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Perspectives in Medicinal Chemistry

Antibiotics and Bacterial Resistance in the 21st Century

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ABSTRACT: Dangerous, antibiotic resistant bacteria have been observed with increasing frequency over the past several decades. In this review the factors that have been linked to this phenomenon are addressed. Profiles of bacterial species that are deemed to be particularly concerning at the present time are illustrated. Factors including economic impact, intrinsic and acquired drug resistance, morbidity and mortality rates, and means of infection are taken into account. Synchronously with the waxing of bacterial resistance there has been waning antibiotic development. The approaches that scientists are employing in the pursuit of new antibacterial agents are briefly described. The standings of established antibiotic classes as well as potentially emerging classes are assessed with an emphasis on molecules that have been clinically approved or are in advanced stages of development. Historical perspectives, mechanisms of action and resistance, spectrum of activity, and preeminent members of each class are discussed.

KEYWORDS: antibiotics, antibiotic resistance mechanisms, drug-resistant bacteria, novel antibiotic targets

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The Rise of Antibiotic Resistance

Antibiotic resistance fast facts

- **30%** – All deaths that were bacterial infection related in pre-antibiotic America[2]
- **$20 / 1.6€ billion** – Excess healthcare costs of resistant infections in the US/EU[3–5]
- **8 / 2.5 million** – Excess hospital days caused by resistant infections in the US / EU[3–5]
- **30%** – Antimicrobial component of pharmaceutical budgets in the US[6]
- **1.6%** – Antibiotic allotment of all drugs in development by major pharmaceutical companies[7]
- **4** – Multinational pharmaceutical companies with antibiotics divisions[11]
- **$1.1 billion** – Cost of unnecessarily prescribed antibiotics in the US[3,4]
- **48%** – Proportion of US hospitals that have adopted stewardship policies[23]
- **36%** – People that correctly answered that antibiotics do not kill viruses in an EU survey[31]
- **24.6 million** – Pounds of antibiotics used non-therapeutically on animals in the US per year in early 2000's[33]
- **300,000** – Pounds of streptomycin (**24**) and oxytetracycline (**40**) sprayed on produce in the US in 1996[42]

Bacterial resistance to antibiotics has been a recognized reality almost since the dawn of the antibiotic era, but only within the past twenty years has the emergence of dangerous, resistant strains occurred with a disturbing regularity. This escalating evolution of resistance coupled with a diminished antibiotic pipeline has led some to claim that a post-antibiotic era is eminent.[1] Given that the three main causes of death in pre-antibiotic America were tuberculosis, pneumonia, and gastrointestinal infections, which combined accounted for 30% of all deaths, this is a frightening prospect.[2] Though we are still far from that scenario becoming reality, the trend in

the antibiotics field has decidedly been negative for some time now. The annual impact of resistant infections is estimated to be $20 billion in excess health care costs and 8 million additional hospital days in the United States (US)[3,4] and over 1.6€ billion and 2.5 million additional hospital days in the European Union (EU).[5] Antimicrobials currently account for over 30% of hospital pharmacy budgets in the US.[6]

Diminished Pharmaceutical Investment

A flagging interest in antibiotics by the pharmaceutical industry is one factor that has contributed to an increased occurrence of hard to treat bacterial infections. In 2004 for example, only 1.6% of drugs in clinical development by the world's 15 largest drug companies were antibiotics. This reduced output of antibiotics has several causes.[7] Antibiotics regimens are typically administered for very limited durations making them far less profitable than drugs used to treat chronic ailments. Further, newly approved drugs for most other ailments are immediately prescribed, whereas new antibiotics are typically held in reserve and only prescribed for infections that more established antibiotics can't treat. This policy helps delay the emergence of resistant strains, but it also limits initial investment return. A market saturated with generic competitors and the inevitable growth of bacterial resistance exacerbates this profit disparity as compared to other drugs in the long term.

Regulatory hurdles have also muted the interest of major pharmaceutical companies. The tolerance of adverse side effects has recently been decreased for many drug classes, including antibiotics. Approval requirements during clinical trials have escalated in most cases from demonstration of noninferiority to superiority, and at times a lack of clear trial guidelines for antibiotics, in particular, have stifled development.[8] Pharmaceutical companies are presented with a paradox wherein federal agencies issue calls for antibiotic development while concomitantly other federal agencies enact policies limiting the appeal of that very development.

These factors have made investment in antibiotics development too high risk, and with the cost at an estimated $1.7 billion per drug, with too little potential reward for many large pharmaceutical companies.[9,10] A metric called net present value (NPV) has been developed for pharmaceutical companies to determine the best avenues of investment at a given time. NPV is a risk-adjusted measure of the projected future revenues of a drug discounting initial development investment and other projected future expenses. A characteristic NPV for an injectable antibiotic may be around 100, which is somewhat unattractive compared to a typical cancer drug, around 300, or a neuroscience drug around 720.[9]

Since 1998 AstraZeneca, GlaxoSmithKline, Merck, Johnson & Johnson, and Pfizer/Wyeth have been the only major pharmaceutical companies to develop an antibiotic past phase I clinical trials.[11] Sanofi Aventis, Eli Lilly, Bristol-Myers Squibb, GlaxoSmithKline, Proctor and Gamble, Roche, and Wyeth have all greatly curtailed, eliminated, or spun off their antibiotic R&D divisions.[6] In fact, as of 2013 there are only four multinational pharmaceutical companies with antibiotics divisions left.[11] No government agency has ever successfully discovered and developed an antibiotic and there have been no indications that any will contribute the resources necessary for such an endeavor anytime in the near future.[6] As a consequence much of what is currently being done in antibiotic development in the western world is done in small pharmaceutical companies, biotech entities, and academic institutions. A number of large pharmaceutical companies still play a central role in antibiotic development in Japan, however.[11–13]

Policies have recently been enacted and incentives offered in an effort to reverse this exodus from antibiotic R&D. Agencies including the World Health Organization (WHO), the European Center for Disease Prevention and Control (ECDC), the Infectious Diseases Society of America (IDSA), and even the US Congress have gotten involved.[5,8,11,14–16] In the 111th congress the Generating Antibiotic Incentives Now (GAIN) Act and the Strategies to Address Antimicrobial Resistance (STAAR) Act were introduced.[17] In 2011 the US government gave $94 million in government funding for the development of the structurally novel antibiotic candidate, Anacor's GSK-052 (though it's clinical trials were subsequently halted in 2012) and also $67 million for Teatraphase's TP-434 (eravacycline **44**), a next generation fluorocycline currently in phase III trials.[11] Even the FDA has recently publically acknowledged that there is an antibiotic crisis.[18,19]

Chronic Clinical Over-prescription and Public Misconceptions

The other factor fueling antibiotic resistance is the evolution and dissemination of resistance factors within bacterial populations. There are a plethora of means by which humans have inadvertently accelerated the evolution of bacterial resistance. The over prescription of antibiotics by doctors for symptoms that in many cases may not be caused by bacteria has historically been one such problematic policy. In recent years steps have been taken to limit antibiotic over prescription, however. In surveys of doctor's visits in 1995 compared to 2005, the percentage that resulted in antibiotic prescriptions decreased universally for symptoms including ear infections, colds, bronchitis, sore throats, and sinusitis.[3,4] Despite these positive trends the Center for Disease Control and Prevention (CDC) recently estimated that approximately 50% of antibiotics are still prescribed unnecessarily in the US at a yearly cost of $1.1 billion.[3,4]

