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Mitigating Antibiotic Resistance with DNA Sequence Information**

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Summary
Antibiotic resistance is a growing problem, particularly among gram-negative bacteria (a distinct class of bacteria distinguished by differences in the cell wall) from hospital-acquired infections. There is a clear need for new drugs. However, the pipeline for new drugs, and especially novel molecular entities, is low. Since an outpouring of new drugs is not going to occur in the near future, it will be important to manage the development of resistance to currently available drugs. A possible way to control the spread of resistance is to take advantage of the tremendous improvements in DNA sequencing technology to identify resistance genes in real time in the clinical setting. With real time information on what genes are present in an infecting bacterium, an informed decision on which antibiotic to use for treatment could be made. DNA sequencing is becoming a feasible tool for use in clinical microbiology laboratories and its widespread use as a diagnostic and surveillance tool is a reasonable expectation in the next several years. The successful use of DNA sequencing will release a vast amount of information on antibiotic resistance genes. To take advantage of this information to slow the evolution and spread of antibiotic resistance, it will be critical to have well-designed databases of resistance sequence determinants.

Current realities
Bacterial resistance to antibiotics is increasing and presents a threat to public health. Two major problems that currently restrict antibiotic therapy are infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) and those associated with multidrug-resistant gram-negative bacteria. Among gram-positive organisms, MRSA has become widespread in both hospital and community settings and by 2003, greater than 50% of *S. aureus* strains isolated in hospitals were MRSA. Due to the acquisition of a new target enzyme that is not efficiently inactivated by the available drugs, MRSA bacterial strains are resistant to nearly all penicillin-style antibiotics.

The current situation with hospital-associated (nosocomial) infections with gram-negative bacteria is equally critical in that no new drugs are expected in the near future to effectively treat these infections. Resistance rates have been increasing for certain problematic species such as *Pseudomonas aeruginosa*, *Acinetobacter* spp. and Enterobacteriaceae including *Klebsiella pneumoniae*. Not surprisingly, the presence of multidrug-resistant strains of gram-negative organisms has been associated with prolonged hospital stays, higher health care costs, and increased mortality.

The emergence and spread of the New Delhi metallo-β-lactamase (NDM-1), which destroys many penicillin-style antibiotics, to many gram-negative bacterial species and multiple countries, is an example of how quickly resistance can emerge and become a threat to public health.

The rapid emergence of resistance reveals a need for monitoring the spread of antibiotic resistance genes. DNA sequence information provides unambiguous identification of antibiotic resistance genes and thus is the most rigorous means of tracking the spread of these genes. With thousands of unique resistance genes known, only DNA sequencing can provide the level of detailed genetic information required for a precise conclusion on the presence of a given resistance mechanism.

The above examples also highlight the need for new antibiotics and particularly for novel molecules with unique mechanisms of action. Unfavorable economic and regulatory environments, however,

have led to a reduction of investments by the pharmaceutical industry in antibiotic research and development. There are several economic factors involved, but the limited duration of antibiotic use for any given treatment relative to treatments for chronic disease, as well as the rise of resistance, make them less-profitable drugs. Regulatory issues for antibiotic approval, including stringent requirements for limiting adverse side effects, as well as uncertainty with regard to criteria acceptable to demonstrate safety and efficacy of a candidate antibiotic, have reduced antimicrobial development efforts.

Scientific opportunities and challenges

While antibacterial drug discovery and development activities within the pharmaceutical industry have decreased, our knowledge of the nature and abundance of antibiotic resistance determinants has steadily increased. This is partially due to tremendous increases in throughput from DNA sequencing technologies whereby sequencing of large bacterial plasmids and genomes has become routine for research purposes. This has led to information on the constellation of antibiotic resistance genes within resistant bacterial isolates as well as knowledge of the genetic mechanisms by which these genes can spread to other bacteria.

Based on the DNA sequencing technology recently made available as well as new technologies in development, there is a promise of maintaining the large increases in sequencing capacity while continuing to decrease the cost. This has led to the beginnings of the use of DNA sequencing as a diagnostic tool for clinical microbiology in addition to its widespread use as a microbiology research tool. In turn, the amount of genomic and plasmid sequence information has been rapidly expanding. The end result is that there is a great deal of molecular information in the form of gene sequences and lists of mutations that are available to describe and diagnose a resistance mechanism.

As DNA sequencing technologies continue to improve, it is likely that sequencing will become a routine diagnostic tool in clinical microbiology laboratories to identify bacterial species as well as possible antibiotic resistance mechanisms. This will lead to an opportunity to use DNA sequence information to guide treatment. As DNA sequencing is incorporated as a clinical microbiology tool, it will greatly increase information on which antibiotic resistance genes (or mutations) are most common in a certain bacteria, as well as the geographical location of where bacterial infections associated with resistance genes occur. On a national and international scale, this would present information on the distribution and abundance of antibiotic resistance genes at any moment in time as well as dynamic information on how the distributions change over time. A DNA sequence of a cultured bacterium from a patient would provide obvious information with regard to treatment choice because one would avoid giving an antibiotic for which resistance genes are clearly present. However, widespread sequencing and rapid dissemination of the information could also guide treatment when the sequence of the organism causing illness was not known. In this case, the distribution of resistance genes among bacteria in a geographic region or specific hospital could be used to determine the probability that such a resistance gene would be present in the organism at hand. Information-based, targeted therapies could decrease inappropriate antibiotic use and thereby decrease the spread of antibiotic resistance.

A key element for harvesting information from the application of high throughput sequencing as a clinical microbiology diagnostic tool is a database containing known DNA sequence information on antibiotic resistance genes and mutations associated with resistance. As DNA sequence data is obtained on an organism, the method to determine the resistance mechanism involved is to search databases for sequence matches to known resistance determinants. If a resistance determinant is not present in the database, no knowledge will be obtained from sequencing. Therefore, the quality and completeness of the database is crucial to success in using sequence information to guide treatment. DNA sequence databases that can be used for sequence matching to resistance genes

exist. However, ideally the databases should also contain information on location (i.e., city, state, and hospital) and frequency of occurrence of the resistance determinant. An open source database whereby clinical microbiology laboratories deposit all sequence information obtained as well as information on the organism, location, and date would allow the database to contain current information on the state of resistance gene frequency at any point in time.

Policy Issues

Policy formulation to foster the development of new antibiotics and management of resistance of currently available antibiotics will depend on several issues:

- Policies will need to be developed for participation in data sharing in the form of depositing sequences into a common, international database. The construction and operation of the database should be funded by governments but be independent of any one government.

- As new technologies, particularly the adoption of low-cost DNA sequencing as a diagnostic tool for antibiotic susceptibility, become established it will be critical that an infrastructure for data management and sharing be in place.

- DNA sequencing as a routine diagnostic tool would release a flood of sequence information that needs to be captured to enable its use as guide for treatment. Development of a common database of antibiotic resistance gene information is a large bioinformatics challenge that will need to be undertaken soon to keep pace with information flow from the technology. Automation in data submission would facilitate database development.

- If such a database were to be used for diagnostic purposes there would need to be approval and monitoring of the database by regulatory agencies of participating countries.

- Efficient use of DNA sequence information and databases requires detailed knowledge of antibiotic resistance mechanism. Therefore, basic research on the genetics and biochemistry of antibiotic resistance should be supported.

- Management of antibiotic use via sequence information should be coupled with increased efforts at development of new antibiotics that act on novel targets in bacteria. A combination of effective management of resistance to current antibiotics and the development of entirely new antibiotics could work in synergy to provide improved treatment options.

References

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