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# Combating Antibiotic Resistance in the 21st Century

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# Combating Antibiotic Resistance in the 21st Century

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**Abstract**

Antibiotic resistance is one of the most significant challenges facing the medical community today. In response, the Centers for Disease Control and Prevention (CDC) created a list of the greatest antibiotic resistance threats, a number of which are gram-positive bacteria. The cell wall of these organisms has long been a favored target of antibiotic therapies, but the development of numerous resistance mechanisms has led to widespread resistance against nearly all major antibiotic compounds on the market. The medical community is faced with the task of developing better antibiotic compounds that preclude the spread of bacterial resistance and also increasing the screening of natural antimicrobials from organisms not readily cultured in the laboratory. The iChip is a novel in situ cultivation device that allows researchers to grow cultures of bacterial species that could not otherwise be cultured in a laboratory setting. This technology has already led to the discovery of several promising novel antimicrobial compounds, including teixobactin. This depsipeptide has excellent activity in vitro against gram-positive organisms including Clostridium difficille, Bacillus anthracis, Enterococcus strains (including vancomycin resistant enterococci), Mycobacterium tuberculosis and Staphylococcus aureus. Pharmacists have a significant impact in the education of patients receiving antibiotic therapy about the issue of drug resistance and how alternative courses of treatment may be needed if antibiotic therapy is unsuccessful.

**Key Terms**

Antibiotic Resistance; Bacterial; Cell Wall; Drug Resistance; Gram-Positive Bacteria; iChip; Teixobactin

## An Overview of Antibiotic Resistance

Antibiotic resistance is one of the most significant challenges facing the medical community today. Not only are bacteria adapting at an increasingly rapid rate, but researchers are developing new antibiotics at the slowest rate since the advent of modern antimicrobials.[1] Governments around the world have realized that antibiotic resistance is a serious threat, and in the United States numerous federal and state agencies are now releasing guidelines and plans on how to face this issue. It is generally agreed upon that in order to effectively combat resistance, the medical profession must: 1) prevent infections and further spread of resistance, 2) track resistant bacteria, 3) improve the use of current antibiotics, 4) promote the development of new antibiotics and diagnostic tests for resistant bacteria and 5) promote better public education about proper antibiotic use and antibiotic resistance.[1,2]

According to the Centers for Disease Control and Prevention (CDC), over 2 million illnesses and 23,000 deaths occur each year in the United States due to antibiotic resistant organisms.[1] These infections are also vastly more difficult and expensive to treat. Estimates of the economic impact of antibiotic resistance range up to $20 billion, with an even higher cost in lost productivity. Antibiotics in general are also responsible for nearly one in five emergency department visits due to adverse drug events (ADEs) and are the leading cause of adolescent emergency department visits for ADEs. The CDC created a list of the greatest antibiotic resistance threats categorized as urgent, serious and concerning. Gram-positive Clostridium difficile and carbapenem-resistant enterobacteriaceae (CRE) are two of the three "urgent" threats, and there are also a significant number of gram-positive species in the lower categories.