Antibiotic stewardship programs are becoming more commonplace in hospital settings and have been correlated in many cases to significant reductions in some strains of resistant bacteria.[20–22] Despite these successes only 48% of US hospitals have adopted stewardship policies to date and numbers are unquestionably even lower in the majority of developing countries.[23] Varied methodologies in measuring antibiotic consumption in US hospitals has been an undermining factor even

where stewardship policies are enacted, though.[24] Along with overall reductions to antibiotic usage, cycling usage between antibiotic classes, using combination therapies, and avoiding use of broad spectrum and last resort antibiotics whenever possible, have also been implemented as strategies to avoid the evolutionary pressure that accelerates resistance.[25]

Overly long or improper treatment regimens may also in some cases exert unnecessary evolutionary pressure on bacteria.[26] This can lead to acquired drug resistance in which a minority resistant bacterial phenotype can find themselves in a less competitive, and therefore more advantageous environment as a phenotypically sensitive majority is killed off.[6] Outpatient antibiotic use has been directly tied to macrolide resistance in *Streptococcus pyogens* and penicillin resistance in *Streptoccous pneumoniae*.[27,28] More restrictive policies regarding outpatient regimes have resulted in the decline of certain resistant isolates in both Finland and France.[29,30]

A lack of public knowledge about antibiotics has also led to their overuse. In a 2009 European survey, of those who had taken antibiotics within the last year, 20% claimed to have taken them for influenza, a viral malady, and only 36% of those surveyed answered correctly that antibiotics do not kill viruses.[31] This particular variety of misuse is especially problematic in countries where antibiotics can be obtained without prescriptions.[32] Europe has instituted an Antibiotics Awareness Day annually on November 18th in an effort to raise public knowledge.[23]

Misuse by the Food Industry

The use of antibiotics in animal feed stocks has also exacerbated the spread of resistance. Especially egregious is their use for non-curative reasons such as prophylaxis, metaphylaxis, and growth promotion which by one estimate accounted for 25–50% of all antibiotic consumption in the early 2000s.[25] Other assessments within the US during the same time period estimated agricultural use to be much greater at 24.6 million pounds of antibiotics being given to animals for non-therapeutic purposes, 2 million pounds being used therapeutically on animals, and 3 million pounds being used in humans per year.[33] Antibiotic use for growth promotion has been banned in the European Union (EU) since 2003[34] and finally in 2012 the FDA banned the use of antibiotics in livestock without a veterinary prescription.[35] There are still many countries where this practice remains unlegislated, however.

There is strong evidence that the use fluoroquinolones in food animals has led to the emergence of fluroquinolone resistant *E. coli*,[36,37] *Salmonella*, and *Campylobacter*.[38] The emergence of vancomycin resistant *Enterococci* (VRE) in Europe was tied to the use of the glycopeptide avoparcin in food animals.[39] Avoparcin was banned in the EU in 1997, which resulted in a reduction in VRE there,[40] but many members of critical antibiotic classes are still used for veterinary purposes. In a survey by the European Medicines Agency there was actually an increase in veterinary sales of fluoroquinolones and fourth generation cephalosporins from 2005 to 2009.[41] The food industry's use of antibiotics has not been strictly limited to livestock either. In the US, in 1996 for example, 300,000 pounds of the aminoglycoside streptomycin (**24**), and oxytetracyline were sprayed prophylactically on apples and pears.[42] Waste runoff containing resistant bacteria or antibiotics from large corporate farms or agro-industrial plants is also a concern.[23] This serves as a mobile means of exposure to antibiotics and the terrestrial locale provides an ideal environment for dissemination of resistance elements from pathogenic bacteria and potentially from soil bacteria as well.[23]

Human Independent Resistance

Though there is undoubtedly a significant human contribution to the evolution of bacterial resistance, there is also resistance that has occurred in nature absent human interference.[43] Resistances to first in class antibiotics such as penicillin G (**4**) and streptomycin (**24**), discovered during the golden age of antibiotics, were observed shortly after their initial isolation.[44] Though this is not always the case, this phenomenon is typical when examining the antibiotic arsenal as a whole.[42] With the advent of cloning and sequencing it was possible to trace β-lactamases to a large number of homologous, but distinct genes that were transferred vertically and horizontally throughout many microbial communities, directly between bacteria and indirectly mediated by the many bacteriophages that infect them.[45] Resistance genes can associate in clusters and be transferred together as well.[46] This kind of genetic diversity couldn't have arisen in the time frame since penicillin's discovery and indeed phylogenetic analysis suggested a more ancient root evolution of these enzymes.[47]

Resistance elements have even been found in bacterial DNA that was isolated for 30,000 years in permafrost.[48] Estimates based on the genetic divergence of antibiotic biosynthetic genes have suggested that some antibiotics could have evolved hundreds of millions of years ago.[49] Taken together this evidence suggests that bacteria have likely had a very long time to evolve resistance to many, if not all, natural product antibiotics, and therefore, resistance is highly likely to exist long before their discovery by man. Most soil bacteria exhibit some form of antibiotic resistance and many of them exhibit many resistance mechanisms even to antibiotics that they do not naturally produce.[50] It could be argued that these samples could be contaminated in a variety of ways including antibiotic runoff. However, this evidence is also supported by a number of studies that have found antibiotic resistant (in some cases highly resistant), commensal bacteria on both humans and animals from remote locales that have never been exposed to antibiotics through unnatural means.[51] Evolution of bacterial resistance to antibiotics is therefore a natural process and would exist even absent human mismanagement.

Human use (and misuse) of antibiotics has clearly put unnatural selective pressure on bacteria, which has accelerated their evolutionary process to the detriment of everyone. To

address this problem, faster development of new antibiotics and more responsible use of currently antibiotics are clearly necessary.

Emergent Bacterial Threats

Bacterial threats fast facts

- **20%** – Proportion of people that are persistent carriers of *S. aureus*[52]
- **$3 billion** – Annual healthcare costs associated with MRSA in US[62]
- **19,000** – Deaths per year caused by MRSA in the US[62]
- **61%** – Vancomycin (**52**) resistance rate of *E. faecium* in the US[5]
- **40%** – *S. pneumoniae* strains resistant to penicillin[75,76]
- **50%** – Chance of contracting C. difficile with > 4 week hospital stays[80]
- **1.3 million** – Worldwide deaths caused by TB per year[90]
- **$483,000** – Average cost of XDR-TB treatment[92–93]
- **30%** – Increase in carbapenem resistant A. baumannii strains from 1995–2004[129]
- **30%** – Quinolone resistance rate for *Enterobacter*[138]
- **700,000** – *N. gonnorhoeae* infections in the US per year[3,4]
- **15.5%** – HAI incidence rate in developing countries[144]

There are many species of dangerous gram-positive and gram-negative bacteria. A sampling of some of the most problematic pathogens and their most alarming resistances are reviewed (Table 1). In the 1990s resistant gram positive bacteria materialized as a major threat with methicillin (MRSA) and vancomycin (VRSA) resistant *Staphylococcus aureus*, VRE, penicillin resistant *Streptococcus pneumonia*, and multi-drug resistant (MDR) *Clostridium difficile* dominating the headlines.

