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NEW TARGETS AND STRATEGIES FOR THE DEVELOPMENT OF ANTIBACTERIAL AGENTS

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INTRODUCTION

The discovery, development, and clinical use of antibiotics during the 20th century have reduced substantially the morbidity and mortality from bacterial infections. At present, about 100 antibiotics have been clinically used. Most antibiotics exert strong selective evolutionary pressure on pathogens, thus increasing the probability that resistance will develop. The potential emergence of untreatable "super-bugs" has alarmed scientists and clinicians alike, raising the specter of a post-antibiotic era, such gloomy forecasts have provided compelling impetus to efforts to develop new strategies for managing infectious diseases.

The incidence of nosocomial infections due to resistant gram-positive pathogens, especially, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) is increasing, while emergence of vancomycin resistance is reducing the number of therapeutic options. Some of the most promising to replace vancomycin include semisynthetic glycopeptides, quinupristin-dalfopristin, oxazolidinones, and everninomycins. Nevertheless, the fear that high-level vancomycin resistance will eventually spread to staphylococci places emphasis on the need for vigilance in the continuing war against pathogenic microbes (3).

Current widely used antibiotics are targeted at a surprisingly small number of vital cellular functions: cell wall, nucleic acid, protein synthesis, and metabolism (Table 1), and instances of resistance to these antibiotics are widespread and well documented. There is little doubt that new antibiotics are needed to combat the growing problem of antibiotic-resistant bacteria, and targeting of new pathways will play an important role in discovery of these new antibiotics (6).

In this lecture, the research status of the critical antibiotic class, and current approaches to the discovery of new agents, including the identification of new molecular targets for antibiotic action, will be presented.

EMPIRICAL AND BIOCHEMISTRY-BASED APPROACH

The biochemical mechanism of acquired resistance to most classes of antibiotics has been understood, which has given a significant opportunity to design rational strategies that can be used to counteract resistance. Examples of the various approaches are considered below.

Analogs of existing antibiotics stable to enzymatic inactivation. Enzymatic inactivation of

antibiotics is an important mechanism of bacterial resistance to £]lactam antibiotics, chloramphenicol, aminoglycosides, and macrolide. To solve this problem, enzyme-stable analogs have been developed, most notably with the £]lactams, e.g. 3rd generation cephalosporins and carbapenem.

Inhibition of bacterial enzymes that inactivate antibiotics. The antibacterial enzymes that degrade or modify antibiotics are themselves potential targets for drug action, leading to combination products that contain an antibiotic and a specific inhibitor that protects the antibiotic from enzymatic inactivation, for example, clavulanic acid, sulbactam, and tazobactam.

Analogs of existing antibiotics not recognized by bacterial efflux pumps. In some isolates, resistance to the macrolides, quinolones and tetracyclines is mediated by efflux pumps that prevent antibiotic accumulation by the bacteria. The analog (e.g. glycylcyclines) of these antibiotic classes that are not recognized by efflux pumps, yet that retain antibacterial activity, provides a solution that can be used to circumvent transport mediated resistance (7).

Analogs of existing antibiotics that bind to modified target sites in resistant bacteria. Modification of the target site for antibiotic action results in resistance to a number of antibiotics, including the tetracyclines, £]lactams, and glycopeptides. The chemical synthesis of analogs of these antibiotic classes has yielded new derivatives that bind to the refractory targets. N-alkyl-substituted glycopeptides represent important leads in the generation of new glycopeptide antibiotics with activity against vancomycin- and teicoplanin-resistant gram-positive bacteria.

NEW ANTIBACTERIAL MOLECULAR TARGETS

An alternative approach to the problem of emerging resistance to current antibiotics is to seek structurally novel antibiotics that inhibit new molecular targets. A systematic approach to the identification of new therapeutic molecular targets is now afforded by recent progress made in bacteria genome sequencing. Bacterial genomes are comparatively small (0.6-6 Mb) and it is now cost-effective to sequence entire genome libraries. The first complete bacterial genome sequence was published in 1995 (2). It is anticipated that 100 bacterial genomes will have been sequenced by this year. A genome size of 2.5 Mb (e.g. *S. aureus*) contains 2000 genes, of which ~10% (200) may be essential for bacterial growth in vitro. If new antibiotics that interact with the gene products of only 5% of these 200 potential molecular targets were successfully developed, it would represent ten new antibiotic classes. Schematic view of genomic tools applied to antimicrobial agent discovery and laboratory-based procedures to identify new molecular targets are depicted in FIG. 1 and in Table 2, respectively.

