Microbial “dark matter” and renaissance in antimicrobial discovery

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Problem:

antibiotic resistance in most pathogens
Antibiotic deployment

- Sulfonamides
- Penicillin
- Streptomycin
- Chloramphenicol
- Tetracycline
- Erythromycin
- Ampicillin
- Methicillin
- Cephalosporins
- Vancomycin
- Linezolid
- Daptomycin
Antibiotic deployment

Antibiotic resistance observed
Antimicrobials from Actinomycetes: Back to the Future

Actinomycetes are the source of most clinically relevant antibiotics in use today and may continue to be so

Richard H. Baltz

The two-faced Roman god Janus could see both forward and backward simultaneously. In describing combinatorial chemistry, and high-throughput in vitro screening at our disposal.
Frequency of discovery of new antibiotics as a function of the total antibiotics discovered. Several antibiotics are shown with arrows pointing to their frequencies of discovery among random actinomycetes. Act, actinomycin D; Dap, daptomycin; Ery, erythromycin; Sm, streptomycin; Stn, streptothricin; Tet, tetracycline; and Van, vancomycin.
Problem:

antibiotic resistance in most pathogens
no new antibiotics
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Cause: over-mining of cultivated microorganisms
Problem: antibiotic resistance in most pathogens

Cause: no new antibiotics

over-mining of cultivated microorganisms

Possible solutions:
Problem: antibiotic resistance in most pathogens
no new antibiotics

Cause: over-mining of cultivated microorganisms

Possible solutions: metagenomics
Problem: antibiotic resistance in most pathogens
no new antibiotics

Cause: over-mining of cultivated microorganisms

Possible solutions: metagenomics
screening chemical libraries
Problem:
- antibiotic resistance in most pathogens
- no new antibiotics

Cause:
- over-mining of cultivated microorganisms

Possible solutions:
- metagenomics
- screening chemical libraries
- microbial “dark matter”
  (uncultivated species)
History of Microbiology

Introduction

Medical microbiology is a branch of microbiology that deals with the study of microorganisms including bacteria, viruses, fungi, and parasites of medical importance that are capable of causing diseases in humans. It also includes the study of microbial pathogenesis, disease pathology, immunology, and epidemiology of diseases.

Medical microbiology is among the most widely studied branches of Microbiology. It has given mankind a chance to fight the organisms that, at one point of time, were pure nemesis to us. This has also provided an in-depth knowledge and in-detail understanding of the nature of pathogens that cause disease in humans. This field of microbiology has been the precursor to the wide gamut of immunological innovations in the field of medical science. This field not only has helped to develop vaccines against many invading organisms; it has also, in a more holistic way, given mankind a second shot at life. Deadly and debilitating diseases like smallpox, polio, rabies, plague, etc., have been either eradicated or have become treatable now because of the efforts of scientists and researchers in the field of medical microbiology.

Microbes are the most significant life forms sharing this planet with humans because of their pervasive presence. Depending on their food sources, microbes may have either beneficial roles in maintaining life or undesirable roles in causing human, animal, and plant diseases. These microbes cause frequent and often severe diseases, such as AIDS, cholera, tuberculosis, rabies, malaria, etc. The ubiquitous presence of microbes in large numbers have given rise to the many mutants, which in part are responsible for emerging diseases such as AIDS, Ebola hemorrhagic fever, and multidrug-resistant tuberculosis (MDRTB).

Microorganisms as a Cause of Disease

Among various causes, the causes suggested for the occurrence of disease were the effect of supernatural phenomena like planetary alignments and effect of bad bodily humors; the faulty environment was also implicated.