***Staphylococcus aureus* (MRSA, VISA, and VRSA).** *S. aureus* is a gram positive, facultative anaerobic pathogen with both hospital and community acquired strains. Though traditionally opportunistic, many *S. aureus* strains are now aggressively pathogenic.[42] It is the most common skin bacterium with 60% of humans being intermittent carriers and 20% being persistent carriers, chronically harboring at least one strain.[52]

S. aureus has evolved an arsenal of extracellular proteins and defense factors unassociated with antibiotic resistance. These include hemolysins, proteases, hyaluronidase, collagenase and an enterotoxin that causes gastroenteritis. Approximately 25% of strains express the exotoxin toxic shock syndrome toxin (TSST-1), and in 5% of strains the exotoxin Panton-Valentine leukocidin (PVL), which causes necrotic hemmorhagic pneumonia.[53–56] PVL is encoded by a bacteriophage now found commonly in community acquired MRSA (CA-MRSA).[57] These toxins have made effective protein translation inhibiting antibiotics particularly desirable in the treatment of some *S. aureus* strains.[58] Additionally, the pigment that gives this bacterium is golden color is staphyloxanthin, a carotenoid, antioxidant, virulence factor that aids in immune system evasion.[59,60]

S. aureus also frequently causes chronic infections by forming biofilms. It is the leading cause of chronic infections associated with indwelling medical devices.[61] Methicillin (**5**) resistance is also highly prevalent and though numbers can vary widely by country, its incidence is high in almost all countries where such data exists, and it is the single most commonly observed drug resistance in both the US and Europe.[5,6] MRSA was recently estimated to be responsible for 60–89% of nosocomial infections leading to 19,000 deaths and over $3 billion in health care costs per year in the United States.[8,17,62–64] It was reported in 2009 that MRSA infections kill more people in US hospitals than HIV/AIDS and tuberculosis combined.[58] β-lactam resistance in MRSA is primarily due to expression of the *mecA* gene which encodes the low affinity penicillin binding protein (PBP 2a).[65]

The glycopeptides, vancomycin (**52**) and teicoplanin (**55**), are common treatments for MRSA, however, resistance has now developed towards them as well. Vancomycin intermediate *S. aureus* (VISA), which is also usually insensitive to teicoplanin (**55**), evolved a less permeable cell wall that traps these antibiotics.[66] VISA was first isolated in 1996 in Japan, but has since been encountered globally.[42] VRSA was first reported in 2002 and is far less common.[67] It is caused primarily by an acquired resistance from the VRE *vanA* gene, which alters the terminal sequence of cell wall precursors, making them poor substrates for vancomycin (**52**) and teicoplanin (**55**).[66] VISA and VRSA strains are not strictly opportunistic, making them even more dangerous.[68]

Resistant *Enterococci* Including VRE

Resistant *Enterococci* are primarily comprised of two species, *E. faecalis* and *E. faecium*, both of which are gram-positive, facultative anaerobic, opportunistic pathogens. *Enterococci* are particularly environmentally tolerant with the ability to withstand a wide range of temperatures and pHs, as well as high salt concentration.[69] They are also capable of colonizing a wide range of locales including the gut, skin, and inanimate surfaces. Both have high levels of resistance rates (30–50%) against the aminoglycosides gentamicin (**25**) and streptomycin (**24**).[70] *E. faecium* is usually inherently resistant to β-lactam antibiotics also, making it particularly difficult to treat when it develops vancomycin resistance, which it much more commonly does than *E. faecalis*.[69] The streptogramin combination, quinupristin (**69**) / dalfopristin (**70**) is an effective treatment for *E. faecium*, but is ineffective against *E. faecalis*.[71]

Some VRE isolates express enterococcal surface protein, which allows for the production of thicker, more drug resistant biofilms. Like VRSA, these traits make VRE common in healthcare-associated infections (HAIs), and particularly in the colonization of indwelling medical devices.[72] Vancomycin resistance in *E. faecium* is common in the US at 61% in 2002, however, this resistance is far less common in the EU.[5,23] Though VRE is known to produce several resistance genes, the most common form of vancomycin resistance, as with VRSA, is *vanA*.[65]

Table 1. Emergent bacterial threats.

BACTERIUM	GRAM STAIN	RESPIRATION	PROBLEMATIC RESISTANCES
Staphylococcus aureus	+	Facultative anaerobe	β-lactams, glycopeptides
Enterococci	+	Facultative anaerobe	β-lactams, glycopeptides, aminoglycosides
Streptococcus pneumoniae	+	Aerotolerant anaerobe	β-lactams, macrolides, quinolones
Clostridium difficile	+	Obligate anaerobe	β-lactams, quinolones
Mycobacterium tuberculosis	+	Aerobe	Rifamycins, quinolones, aminoglycosides
Escherichia coli	-	Facultative anaerobe	β-lactams, quinolones, aminoglycosides
Pseudomonas aeruginosa	-	Facultative anaerobe	All classes except polymyxins
Acinetobacter	-	Facultative anaerobe	All classes
Klebsiella pneumoniae	-	Facultative anaerobe	β-lactams, quinolones, aminoglycosides
Enterobacter	-	Facultative anaerobe	β-lactams, quinolones
Neisseria gonorrhoeae	-	Aerobe	β-lactams, quinolones, tetracyclines, macrolides

Streptococcus pneumoniae. *S. pneumoniae* is a gram-positive, aerotolerant, anaerobic, opportunistic pathogen. It is the leading cause of bacterial pneumonia, but it can also cause otitis media, sinusitis, and meningitis among other pathologies.[73] It has a polysaccharide capsule that makes it naturally resistant to phagocytes. It also produces hydrogen peroxide to kill other bacteria.[74] Approximately 40% of strains are no longer susceptible to penicillin, and its penicillin resistance often correlates with resistances to macrolides, sulfamides, older tetracyclines, and early generation cephalosporins.[75,76] Even absent β-lactam resistance, macrolide resistances caused by upregulated efflux encoded by *mef* or *erm* genes is increasing in *S. pneumoniae*.[77] Resistance to the third-generation fluoroquinolone, levofloxacin (**60**), has also been observed recently.[78] Though resistance isn't as prevalent as in some other gram-positive pathogens, the pathologies associated with *S. pneumoniae* infection make the prospects of increased resistance worth particular consideration.

Clostridium difficile. *C. difficile* is a gram-positive, obligate anaerobic, spore forming opportunistic pathogen. Spores are highly environmentally tolerant and are resistant to heat, changes in pH, alcohol based hand sanitizers, and many traditional cleaning products that don't contain bleach.[79] *C. difficile* can be community acquired, but has a particularly high rate of acquisition in hospitals. Patients hospitalized for over four weeks have an approximately 50% chance of contracting *C. difficile*.[80] It is probably best known for causing antibiotic associated diarrhea. This pathology results when *C. difficile* is contracted and antibiotics that it is resistant to kill all other bacteria in the gastrointestinal tract. This subsequently causes *C. difficile* overgrowths as they spread to inhabit these now vacant niches. It produces an enterotoxin (toxin A) and a cytotoxin (toxin B) which play a role in the resultant symptoms and can lead to colitis, as well as life threatening complications.[81] Prophylactic cephalosporin use in surgeries has been linked to this condition and their use for this purpose is now restricted in certain at risk patient populations.[42] In 2005 a hypervirulent strain of fluoroquinolone resistant *C. difficile* emerged and quickly spread across North America.[82,83] As serious *C. difficile* infections rarely emerge without the use of antibiotics, eliminating unnecessary usage becomes especially important with the advent of resistant strains of this pathogen.