New molecular targets involved in bacterial growth. Nucleic acid, protein, and cell wall synthesis are already the targets of existing antibacterial agents. Nevertheless, the complexities

of these processes provide a rationale for the search of inhibitors acting on new molecular targets within these pathways. Indeed, this position is supported by the discovery and current development of the oxazolidinones, which inhibit bacterial protein synthesis by a novel mechanism.

The discovery and development of clinically useful antibiotic classes, such as aminoglycosides, macrolides and tetracylclines, have already demonstrated that bacterial protein synthesis is a suitable target for drug intervention. Recent progress in the discovery and development of bacterial protein synthesis inhibitors is illustrated by consideration of the glycylcyclines (DMG-DMDOT), ketolides (RU64004), oxazolidinones (Linezolid), and streptogramins (Synercid). A wide diversity of their chemical structures reflects a multiplicity of different interactions with target 16s and 23s rRNA molecules.

New molecular targets involved in bacterial infection. Recent interest in the mechanisms by which pathogenic bacteria cause disease has raised the possibility of designing new agents that act against gene products expressed primarily or exclusively during infection (1). An anticipated advantage of developing such agents will be the likely absence of preexisting resistance mechanisms. The search for new microbial targets in connection with infection will be substantially associated by new techniques designed to detect bacterial genes expressed selectively in vivo. Targeting of in vivo-expressed functions may lead to narrow-spectrum agents because the targets could be highly specific for each pathogen.

(a) Gene products known to have a role in infection. Assimilation of iron by pathogenic bacteria is essential for their growth in vivo. It was one of the first infection-related processes to be suggested as a potential target for antibiotic action.

Surface-expressed bacterial proteins play a central role in the pathogenicity of many bacteria, particularly in gram-positive species, where they promote bacterial adhesion to host tissues, facilitate subsequent invasion of the tissue, and confer resistance to phagocytosis (4). Inhibition of the anchoring process to the cell wall by an antibiotic should prevent the pathogen from establishing disease or render it susceptible to the host defense system.

(b) IVET. In vivo gene expression technology (IVET) is a new technique which distinguishes genes expressed during growth in vitro and in vivo from those that are expressed selectively during infection in vivo (virulence genes or in vivo-induced genes). The IVET approach uses the host to enrich for genes that are expressed in host tissues during the pathogenesis of infections and can be applied, in principle, to any pathogen (5).

(c) STM. Signature-tagged mutagenesis (STM) technique contains a negative selection procedure whereby transposons containing unique sequence tags are used for mutagenesis to inactivate genes with a role in infection. Inactivation of the virulence genes renders the mutants avirulent in experimental infections, but their capacity to grow in vitro is not impaired.

Avirulent mutants are revealed by detection of signature (sequence) tags in organisms present in the inoculum, but not in organisms recovered from experimental infections.

(d) RNA transcript analysis. The foundation of the identification of new genes with a role in infection by analysis of bacterial transcripts expressed in vivo arise from the demonstration that RNA transcripts can be used to detect expression of bacterial genes responding to various stimuli imposed during laboratory culture.

Little doubt remains about the need to develop new classes of antibacterial drug to combat the increasing number of resistant infections. With the success of a genomic approach to design neuraminidase inhibitors against flu virus, expectations are growing that these avenues will soon begin to pay off for antimicrobials.

CONCLUSIONS

The development of antibiotics for the chemotherapy of bacterial infections represents one of the most remarkable achievements of 20th century. Unfortunately, the increasing emergence of acquired resistance to antibiotics seriously threatens their effectiveness for the therapy of nosocomial and community-acquired infection. The development of new prophylactic and therapeutic procedures is urgently required to meet the challenges imposed by the emergence of bacterial resistance.

With the advent of automated, high-throughput DNA sequencing, the availability of bacterial genome sequences to discover new antibiotics has been accelerated. New molecular techniques are providing investigators opportunities to discover families of novel antibiotics.