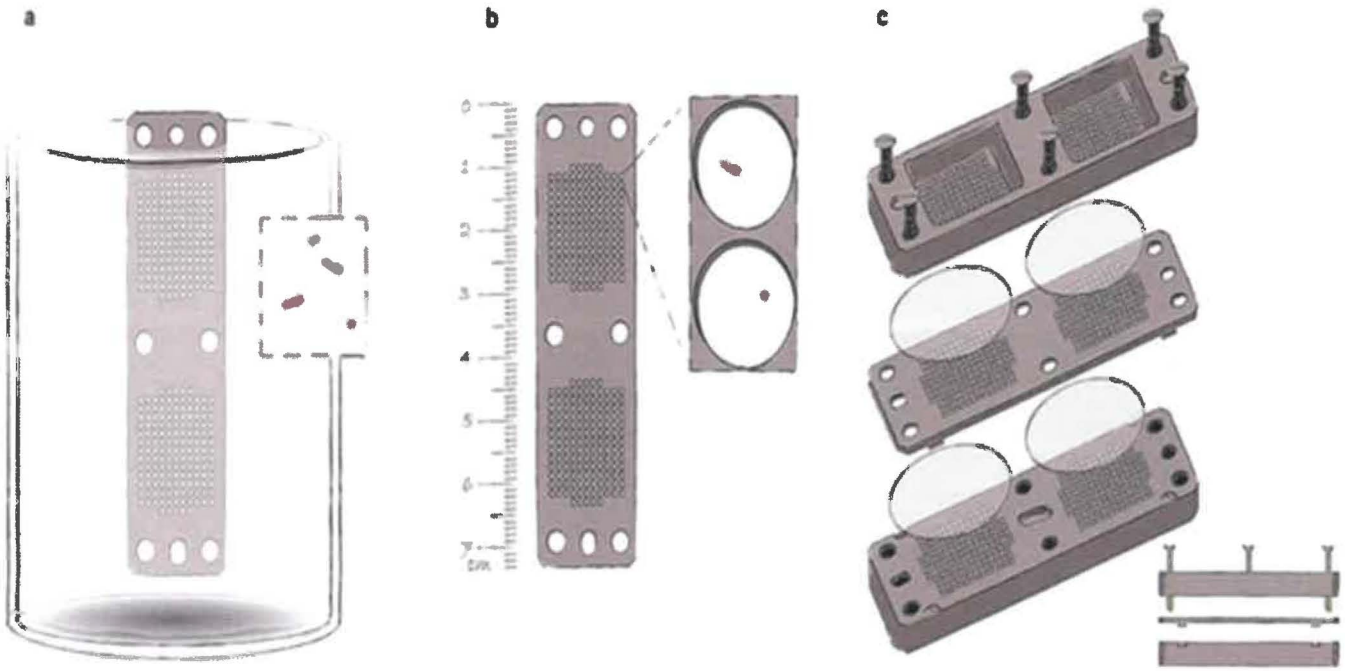
A brief examination of the history of antibiotic development, as compared to antibiotic resistance, helps illustrate the increasing difficulty of not only developing new antimicrobial agents but also fostering proper and effective use of currently available compounds.[1] Several years before the widespread use of penicillin in 1943, there were already known isolates of resistant Staphylococcus species. With the development of additional β-lactams and tetracyclines, bacterial species resistant to these compounds also rapidly emerged. As the 20th century closed, every major antimicrobial compound had known resistant isolates. The first decade of the 21st century has seen relatively few new antibiotics introduced to market, yet even these compounds are already associated with resistant species.

## Common Cell Wall Associated Mechanisms of Resistance in Gram-Positive Bacterial Species

The bacterial cell wall and its synthesis are common targets among natural and synthetic antibacterial compounds. This section discusses numerous drug targets associated with cell wall synthesis as well as resistance mechanisms.

The oldest and largest class of modern antibiotics are the β-lactams, some examples of which include the penicillins, cephalosporins and carbapenems.[3] Transpeptidases involved in cell wall synthesis act as penicillin binding proteins (PBPs) due to their affinity for the β-lactam ring. By mimicking endogenous precursors, β-lactams inhibit transpeptidase activity. However, bacterial species that produce enzymes capable of hydrolyzing the β-lactam ring are able to inactivate the drug. β-lactamase associated resistance is a leading cause of resistance to the β-lactams.[4] Another common cause of β-lactam resistance is the protein PBP2a. Bacteria that acquire and express the *mecA* gene are able to synthesize PBP2a, which then crosslinks peptidoglycan (PG) when the normal transpeptidase PBPs are inactivated by β-lactams.[5]

**Figure 1. Isolation Chip (iChip) Design.**




(a & b) sample illustration of how the iChip is placed in suspension to capture single cells per each through-hole; (c) two outer plates are fastened to the center plate in the assembly of the iChip. Adapted from: Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, et al. A new antibiotic kills pathogens without detectable resistance. *Nature*. 2015 Jan 22;517(7535):455-9. Used with permission of the authors.

Wall teichoic acid (WTA) and its synthesizing enzyme TarO are also viable targets for antibiotic compounds.[5] It is hypothesized that WTAs play a role in stability of the cell wall during division. Inhibition of WTA synthesis via gene deletion or pharmacological intervention results in defective and inefficient bacterial cell division. Furthermore, Campbell et al. demonstrated a "synthetic lethality" when they combined an inhibitor of TarO with β-lactam antibiotics. In one of the experiments, investigators observed that methicillin resistant Staphylococcus aureus (MRSA) strains treated with tunicamycin developed a significant sensitivity to a number of β-lactam antibiotics. However, this synergistic effect did not carry over to other classes. A possible reason for this specific synergism is that inhibition of WTAs results in misplacement of PBP targets of the β-lactam class, allowing these drugs to overcome resistance mechanisms.