A New Wave of Resistant Gram Negative Infections

These gram-positive threats are still widespread and destructive, accounting for the majority of bacteria-related deaths in the United States by a significant margin (Fig. 1).[4] In recent years, however, resistance rates have stabilized or decreased for many of them, including MRSA and VRE, according to the European Antimicrobial Resistance Surveillance Network (EARS-Net).[15] Unfortunately, within the past ten years, as antibiotic development has focused on these threats,[84] drug resistant tuberculosis and a wave of new gram-negative strains just as perilous as their gram-positive counterparts have evolved. At least one analysis has suggested that the resistant gram-negatives may now be even more costly then gram-positives, MRSA included.[85] Also, in a European survey a compilation of some of the most common gram-negative infections were found to slightly outnumber common gram-positive infections.[5] In some ways the new resistant gram-negative pathogens are even more worrying, as their more difficult to penetrate outer membranes and higher prevalence of efflux pumps make them naturally resistant to many antibiotics. Carbapenem resistant gram negative strains, particularly *Enterobacteriaceae* (CRE), are becoming increasingly common place. The main gram-negative threats are multi- (MDR) and pan- (PDR) drug resistant *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter*, *Klebsiella pneumoniae*, *Enterobacter*, and most recently *Neisseria gonorrhoeae*.[86,87]

Mycobacterium tuberculosis **(MDR-TB and XDR-TB).** *Mycobacterium tuberculosis* is a highly aerobic, pathogenic bacterium that is the main cause of tuberculosis (TB). Though it doesn't typically gram stain because of a high lipid content

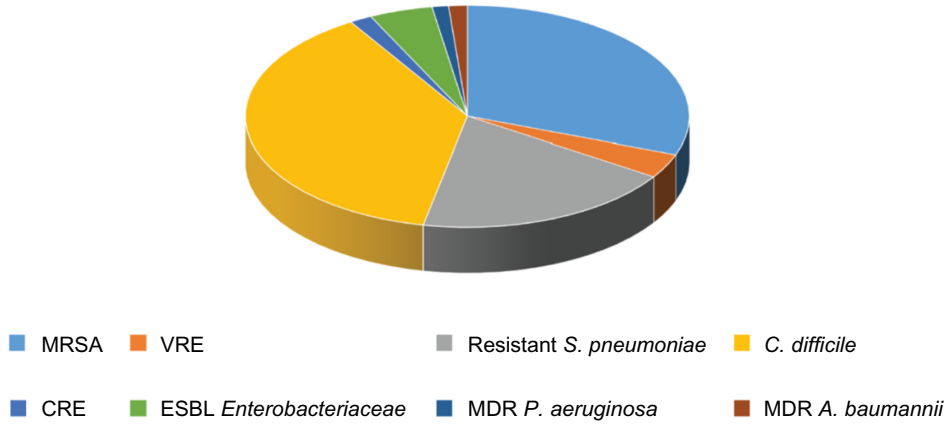


Figure 1. Deaths caused by select bacteria in the United States.

in its cell wall, it is classified as gram-positive because of the lack of an outer membrane present in gram-negative bacteria. This atypical cell wall protects it from macrophage digestion and gives it an inherent resistance to many antibiotics.[88,89] An estimated one third of the world's population is infected with latent TB. Many will not have the disease progress to an active state, however enough do to make TB unquestionably one of the greatest bacterial threats.[90] TB is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent with 1.3 million deaths from 8.6 million new infections in 2012 largely in developing countries.[90] MDR-TB, resistant to the first line combination therapy of rifamycin, isoniazid, and pyrazinamide is becoming fairly commonplace with about 450,000 people in the world developing cases in 2012. Of those cases 9.6% are estimated to be extensively drug resistant (XDR-TB), which is further resistant to at least one second-line fluoroquinolone and aminoglycoside.[90,91] XDR-TB sometimes requires a two-year course of antibiotics at a staggering average cost of $483,000 and can be fatal even with proper treatment.[3,4,92,93]

Resistant *Escherichia coli*

E. coli are gram-negative, facultative anaerobes that are most commonly commensal, but can also be pathogenic. Pathogenic strains can produce potentially deadly toxins including enterohemmorhagic verotoxin (Shiga-like toxin), which causes hemolytic-uremic syndrome and renal failure.[94] This toxin was originally gained from a prophage.[95]

Traditionally *E. Coli* has been one of the most widely antibiotic susceptible of the *Enterobacteriaceae* family. Recently, though, horizontal gene transfer has allowed for the rise of highly resistant strains.[96] *E. coli* resistance is worrying because they are the most common gram-negative bacterial infections in humans and occurrence of strains with extended spectrum β-lactamases (ESBLs) conferring resistance to third generation cephalosporins has been steadily rising in Europe.[5] ESBL positive strains in bacteraemias have also shown high cross resistance to fluoroquinolones (>80%) and gentamicin (**25**) (>40%).[96] Although still fairly uncommon, *E. coli* on multiple continents have also acquired the New Delhi Metallo-β-lactamase-1 (NDM-1) enzyme from *K. pneumoniae*, which confers a broad resistance to all β-lactams including carbapenems with the exception of the monobactam, aztreonam (**18**).[97–100] Fluoroquinolone resistance is also common among *E. coli*.[101,102] Bacteria overexpressing *FomA* and *FomB* plasmidic genes are capable of inactivating fosfomycin through phosphorylation.[103] *E. coli* are also the most commonly zoonotic pathogens discussed herein. *E. coli* O157:H7, an enterohemmorhagic strain, has been associated with many zoonotic outbreaks and incidences of food borne illness including a 1999 outbreak in the US that infected at least 127 people.[104] Another enterohemmorhagic strain, *E. coli* O104:H4, infected over 3,800 people in Germany in 2011 causing 54 fatalities.[105]

MDR *Pseudomonas Aeruginosa*

P. aeruginosa is a gram-negative, facultative anaerobic, opportunistic pathogen. It is the most common cause of chronic lung infections in cystic fibrosis (CF) patients.[106] These strains are frequently highly resistant and it is no longer uncommon to see CF related infections that are resistant to all antibiotics except polymyxins.[107,108] *P. aeruginosa* employs a type III secretion system to extrude a host of potent cytotoxins directly into host cells.[109] It has a high environmental tolerance especially with respect to nutritional requirements and has been known to survive in such diverse environments as jet fuel and disinfectant.[110] *P. aeruginosa* naturally has a host of siderophores (Fe^{3+} carriers) and pigments that allow it to evade the innate immune system. Additionally it has particularly discriminating outer membrane porins that make its outer membrane impermeable and thus naturally resistant to many antibiotics, and a high propensity to form biofilms that can increase resistances to antibiotics 100 to 1000 fold.[111,112]

Further antibiotic resistance occurs thorough a wide variety of mechanisms. Some strains have acquired a variety of β-lactamases including ESBLs, *K. pneumoniae* carbapenemase (KPC), and metallo-β-lactamases (MBLs).[113] *P. aeruginosa* also has an extremely comprehensive efflux pump

systems. Mutations resulting in loss of the OprD porin coupled with upregulation of MexEF-OprN efflux pumps result in resistance to carbapenems and fluoroquinolones. MexCD-OprJ upregulation also results in resistance to fluorquinolones and some β-lactams. MexAB-OprM upregulation confers resistance to sulfonamides, β-lactams, cephalosporins, fluoroquinolones, macrolides, novobiocin, tetracycline (**39**), chloramphenicol (**30**), and some detergents. MexXY-OprM results in aminoglycoside efflux.[114] Fluoroquinolone resistance can also occur through DNA gyrase and topoisomerase IV mutations. While rare, mutations to both are found in many persistent infections.[115] Pan-resistant *P. aeruginosa* susceptible only to polymyxins strains have been isolated. In one case the isolated strain produced AmpC β-lactamases, decreased OprD porin expression, and upregulation of MexXY efflux.[116] Another strain produced an MBL, AmpC β-lactamase, and two aminoglycoside acetylating enzymes (AACs).[117]

MDR *Acinetobacter*

Resistant *P. aeruginosa* and *Acinetobacter* could be especially dangerous in the long term because of their intrinsic resistance to some gram-negative antibiotics and their ready acquisition of DNA from other bacteria ensuring the spread of additional resistances.[111,118] In a survey of select European countries they currently have the highest resistance rates to both carbapenems and aminoglycosides, two traditionally last resort antibiotics.[101,102] *P. aeruginosa* resistance has been stabilizing in the US, while unfortunately *Acinetobacter* resistance has been increasing.[119] The mortality rate for the latter is notably higher as well.[120]