Even before microorganisms were seen, some investigators suspected their existence and responsibility for disease. Among others, the Roman philosopher Lucretius (about 98-55 BC) and the physician Girolamo Fracastoro (1478-1553 AD) suggested that disease was caused by invisible living creatures. Fracastoro was much more than an author of the popular poem on syphilis. In his book “De Contagion, contagionis natura et cannione (On Contagion, Contagious Diseases, and their Treatment),” published in 1546, he proposed the revolutionary theory that infectious diseases are transmitted from person to person by minute invisible particles. He further suggested that infections spread from person to person by minute invisible seeds, or somatit, that are self-replicating and act on the humors of the body to cause disease. His theories were ahead of their time and it took about 200 years for the microscope to be invented and his theories to be proved.

Historical Background

Microbial diseases have undoubtedly played a major role in historical events, such as the decline of the Roman Empire and the conquests of the New World. In 1347, plague or Black Death struck Europe with a brutal force. By 1351, about 4 years later, the plague had killed one-third of the population (about 25 million people). Over the next 80 years, the disease has struck repeatedly, eventually wiping out 75% of the European population. Some historians believe that this disease changed European culture and prepared the way for the Renaissance. This is just an example from many such epidemics, which while being devastating in their scope spared not even the high and mighty of the times.

Apart from the bubonic plague, measles (now thankfully extinct) and smallpox too played their roles as epidemic diseases causing high mortality and morbidity. The first recorded epidemic of smallpox was in the year 1350 BC in Egypt. The disease was unknown in the population of the New World until the Portuguese and Spanish explorers made their appearance. Smallpox then traveled across America, devastating the previously unexposed population. It was already known at that time that the disease spreads through the skin lesions and scabs, and that survivors of the infection were immune to reinfection on further exposure. Though adopted much later in America and Europe, the practice of inoculation or variolation, whereby people were intentionally exposed to smallpox to make them immune, was already being practiced in India, China, and Africa for centuries.
Introduction

Pathogenesis

Immunological knowledge and survival of pathogens

Epidemics

Historical epitaphs:

self-propagating organisms

1847 plague

AVS
0.022 μm membrane
Agar containing Bacteria

0.022 µm membrane

Agar containing Bacteria
In theory, there is no difference between practice and theory, but in practice, there is.
Microbial recovery in diffusion chambers

Growth recovery

- February
- March
- June
- July
- August
- September

Control
However beautiful the strategy, you should occasionally look at the results.

Winston Churchill.
Antibiotic nursery

Trying to grow soil bacteria in the lab almost always ends in failure, so instead the iChip grows them right where they feel at home.

1. Take a sample of bacteria from the soil.
2. Dilute the sample so the concentration of bacterial cells is low. Then wash it over the iChip so there is one cell per well.
3. Close up the wells with a permeable membrane. The bacteria won’t be able to escape, but all the chemicals from the environment can seep in.
4. Place the device back in the soil. Pure colonies of as many as 30 per cent of the species will grow.
z-stack
Frequency of discovery of new antibiotics as a function of the total antibiotics discovered. Several antibiotics are shown with arrows pointing to their frequencies of discovery among random actinomycetes. Act, actinomycin D; Dap, daptomycin; Ery, erythromycin; Sm, streptomycin; Stn, streptothricin; Tet, tetracycline; and Van, vancomycin.
NovoBiotic Pharmaceuticals is a privately held, early stage biotechnology company focused on the discovery and development of new drugs from natural sources.
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60,000 “uncultivable” bacterial strains from soils grown by *in situ* cultivation approaches
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28 novel antibiotics: odds are 1:2000
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Recent NIH supported project: 10,000 strains grown by ichip technology
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Several novel antibiotics, among them teixobactin
A new antibiotic kills pathogens without detectable resistance


Antibiotic resistance is spreading faster than the introduction of new compounds into clinical practice, causing a public health crisis. Most antibiotics were produced by screening soil microorganisms, but this limited resource of cultivable bacteria was overmined by the 1960s. Synthetic approaches to produce antibiotics have been unable to replace this platform. Uncultured bacteria make up approximately 99% of all species in external environments, and are an untapped source of new antibiotics. We developed several methods to grow uncultured organisms by cultivation in situ or by using specific growth factors. Here we report a new antibiotic that we term teixobactin, discovered in a screen of uncultured bacteria. Teixobactin inhibits cell wall synthesis by binding to a highly conserved motif of lipid II (precursor of peptidoglycan) and lipid III (precursor of cell wall teichoic acid). We did not obtain any mutants of Staphylococcus