Two additional targets for inhibiting cell wall synthesis are Lipid II and Lipid III. These compounds are associated with the movement of PG building blocks across the cell membrane.[3,6] Lipid II is a precursor of PG that is transported across the cell membrane by flippase-type transporters.[7,8] Once outside the cell, Lipid II is incorporated into the cell wall. Glycopeptide antibiotics, such as vancomycin and teicoplanin, are known to inhibit Lipid II. Despite the large number of naturally occurring antimicrobials that favor this target, vancomycin resistant organisms are becoming increasingly common.[1] This is due to the presence of the *vanA*-type resistance operon which changes the terminal amino acids on Lipid II from D-alanine-D-alanine to D-alanine-D-lactate.[3] This change adversely impacts the binding affinity of vancomycin for Lipid II, resulting in resistance. Lipid III is involved in WTA synthesis. As mentioned previously, WTAs are not essential to cell survival, however, inhibition of WTA synthesis at Lipid III can lead to the buildup of toxic intermediates as well as autolysin mediated degradation of PG.[5,6]

## The Need for Better Antibiotics and Techniques of Discovery

As the above sections highlight, there are numerous and diverse pathways by which antibiotic drugs target gram-positive bacteria. However, these organisms have developed highly efficient means of evading both natural and synthetic antimicrobial compounds, and this capability threatens to render the vast majority of current antibiotics useless. Moving forward, the medical community has several options: develop better antibiotic compounds that preclude the development of bacterial resistance and/or find ways to increase the screening of natural antimicrobials from organisms not readily cultured in the laboratory.[1,6]

One of the greatest limitations in modern antimicrobial discovery is that traditional, yet current cultivation methodologies, such as petri dish cultivation, are unable to effectively cultivate certain microbial phyla with known or suspected cultivable representatives. As a result, both the potential to

**Table 1. Bactericidal Activity of Teixobactin.[9]**

| Organism and Genotype | Teixobactin MIC (μg/mL) |
|---|---|
| Staphylococcus aureus (MSSA) | 0.25 |
| Staphylococcus aureus (MRSA) | 0.25 |
| Enterococcus faecalis (VRE) | 0.5 |
| Bacillus anthracis | ≤ 0.06 |
| Clostridium difficile | 0.005 |
| Haemophilus influenzae | 4 |
| Escherichia coli | 25 |
| Escherichia coli (asmB1) | 2.5 |
| Pseudomonas aeruginosa | > 32 |
| Klebsiella pneumoniae | > 32 |

MSSA = methicillin-sensitive Staphylococcus aureus; MRSA = methicillin-resistant Staphylococcus aureus; VRE = vancomycin-resistant Enterococci; asmB1 = a cell membrane assembly suppressor mutation. Minimum inhibitory concentration (MIC) values were determined by broth microdilution. Adapted from: Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, et al. A new antibiotic kills pathogens without detectable resistance. *Nature*. 2015 Jan 22;517(7535):455-9. Used with permission of the authors.

screen for and culture new anti-infective agents is limited in the drug discovery process.[9] In 2009, however, the development and introduction of a novel in situ cultivation method, referred to as an isolation chip (iChip), demonstrated a promising capacity to cultivate environmental microorganisms that were formerly considered "uncultivable." Specifically, the iChip (Figure 1) is an assembly of three sealed hydrophobic plastic polyoxymethylene plates (one central plate and two outer panels), with each containing several hundred "through-holes," which permit the capture of single microbial cells when suspended in an agar-based liquid medium. On each side of the central plate and between each of the outer panels are two 47-mm polycarbonate membranes which prohibit the migration of single cells captured in the through-holes. Collectively, these technological features enable the isolation of monospecific cultures, particularly of microbial phyla that could not be previously cultured in conventional methods such as petri dishes.