The most common resistant *Acinetobacter* species is *A. baumannii*, a gram-negative, facultative anaerobic, opportunistic pathogen. This bacterium is also colloquially referred to as "Iraqibacter" because of its rapid emergence as a problem pathogen in wounded soldiers during the Iraq war. Many gram-negative bacteria are known for environmental persistence and *Acinetobacter* are particularly adept. With especially thick cell walls that protect them from dry conditions and high tolerance to temperature, pH, and nutrient changes, they are capable of surviving for up to 5 months on inanimate objects.[121–123] *A. baumannii* is naturally resistant to many antibiotics due to both poor membrane penetration and active efflux pumps as well.[121] Overexpression of the AdeABC efflux pump causes broad resistances to cephalosporins, fluoroquinolones, and tigecycline (**46**), the first member of a new tetracycline subclass called glycylcyclines. The AbeM efflux pump leads to aminoglycoside and fluoroquinolone resistance.[124] More specific efflux pumps, Tet(A) and Tet(B) for tetracyclines and CmlA for chloramphenicols also exist.[96a] Further hindering uptake, 30% of *A. baumannii* isolates produce an exopolysaccharide capable of forming biofilms.[125] It also expresses a powerful, epithelial cell targeting cytotoxin that aids in its colonization.[126]

MDR *A. baumannii* has two main modes of resistance. The first is the aforementioned efflux pumps, which also impart resistance to ammonia based disinfectants.[121] The second is a variety of β-lactamases including ESBLs and carbapenemases including imipenem MBLs and oxacillinases (OXAs).[127] These antibiotic resistances factors coupled with *Acinetobacter* natural resistances have combined to produce *A. baumannii* strains with resistance to all known antibiotics including colistin (**71**).[121] MDR *Acinetobacter* are also already particularly common with greater than 60% of all hospital acquired *Acinetobacter* strains resistant to multiple drugs including carbapenems.[128] Moreover this resistance emerged over a remarkably short time period with a greater than 30% increase in carbapenem resistant *A. baumannii* strains from 1995 and 2004, which coincides closely with the rapid spread of OXAs.[129] During the same time period resistance to the aminoglycoside amikacin (**28**), the quinolone ciprofloxacin (**59**), and the β-lactam / β-lactamase inhibitor combination piperacillin (**9**)-tazobactam (**21**) also increased steadily.[124] MDR *Acinetobacter* and *Klebsiella* are so dangerous that their outbreaks have resulted in hospital ward closures on multiple occasions.[130,131]

MDR and Pan-drug-resistant *Klebsiella pneumoniae*

K. pneumoniae is a gram-negative, facultative anaerobic, primarily opportunistic bacterium that can be nosocomial or community acquired. Community acquired *K. pneumoniae* most commonly causes pneumonia, like *S. pneumoniae*, there are a variety of other pathologies it can cause as well and only about 5% of pneumonia cases are caused by *K. pneumoniae*. This bacterium has a thick polysaccharide capsule that acts as an antiphagocytic factor,[132] and was the first species that *qnr* quinolone resistance genes were isolated from.[133]

This species also commonly acquires MDR determinants, and in particular an impressive array of β-lactamases. Most worrying are ESBLs, KPC, and most recently NDM–1. The latter two have caused multiple epidemics and even more troublingly are capable propagation to other species.[97,127,134] Carbapenem resistances are a serious problem since carbapenems were highly resistant to most other β-lactamases prior to the advent of KPC and were often used as drugs of last resort for serious gram-negative infections.[113] NDM-1 genes have commonly been found on plasmids and since the first identification of NDM-1 in 2007, producer strains have quickly spread around the world.[87,100] NDM-1 producing strains are typically highly resistant with other resistance mechanisms commonly including ESBLs, AAC AMEs and ribosomal methylases for aminoglycoside resistance, and fluoroquinolone resistant topoisomerase mutations, among others. The majority of these strains are only susceptible to the glycylcycline tigecycline (**46**) and the polymyxin colistin (**71**).[87,135] Pan-resistant strains have even been reported.[136] Though NDM-1 producing strains have so far remained relatively rare,[100] their rapid globalization coupled with their extreme resistance profiles warrants close monitoring in years to come.

β-lactam and Quinolone Resistant *Enterobacter*

Enterobacter is a genus of gram-negative, facultative anaerobic, opportunistic pathogens. They are mainly known to exhibit

antibiotic resistance through expression of an extensive variety of ESBLs and carbapenemases including, KPC, OXA, and several MBLs.[137] They are also the pathogen to most commonly have *qnr* quinolone resistance genes at over 30% occurrence in isolates.[138] Their outlook is less grim then some of the aforementioned pathogens, however. Colistin (**71**), tigecycline (**46**), amikacin (**28**), and some fluoroquinolones remain effective treatment options even for most MDR strains.

Resistant *Neisseria Gonorrhoeae*

N. gonorrhoeae is a gram-negative, aerobic, fastidious, sexually transmitted pathogen. It has several methods of avoiding the immune system including Opa proteins that bind immune cell receptors to prevent a response and antigenic variation, which prevents the host from developing immunological memory against them.[139] Like *E. coli*, *N. gonorrhoeae* has traditionally been an easy to treat pathogen, but progressive accumulation of resistance mechanisms has gradually led to highly resistant strains. Penicillin and ciprofloxacin resistances acquired by plasmid, are now widespread, with resistances to the commonly used macrolide azithromycin (**32**) and some cephalosporin becoming increasingly common.[140,141] High level tetracycline resistance via TetM efflux proteins is also common.[142] Most recently a MDR *N. gonorrhoeae* strain with high level resistances to the third-generation cephalosporins cefixime and ceftriaxone has been identified as well.[128] MDR *N. gonorrhoeae* is particularly worrying as this bacterium is community acquired and very commonly pathogenic, infecting 700,000 per year in the United States alone.[3,4]

Hospital Acquired Infections

Bacteria are responsible for approximately 90% of all HAIs.[128] HAIs are a major problem in the industrialized world having 5% and 7.1% incidence rates in the US and the EU, respectively.[143,144] In developing countries, where sterile practices are less stringent, the problem is much worse, with an estimated 15.5% incidence.[144] Additionally, the often immunocompromised patients that these infections target obviously have higher mortality rates than those with healthy immune systems. The risk of fatality associated with infections caused by resistant bacteria as compared to antibiotic sensitive bacteria is much higher as well. In most cases this is not because of any increased virulence, but rather because of prolonged bacterial exposure due to delayed, or a lack of an appropriate therapy.[6,145]

The Search for New Antibacterial Agents

The number of new antibacterial agents has decreased steadily in the United States over the last several decades.[8,146] Historically there has been a higher chance of success with the development of compounds that belong to already established antibiotic classes.[147] Developmental risks are lower because of already proven microbiological assays to determine efficacy, known toxicological issues, and established regulatory routes for approval.[148] Some scaffolds have been used particularly extensively. Between 1981 and 2005 cephalosporins, penicillins, quinolones, and macrolides accounted for 73% of all new antibiotics.[149]

There is also a lack of diversity in the cellular target of all known antibiotics. Almost all clinically used antibiotics inhibit DNA, RNA, protein, or cell wall synthesis, and there are less than twenty-five molecular targets that account for their activity. Approximately half of all antibiotics target the cell wall.[150] In some cases structurally distinct antibiotics, even from separate gene clusters, are known to bind the same sites.[151,152] Comparative analysis of bacterial genomes has indicated that there are around 300 essential, highly conserved proteins that could potentially be new, broad spectrum drug targets.[153–157] Though studies have recently begun to identify many antibacterial agents with novel molecular targets, activity is insufficient for many of these to be developed without further modification.

