dozens of penicillins and cephalosporins were introduced with altered and improved properties to such an extent that cephalosporin compounds were grouped into "generations" first, second, and third consistent with their antimicrobial properties and spectrum. This effort in several antibiotic classes was highly successful and led to view held by many people in the mid-1980's that there were sufficient antibiotics to treat all important human bacterial diseases.

By the mid-1980's the success of the whole cell screening effort had led to the discovery and characterization of more than 6,000 antibiotics derived from natural products or fermentations. Whole cell screening typically began with a natural product extract or fermentation broth. The broth or fluid was introduced onto paper disks and these were placed onto solid agar cultures of important pathogens. Following incubation, a clear zone around the disk but heavy bacterial growth outside of the zone indicated the presence of an antimicrobial activity in the extract or broth. The pattern of activity or lack of it against many different bacteria indicated whether further interest was warranted. If so, then re-fermentation or re-extraction was undertaken and if the activity was confirmed and was reproducible then a natural products chemist usually undertook isolation and structural identification of the observed activity. This generalized process was invented in the first two decades of the antibiotic era and depended upon patterns of bacterial inhibition and attempts such as paper chromatography at about the level of re-fermentation to determine if the activity was novel and deserved more work. The sheer number of antibiotics discovered by such means eventually completely overwhelmed attempts to determine if an given activity was novel early in the process.

Many pharmaceutical companies undertook the challenge of discovering new antimicrobial agents through the application of molecular biology/genetics approaches. Recognition that many useful marketed antibiotics existed but only represented 4 or 5 specific basic biochemical mechanisms of bacterial inhibition led to the new strategy of screening based on desired mechanism of action. In this process, a study group decides what biochemical process or specific molecule such as a specific enzyme might make a good target for inhibition, leading to death of the microbe. Genetic or molecular biology approaches are used to isolate a specific biochemical pathway or the gene for a specific molecule is overexpressed or cloned and expressed in a host cell. Once the biochemical pathway or specific molecule is available then a high-throughput assay is devised which measures the activity of that pathway or molecule through the generation of measurable product. Compounds from chemical collections, usually many thousands with known structures, can be added individually to the assay to determine if inhibition occurs. From such assays, inhibitors of known chemical structure can be identified and prioritized for subsequent synthetic chemistry efforts aimed at improving properties. The significant advantage to this approach as opposed to whole cell screening is that the process is based solely on desired mechanism of action. The new templates discovered are very likely to be chemically novel, their novelty will be known at the beginning of effort and not the end, and they will not likely be cross

resistant with existing antibiotics. This process is trivially-known as mechanistic screening or molecular-based screening and in its many variants is being undertaken with the aim of bringing antimicrobial agents with unique mechanisms of action to the market.

Mechanism-based screening will in the longer term provide antimicrobial agents active against resistant bacteria. In the shorter term, that is the next few years, several compounds are making their way toward the market which will address our need for antimicrobial agents active against resistant gram-positive pathogens. For example, drugs in development include everninomycin which is a unique oligosaccharide produced in a micromonospora fermentation with potent antibacterial activity against resistant staphylococci, streptococci, and enterococci¹. From a rational synthesis program aimed at improving the antibacterial spectrum of tetracycline, the glycylcyclines have emerged which possess exceptional activity against efflux-pump containing gram-positive and -negative pathogens². Vancomycin activity even against resistant gram-positive organisms has been substantively improved upon in a glycopeptide synthetic program culminating with LY333328³. Several new quinolone and macrolide antibiotics derived from synthetic efforts and possessing enhanced activity against the resistant streptococci in particular, have either recently been approved for marketing or their approval is pending. Scientists at Pharmacia & Upjohn Inc. have for several years been engaged in a synthetic chemistry effort with a group of compounds known as oxazolidinones^{4,5}. The oxazolidinones are of particular interest because a lead compound, linezolid, is in phase III human clinical development and among other characteristics it possesses exceptional antibacterial activity against antibiotic-sensitive and -resistant staphylococci, enterococci, and streptococci. It has a unique mechanism of action, acting at the initiation site in protein synthesis, and in laboratory studies it has proven exceptionally difficult to develop bacteria resistant to it. Although the result of a synthetic chemistry effort, the oxazolidinones also typify current thinking with regard to the strategic goals of mechanistic screening in that they are a structurally-unique chemical class of antimicrobial agents possessing a unique mechanism of action and thereby completely lacking cross-resistance with marketed antibiotics.

Resistance development in the streptococci, staphylococci, and enterococci is a grave concern and a current serious clinical problem. The pharmaceutical industry is meeting the challenge of gram-positive resistance with the introduction in the next few years of antimicrobial agents derived from existing classes of antibiotics, new fermentation products, and totally synthetic drugs. In the

longer term, mechanistic screening has been devised as an entirely new approach to identifying molecular templates suitable for synthetic chemistry efforts which will result in new antimicrobial agents unaffected by existing bacterial resistance patterns.

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