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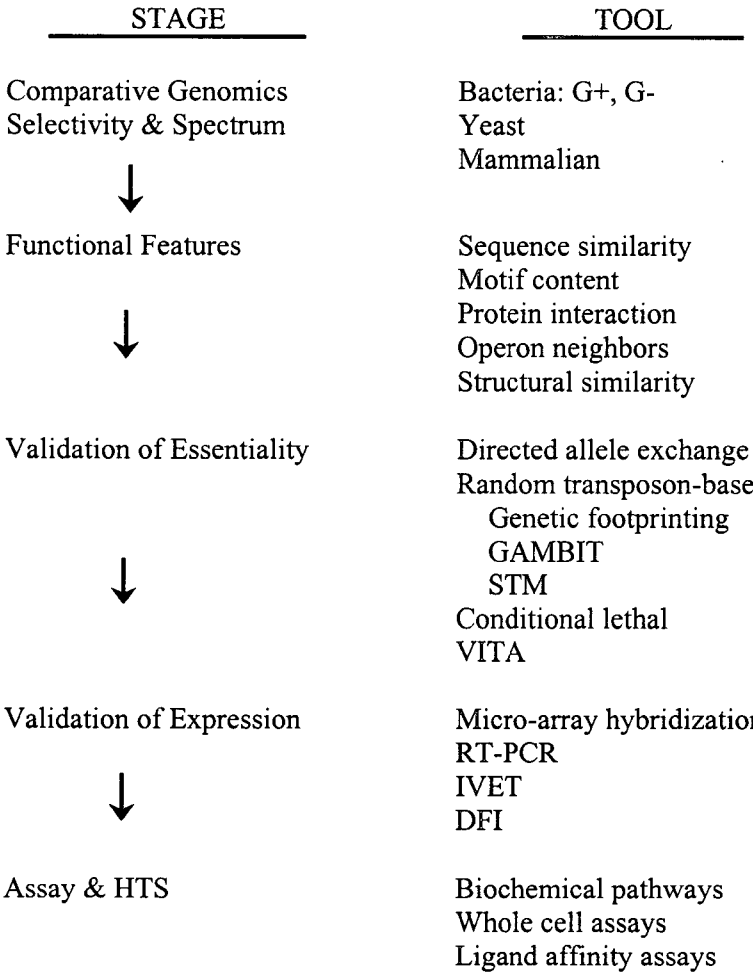

FIG. 1. Schematic view of genomic tools applied to antimicrobial agent discovery.

Table 1. Molecular targets of widely used antibiotics

Target	Antibiotic class
Protein synthesis	
30S ribosomal subunit	Aminoglycosides, tetracyclines
50S ribosomal subunit	MLSs, chloramphenicol
$tRNA^{Ile}$ synthetase	Mupirocin
Elongation factor G	Fusidic acid
Nucleic acid synthesis	
DNA gyrase A subunit	Quinolones
DNA gyrase B subunit	Novobiocin
RNA polymerase beta subunit	Rifampin
DNA	Metronidazole
Cell wall peptidoglycan synthesis	
Transpeptidases	Beta-lactams
D-Ala-D-Ala ligase	Glycopeptides
Antimetabolites	
Dihydrofolate reductase	Trimethoprim
Dihydropteroate synthesis	Sulfonamides
Fatty acid synthesis	Isoniazid

Table 2. Laboratory-based procedure to identify new antibacterial molecular targets

Technique	Basis of technique
IVET	Positive selection of genes expressed throughout infection Purine auxotrophy Chloramphenicol resistance Gamma delta resolvase expression
STM	Negative selection for genes essential for infection
Subtractive hybridization	Enrichment for genes expressed under ex vivo conditions
RNA transcript analysis	Global gene expression patterns during infection Large-scale, multiplex PCR amplification of mRNA
Reporter gene constructs	Reporter gene expression during infection
DFI	Selection of bacterial fusions that show altered levels of expression in host tissues, using FACS
GAMBIT	Transposition mutagenesis on PCR-amplified genomic segments from bacteria in vitro, followed by introduction into naturally competent host bacteria by transformation

IVET, in vivo expression technology; STM, signature-tagged mutagenesis; DFI, differential fluorescence induction; FACS, fluorescence-activated cell sorter; GAMBIT, genomic analysis and mapping by in vitro transposition.