The development of new antibiotics in existing classes is an absolutely essential exercise that has been encouraged even by the IDSA, a principal entity in the push for new scaffold development.[11] However, new antibiotics that conform to established classes are often subject to at least some of the same resistances observed in previous members of the class. It is therefore also necessary to develop new antibiotic classes. There have only been six first in class antibiotics with totally novel scaffolds approved since the 1960s and all of these have been introduced in the past fifteen years, a thirty year innovation gap (Fig. 2). It is worth noting that all of these were developed to combat gram-positive pathogens including *M. tuberculosis* and they all have very little or no activity against gram-negatives. The innovation gap remains for novel antibiotics with potent gram-negative activity.

The new first in class antibiotics introduced for human use are the streptogramin combination quinupristin (**69**) / dalfopristin (**70**) in 1999, the oxazolidinone linezolid (**74**) in 2000, the lipopeptide daptomycin (**79**) in 2003, the pleuromutilin retapmulin (**81**) in 2007, the macrolactone fidaxomicin (**83**) in 2011, and the diarylquiniline bedaquiline (**84**) in 2012. Linezolid (**74**) and bedaquiline (**84**) are fully synthetic molecules while the others are natural products. Though they were only recently developed for approval these molecules or their leads were all discovered much earlier with the exception of the diarylquinolines. Streptogramins were discovered in the 1960s, the leads for linezolid in the 1970s, daptomycin (**79**) in the 1980s, pleuromutilins were isolated in the 1950s, and macrolactones similar to fidxomicin were found in the 1970s.[12]

The early successes of many of these newer antibiotic classes suggest that scaffolds originally discarded during the heyday of antibiotic discovery because of liabilities such as narrow activity spectrum, like fidaxomicin (**83**) and bedaquiline (**84**), or higher toxicity, like the recently resurrected polymyxins, may need to be revisited given the desperate situation we are experiencing. It has even been argued that species-specific antibiotics may offer some significant advantages.[62]

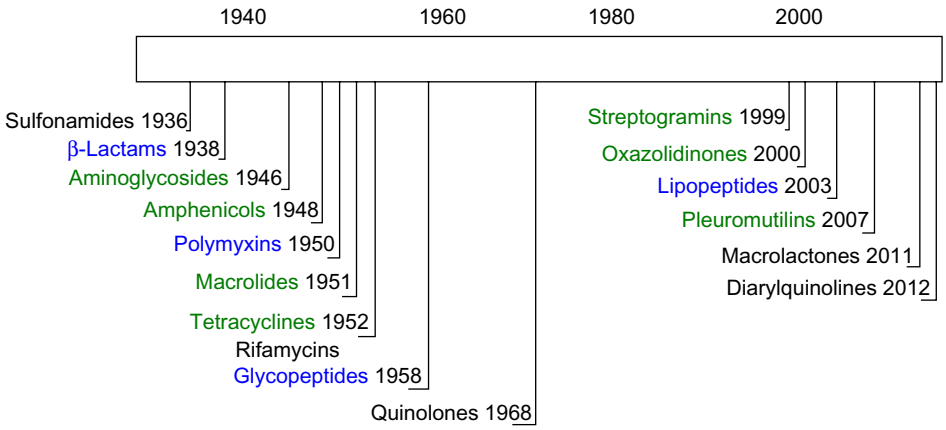


Figure 2. Timeline of first clinical introduction of antibiotic classes.
Notes: Classes targeting the cell wall or membrane are highlighted in blue. Classes targeting the ribosome are highlighted in green.

Their cellular targets are less likely to overlap with those of eukaryotic cells or mutualistic gut bacteria. Also, resistance will likely be slower to develop, as resistance genes would likely have to originate in the target species since there would be no evolutionary pressure to produce resistance determinants in bacteria that are naturally resistant to the compound.

In response to this lack of innovation the IDSA issued the 10 × '20 initiative in 2010 that calls for the development of 10 novel, effective antibiotics by 2020.[158] A 2013 update showed little progress towards that goal though and in a 2011 survey of all potential antibiotics in clinical trials only two out of the twenty one had totally novel scaffolds.[11,12] There is a particular need for antibiotics effective against gram-negative pathogens as they comprise most of the currently emerging threats and the majority of recently developed antibiotics are not effective against them.[5,6] According to the IDSA, of the drugs currently in clinical trials there is in particular a deficiency in antibiotics that have good activity against MBL producing gram-negatives and broad activity against *Acinetobacter* strains.[11]

Semi-synthetic Approaches

Improved biophysical techniques have garnered a wealth of information about cellular targets and binding modes of many established antibiotics frequently making the rational design of semi-synthetic analogs of natural products a fruitful exercise. Precursor directed biosynthesis, mutasynthesis, and chemoenzymatic approaches are also increasingly being investigated to diversify certain established scaffolds.[159] These modifications, implemented to circumvent bacterial resistance mechanisms, have allowed many scaffolds to continue to be useful long after clinical resistance has become predominant to early members of the classes.

The majority of the antibiotics introduced within the last 30 years are semi-synthetically derived. There are now semi-synthetic members of most antibiotic classes that are founded on natural products and there are many examples of highly utilized or extremely promising antibiotics that are semi-synthetically derived. These include the β-lactams meropenem (**14**) and tazobactam (**21**), the aminoglycoside amikacin (**28**), the macrolide azithromycin (**32**), the tetracycline tigecycline (**46**), the rifamycin rifampicin (**47**), the glycopeptide telavancin (**53**), and the streptogramin combination quinupristin (**69**) / dalfoprisitin (**26**).[149]

Synthetic Development

Given the length of time bacteria as a whole have likely had to develop resistance to many natural product antibiotics coupled with the apparent ease with which many resistance genes are disseminated, developing totally synthetic antibiotics would appear to be an attractive strategy. To date, however, synthetic antibiotics are still extremely rare with the sulfa drugs, quinolones, oxazolidinones, and diarylquinolines being the only examples. They are outnumbered two to one by natural product antibiotics and their semi-synthetic derivatives,[42,160] with development over the past few decades focused especially on the latter.[149] Historically all synthetic classes, with the exception of diarylquinolines, were originally discovered outside of traditional antibiotic discovery programs. Sulfa drugs were originally developed as dyes, the first quinolone was an intermediate in the synthesis of chloroquine, a malaria drug, and oxazolidinones were originally developed to treat foliage diseases in plants.[62]

Without a doubt one of the greatest challenges to finding new synthetic scaffolds is the issue of bacterial cell penetration. This is particularly true of gram-negatives, which are naturally resistant to many antibiotics because of outer membranes that keep many amphipathic drugs out as well as inner membranes and highly active efflux pumps that often recognize highly hydrophilic molecules.[161,162] The difficulties of prokaryotic uptake often mean that antibiotics have to be administered at concentrations two to three orders of magnitude higher than therapeutics prescribed for most other diseases. This can impact the therapeutic window and lead to additional toxicity concerns.[163]

The majority of antibiotics do not strictly adhere to the Lipinski rules, a series of soft rules governing the likelihood of a compound's oral bioavailability and "drug like" character. In fact, several major antibiotic classes routinely break all of them. Notably, these rules were designed in the context of treating eukaryotic maladies. The establishment of a similar set of rules for antibacterials would greatly aid in antibiotics rational design and in the formation of compound libraries better suited for antibiotic screening purposes. There are no rules that have been routinely applied as of yet; however, some insights have started to be noted. Relatively hydrophilic compounds with masses below 600 Da tend to have good penetrance probably because of their ability to pass through outer membrane porins.[164,165] MDR efflux pumps tend to recognize cations and hydrophobic compounds particularly well, whereas anions are generally not recognized.[166–168] The inclusion of atoms not usually found in nature like boron and fluorine have had successes, possibly again because of efflux pump evasion.[169] Fluoroquinlones, at this point probably the most successful fully synthetic antibiotics, adhere to all of these observations.