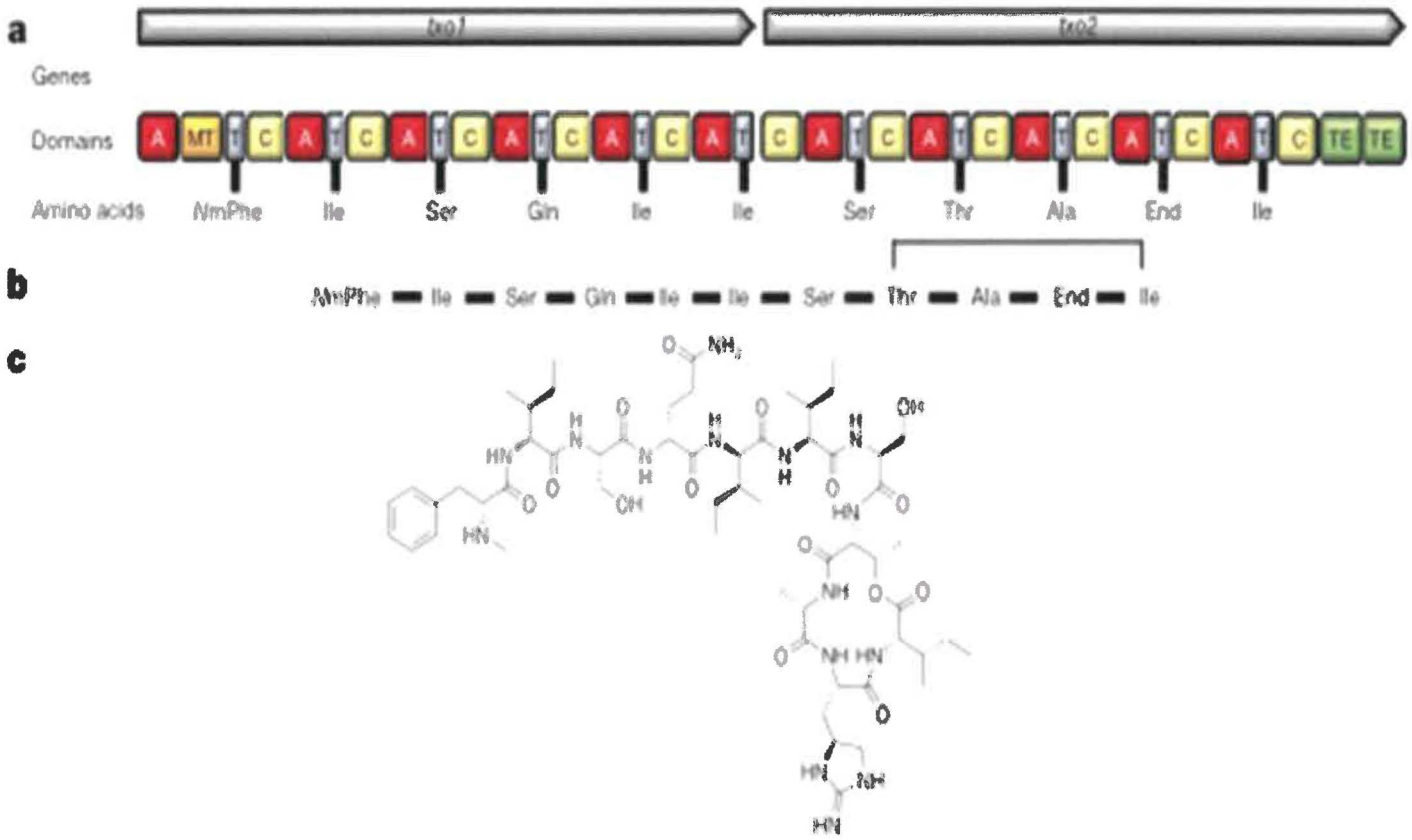
### Isolation of Teixobactin Using iChip Cultivation

The ability of iChip to isolate formerly uncultivable microorganisms indicates its promising potential to expand the drug discovery effort of anti-infective agents, especially those that may be able to combat the problem of drug resistance among current anti-infective agents available in the United States' pharmaceutical market. In fact, in January 2015, Ling et al. of NovoBiotic Pharmaceuticals in Cambridge, Massachusetts, utilized iChip cultivation among 10,000 different microbial isolates and identified a new antibiotic—teixobactin—that demonstrated notable bactericidal activity against commonly known drug-resistant microbes, including Staphylococcus aureus, difficult-to-treat enterococci, Mycobacterium tuberculosis, Clostridium difficile and Bacillus anthracis (Table 1).[9] Teixobactin (Figure 2), an unusual depsipeptide which contains enduracididine, methylphenylalanine and four D-amino groups, is proposed to inhibit bacterial cell wall synthesis in gram-positive microbes by binding to Lipid II and Lipid III, precursors of peptidoglycan and teichoic acid, respectively.