Caption: A previously uncultured bacterium, Eleftheria terrae, makes a new antibiotic, teixobactin, for which there is no detectable resistance.
Teixobactin

Eleftheria terrae

Credit: William Fowle, Northeastern University

Caption: A previously uncultured bacterium, *Eleftheria terrae*, makes a new antibiotic, teixobactin, for which there is no detectable resistance.
Table 1 | Activity of teixobactin against pathogenic microorganisms

<table>
<thead>
<tr>
<th>Organism and genotype</th>
<th>Teixobactin MIC (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus (MSSA)</td>
<td>0.25</td>
</tr>
<tr>
<td>S. aureus + 10% serum</td>
<td>0.25</td>
</tr>
<tr>
<td>S. aureus (MRSA)</td>
<td>0.25</td>
</tr>
<tr>
<td>Enterococcus faecalis (VRE)</td>
<td>0.5</td>
</tr>
<tr>
<td>Enterococcus faecium (VRE)</td>
<td>0.5</td>
</tr>
<tr>
<td>Streptococcus pneumoniae (penicillin^R)</td>
<td>≤ 0.03</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>0.06</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>0.12</td>
</tr>
<tr>
<td>Viridans group streptococci</td>
<td>0.12</td>
</tr>
<tr>
<td>B. anthracis</td>
<td>≤ 0.06</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>0.005</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>0.08</td>
</tr>
<tr>
<td>M. tuberculosis H37Rv</td>
<td>0.125</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>4</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25</td>
</tr>
<tr>
<td>Escherichia coli (asmB1)</td>
<td>2.5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

The MIC was determined by broth microdilution. MSSA, methicillin-sensitive S. aureus; VRE, vancomycin-resistant enterococci.
ichip and the discoveries it enabled were named
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- one of the top 100 greatest innovations by Popular Science
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- one of the five most important medical developments by BBC
The 2015 Top 20 Drugs In The Pipeline
Novel Antibiotic

12. Teixobactin
Northeastern University
Bacterial infections

A new antibacterial molecule with a novel mechanism of action has been unearthed by U.S. academics at Northeastern University. Teixobactin—a cyclic depsipeptide—is believed to work in a way that makes it difficult for bacteria to develop resistance to it. This is being viewed by many as a step towards a new class of antibiotics that could potentially tackle the growing problem of antibiotic resistance.
Teixobactin isn’t even the most promising part of its own story. That honour falls on the iChip—the tool that the team used to discover the compound. Teixobactin is a fish; the iChip is the rod. Having the rod guarantees that we’ll get more fish—and we desperately need more.
Replace standard laboratory courses with discovery-based research courses.
- PCAST, Engage to Excel Report

The Small World Initiative is an innovative undergraduate research collaborative that presents a unique combination of science education, scientific research, and science diplomacy. Using authentic scientific research as a driver, this initiative focuses on addressing a worldwide health threat, the diminishing supply of effective antibiotics. Students will contribute to discovering new antibiotics from soil bacteria in their local environment. The research course will provide the venue for the investigation of biological and chemical soil diversity. It will also be a platform from which to teach a number of different biological concepts in the context of four distinct introductory courses. The goal of this project is to increase retention in college, inspire students to pursue science, and train future scientists.
Upcoming Events

June 1-5, 2017 – SWI Symposium, LA
SWI’s 4th Annual Symposium at ASM Microbe in New Orleans, LA

July 19-23, 2017 – Training Workshop, CT
SWI’s next instructor training will be hosted at UConn in Storrs, CT

WHO WE ARE

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  - Lab for Non-Science Majors
  - Lab for Science Majors
  - Microbiology Lab
JOIN US

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Apply

Majors
Thank you!