Screening for synthetic leads has not conventionally been a successful method of discovery. Major high throughput synthetic screens and rational design campaigns of synthetic molecules have failed utterly in many cases to identify a single antibiotic.[170] The wide spread failure of cell free target based screens in particular, which were an industry standard, has been implicated as one of the primary reasons for many major pharmaceutical companies movement away from antibiotics development.[170–171] Most scaffolds found at major pharmaceutical companies are optimized more for human eukaryotic targets and are not up to the disparate challenges associated with prokaryotic cellular uptake and evasion of rampant bacterial efflux mechanisms, particularly in gram-negative bacteria. Therefore target based screens of synthetic molecules will often lead to hits with high potency, but no real world utility.[43, 62] This is a drawback that rational design of synthetic molecules suffers from as well.[163] Whole cell screens do not suffer from this disadvantage though.

Taking note of fact that all current synthetic antibiotics were originally discovered for other purposes, whole cell screens of libraries originally created by non-antibiotics programs have been done recently. These have been used to identify some promising new leads.[172,173] Many hits on whole cell screens may exhibit narrow or even genus specific activity, as in the case of bedaquiline (**84**) though, which notably is the only synthetic, clinically approved antibiotic to our knowledge that was discovered by high throughput screens actually designed to identify antibiotics.[163] For some particularly hard to treat pathogens this may be acceptable at this point though.

Taking whole cell screens a step further, in vivo screens have also gained some interest with the rationalization that metabolically activated prodrugs, like sulfonamides, may be overlooked in traditional in vitro screens. Using animal models would of course be prohibitive in any large screening process for obvious reasons. However, *Caenorhabditis elegans*, a nematode, can be infected with human pathogens to making it a passable model organism for in vivo screens. Screens using *C. elegans* have had hits, including some that have no in vitro activity.[174,175]

Diversity oriented synthesis based approaches have been used to create promising totally synthetic molecules that more closely mimic microbial natural products.[176] Combinatorial chemistry can be done to create libraries around known privileged scaffolds. Another approach is to do unbiased diversity oriented synthesis. This approach, coupled with subsequent SAR, has been used to find promising antibiotic candidates.[177,178]

Natural Product Development

Natural products are a historically successful and still a very much viable option as new antibiotics. During the "golden age" of antibiotics many of the current antibiotic classes were discovered by systematic screening methods of *Streptomyces* introduced by Selman Waksman in the 1940s. There is reason to believe that many natural products are still as yet undiscovered. One recent estimate puts the number of discovered antibiotics as only 10% of the total from screened bacterial strains and only 1% from all microbes.[179] Approximately two thirds of natural product antibiotics are isolated from terrestrial soil actinomycetes.[180,181] Multiple classes of antibiotics are even known to be encountered within the same gene clusters.[182] Finding a useable antibiotic in the milieu of compounds produced by these organisms is no easy feat though, especially given that the most commonly produced antibacterial molecules for these particular bacteria have all likely been already identified. It was recently estimated that with current technology 10^7 actinomycete strains would have to be screened to discover the next novel antibiotics class.[49] Given that a strain collection at a large pharmaceutical company may be around 50,000 isolates, this is no longer a feasible approach.[163]

Exploration of bacteria from other ecological niches has recently yielded many promising new lead compounds, however. The producers of these include deep sea sediment actinomycetes,[183] marine sponges and seaweeds (though these seem to actually be made by colonizing bacteria),[184,185] bacterial symbionts of insects, ascidians, fungi,[186–189] and myxobacteria.[190]

With the colossal advances in gene sequencing technology within last several decades, genomic prospecting has also begun. Genomic sequencing has in several cases identified silent operons that code for secondary metabolites within *Streptomyces*, some of which are not currently known to produce antimicrobial compounds.[191,192] The proper conditions for realizing expression of these potential antibiotics in cultures can be difficult as antibiotic production may depend on a variety of factors including proper concentration of quorum sensing factors, which may be difficult to replicate.[193–195] Methods of

manipulating these silent operons represent an active area of research. These approaches have thus far never realized more than the identification of several lead compounds per year though.[192, 196–199]

Natural products screens have been touted over synthetic molecule screens both for the obviously superior number of compounds available and the fact that natural products have already been "prescreened" by evolution.[163] It has traditionally been an often used approach, with whole cell empirical screening being the method of discovery of the majority of antibiotics used today.[171] Whole cell screening does not aid in identification of mode of action, however, and this approach can be expensive. It is made even more so in the realm of natural products screening, particularly for antibiotics, as many antibiotic producing bacteria are difficult to culture (an estimated 99% of microbial species are uncultured).[200,201]

Even more importantly, in current screens, many hits are actually previously discovered compounds. This is because of the pervasiveness of lateral transfer of antibiotic producing genes amongst terrestrial soil bacteria. One study estimated that 1 in 100 actinomycete*s* produce streptomycin (**24**), 1 in 250 tetracycline (**39**), 1 in 66,000 vancomycin (**52**), and 1 in 200,000 erythromycin (**31**).[49] This phenomenon is not strictly limited to actinomycetes either.[202–206] Some members of the same antibiotic subclass can even be produced by extremely disparate organisms. Cephalosporins, for example, are produced by actinomycetes, proteobacteria, and fungi.[207,208] Several methods have been developed to alleviate the problem of rediscovery. One strategy is to use strains resistant to commonly "rediscovered" antibiotics in the screening process. This has been done with wild type MRSA and with MDR *E. faecium*, which led to the discovery of many new promising compounds.[209,210]

Target based natural products screens offer a useful counterpoint to whole cell screening. Target based screens do not suffer from all the drawbacks that these screens have when applied to synthetic molecules. Recently, several bioinformatics based, genome screening approaches have been used with some success.[211–213] It was previously mentioned that through genomic screening it has been estimated that there are hundreds of potential broad spectrum targets that no antibiotics have ever been developed for. Screens developed for these targets have the advantage that there are no false positives caused by already discovered antibiotics. Also, drugs developed for these targets may have less initial bacterial resistance than targets that already have selective pressure from many current antibiotics.

Hybrids of whole cell and cell free target based assays now exist as well. Whole cell reporter assays or whole cell target-based assays employ either cells or conditions that are engineered to report specific molecular events at sub-bactericidal concentrations, unlike traditional whole cell screens that simply look at cell death upon introduction of a compound or isolate. Antisense technology has also been used successfully to identify promising natural products. One particularly interesting discovery made using these techniques was platensimycin and other potent fatty acid biosynthesis inhibitors.[214]

A History of Established Antibiotic Classes

The following sections present profiles of the major established classes of antibiotics with a focus on new or exceptional

Table 2. Antibiotics of the 21st century.