### Therapeutic Implications of Teixobactin in Combating Antibiotic Resistance

The bactericidal activity and undeveloped resistance of teixobactin gives the compound an edge over anti-infective agents displaying resistance profiles and bacteriostatic properties. Researchers found teixobactin had excellent activity in vitro against gram-positive organisms including drug-resistant strains.[6] Data results showed the compound had exceptional activity against Clostridium difficille and Bacillus anthracis, and also displayed excellent activity against Enterococcus strains, Mycobacterium tuberculosis and Staphylococcus aureus. The advantage of teixobactin compared to current anti-

**Figure 2. Teixobactin Predicted Structure and Biosynthetic Gene Cluster.**




Adapted from: Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, et al. A new antibiotic kills pathogens without detectable resistance. *Nature*. 2015 Jan 22;517(7535):455-9. Used with permission of the authors.

infective agents is based on the inability of bacteria to develop resistance. Teixobactin's major targets, Lipid II and Lipid III, are a new way to inhibit cell wall biosynthesis, which is why bacteria have not been able to show resistance yet. Researchers were unable to obtain mutants of Staphylococcus aureus or Myobacterium tuberculosis resistant to teixobactin, even when plating on media with a low dose (four times Minimum Inhibitory Concentration) of the compound. Table 2 shows a summary of common antibiotic drugs, their targets and mechanisms of resistance. Although the table is not comprehensive, it displays the mechanisms of resistance relative to drug targets. It also shows teixobactin targets a slightly different part of the bacterial cell wall than what has been targeted in the past by other anti-infective agents. Unfortunately, teixobactin is mostly ineffective against gram-negative bacteria because the compound does not target the components in the gram-negative wall. The bacteria from which teixobactin was isolated, Eleftheria terrae (beta-proteobacteria), is a gram-negative bacteria, and the organism would not survive if it inhibited its own cell wall. Animal studies establishing safety and efficacy data are the next step in pushing teixobactin toward approval by the U.S. Food and Drug Administration (FDA). Researchers have shown that teixobactin had no toxicity against mammalian cells at 100 µg/mL (the highest dose tested) in vitro. Teixobactin showed no hemolytic activity, did not bind DNA, and was tested in vivo in mice infected with Streptococcus pneumonia; it was shown to be highly efficacious because it caused a 6 $\log_{10}$ reduction of colony forming units in the lungs.

## The Role of the Pharmacist

The role of pharmacists is to prevent antibiotic resistance from progressing by thoroughly explaining antibiotic drug regimens to patients and educating patients on the importance of finishing a regimen although symptoms may have subsided. Furthermore, pharmacists can educate patients on antibiotic resistance in the community and can help patients understand that antibiotics do not work against viral infections. Lastly, pharmacists can stay informed on the latest news for antibiotic discovery so that they understand the indications and mechanisms of new antibiotic compounds for when these drugs are placed on the shelf in the pharmacy for the first time. This will allow pharmacists to give physicians and other health care professionals with pre-

**Table 2. Summary of Select Antibiotic Drugs, Targets and Resistance.** [3,5,6]

| Drug Class | Select Compounds | Drug Targets / Mechanism of Action | Common Resistance Mechanisms |
|---|---|---|---|
| **ß-lactams** | penicillins, cephalosporins, carbapenems | PBPs / inhibition of transpeptidases | ß-lactamase, PBP2a activity |
| **Sugar substrate analogue** | tunicamycin | WTA synthesis, TarO / decreased efficiency of cell division, increased susceptibility to ß-lactams | "Synthetic lethality" unique to combination TarO inhibitor and ß-lactam. Not effective with other classes. |
| **Glycopeptides** | vancomycin, teicoplanin | Lipid II / incomplete cell wall biosynthesis | Alteration of Lipid II terminal amino acids |
| **Novel depsipeptide** | teixobactin | Lipid II, Lipid III / incomplete cell wall biosynthesis, toxic intermediates of WTA synthesis | None yet observed |

scribing abilities expert advice in choosing the best antibiotic regimen possible for patients.

## Conclusion

Due to the fact that bacteria are becoming more resistant to antibiotic compounds at an increasing rate, it is vital for the well being of patients presenting with infections to discover new antibiotics that can help better fight off these resistant pathogenic organisms. These organisms have developed highly efficient means of evading both natural and synthetic antimicrobial compounds, and this ability threatens to make the vast majority of current antibiotics useless. The development of iChip as a novel in situ cultivation method for environmental microorganisms that were once thought to be uncultivable demonstrates a promising capacity to discover antibiotic compounds that can be used to combat highly resistant bacterial infections.

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