CLASS	CLINICALLY INTRODUCED SINCE 2000	IN PHASE II OR III TRIALS
Sulfonamides	None	None
β-lactams	Biapenem (**16**), ceftaroline (**11**), doripenem (**15**), ertapenem	Ceftobiprole, ceftolozane (**12**), razupenem (**17**)
Aminoglycosides	None	Plazomicin (**29**)
Amphenicols	None	None
Macrolides	Telithromycin (**35**)	Cethromycin (**36**), solithromycin (**37**)
Tetracyclines	Tigecycline (**46**)	Eravacycline (**44**), omadacycline (**45**)
Rifamycins	Rifaximin (**51**)	None
Glycopeptides	Telavancin (**53**)	Dalbavancin (**56**), oritavancin (**54**), ramoplanin (**57**)
Quinolones	Balafloxacin, gemifloxacin, pazufloxacin, prulifloxacin	Avarofloxacin (**65**), delafloxacin (**63**), finafloxacin (**66**), JNJ-Q2, levonadifloxacin, nemonoxacin (**64**)
Streptogramins	None	None
Polymyxins	None	None
Oxazolidinones	Linezolid (**74**)	AZD5847 (**78**), radezolid (**76**), sutezolid (**77**), tedizolid (**75**)
Lipopeptides	Daptomycin (**79**)	Surotomycin (**80**)
Pleuromutilins	Retapmulin (**81**)	BC-3781 (**82**)
Macrolactones	Fidaxomicin (**83**)	None
Diarylquinolines	Bedaquiline (**84**)	None

members. What are thought to be emerging classes are also discussed even though some may only have one currently clinically approved member. Discussion of many structurally unique antibiotics has been avoided for brevity. Promising antibiotics in clinical trials are also discussed with a focus primarily on phase II and III candidates (Table 2).

Sulfonamides

Sulfonamides are a structurally diverse class of antibiotics that all have an aryl sulfonamide moiety in common (Fig. 3). The first sulfonamide discovered was prontosil (**1**) in 1932.[163] Sulfonamides were first used clinically in 1936. They are synthetic antimetabolites that inhibit dihydropteroate synthetase, an enzyme totally absent human cells used in folic acid metabolism. Inhibition of this enzyme ultimately leads to repressed DNA replication and bacteriostatic activity against aerobic gram-positive and negative bacteria. Due to their broad spectrum activity sulfonamides were once popular antibiotics.[215] Increases in resistance, allergic reactions, and rare, but serious side effects including Stevens-Johnson syndrome and blood dyscrasias led to declines in their use many years ago.[216] However, interest has recently been rekindled in the use of sulfamethoxazole (**2**) in a combination therapy with trimethoprim (**3**), a compound that inhibits DNA replication by binding dihydrofolate reductase, another enzyme involved in folic acid metabolism. Trimethoprim (**3**) has been found to have an up to 100 fold synergistic effect in combination with sulfonamides.[42] This combination has good activity against some MRSA strains and evidence has suggested that resistance has actually decreased to these compounds in recent years likely because of many years of infrequent usage.[217,218]

β-lactams

β-lactam antibiotics are diverse in their structure, but they share a common four-membered β-lactam ring, which functions as the active pharmacophore for this class. The first β-lactam antibiotic to be discovered was benzylpenicillin (penicillin G) (**4**) in 1928 though it wasn't used clinically until 1938.[163] β-lactams are the class with by far the most FDA approved members. They are also the most populous class on the WHOs list of critically important antibiotics to human medicine. There are 28 members, including antibiotic/β-lactamase inhibitor combinations, from three subclasses: penicillins, cephalosporins, and carbapenems that are listed as critically important (Table 3).[219] They exhibit antibacterial activity by acting as suicide substrates for penicillin binding proteins (PBPs) (transpeptidases) inhibiting cell wall biosynthesis, specifically maintenance of peptidoglycan. This leads to cell stress responses that result in cell lysis.[220] Many currently used β-lactams have very broad spectrum activity against most aerobic and anaerobic gram-positive and negative bacteria as well as low toxicity profiles making them popular first line antibiotics.[42] Resistance to older members of this class, especially the penicillin subclass, has proliferated dramatically though.

Resistance usually occurs via hydrolysis of the β-lactam ring mediated by a wide range of β-lactamases. These enzymes have been divided into four classes by the Ambler classification system: class A (KPCs and most ESBLs), class B (MBLs), class C (AmpC β-lactamases), and class D (OXAs). Class A includes many enzymes that can hydrolyze penicillins and cephalosporins as well as some that can hydrolyze monobactams and KPCs that are capable of hydrolyzing carbapenems.[221] The ESBLs from this class are plasmid mediated which has aided in their intra- and interspecies diffusion.[222] MBLs use divalent cations such as zinc as cofactors. Many are encoded in class 1 integrons, typically on gene cassettes also coding for aminoglycoside modifying enzymes (AMEs), found on transposons facilitating their spread.[223] MBLs inactivate many β-lactams including carbapenems and there are no currently improved inhibitors for them, but they have no activity against aztreonam (**18**), a monobactam.[224] AmpC β-lactamases are typically chromosomally encoded. AmpC and other class C β-lactamases can inactivate many β-lactams including aztreonam (**18**) with preferential activity against cephalosporins, but they have no activity against carbapenems.[221,225] Many OXAs are encoded on integrons.[226–229] Class D which is solely comprised of OXAs can hydrolyze cephalosporins and aztreonam (**18**) and some have carbapenemase activity as well.[221,230] Though their activity isn't as great as MBLs they are the most commonly found β-lactamase in *Acinetobacter*, which makes them particularly problematic.[222]

Altered PBPs, especially in *Streptococci*, also occur.[231] Methicillin (**5**) and other β-lactam resistances in MRSA are caused by production of low affinity PBP2a in greater than 90% of isolates.[232] Likewise in *S. pneumoniae* resistance to β-lactams is commonly caused by expression of a variety of

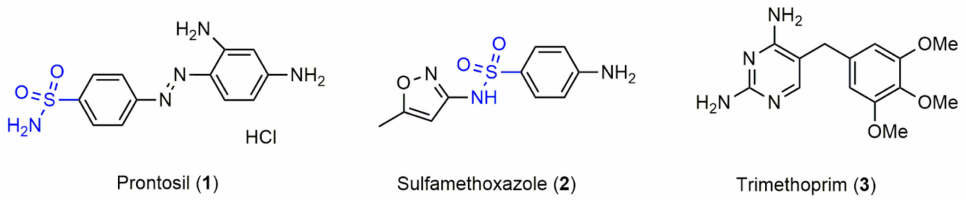


Figure 3. Sulfonamides and trimethoprim (**3**).
Note: The sulfonamide moiety is highlighted in blue.

Table 3. Critically important antibiotics.

CLASS OR SUBCLASS	PRIMARY TARGET	WHO CRITICALLY IMPORTANT MEMBERS [219]
Penicillins	Penicillin binding proteins	Penicillin G (**4**) and V, ampicillin (**7**), ampicillin (**7**) / sulbactam (**22**), amoxicillin (**8**), amoxicillin (**8**) / clavulanate, piperacillin (**9**), piperacillin (**9**) / tazobactam (**21**), azlocillin, carbenacillin, mezlocillin, ticarcillin, ticarcillin / clavulanate
Cephalosporins	Penicillin binding proteins	Cefixime, cefotaxime, cefpodoxime, ceftazidime, ceftizoxime, cefoperazone, cefoperazone / sulbactam (**22**), ceftriaxone, cefepime, cefpirome, cefoselis
Carbapenems	Penicillin binding proteins	Ertapenem, faropenem, imipenem (**13**), meropenem (**14**)
Aminoglycosides	30S ribosomal subunit	Amikacin (**28**), arbekacin, gentamicin (**25**), netilmicin, tobramycin (**27**), streptomycin (**24**)
Macrolides	50S ribosomal subunit	Azithromycin (**32**), clarithromycin (**33**), erythromycin (**31**), midecamycin, roxithromycin (**34**), spiramycin, telithromycin (**35**)
Tetracyclines	30S ribosomal subunit	Tigecycline (**46**)
Rifamycins	RNA polymerase	Rifabutin (**49**), rifampin, rifaximin (**51**)
Glycopeptides	Peptidoglycan units	Teicoplanin (**55**), vancomycin (**52**)
Quinolones	Topoisomerase II and IV	Cinoxacin, nalidixic acid (**58**), pipemidic acid, ciprofloxacin (**59**), enoxacin, gatifloxacin, gemifloxacin,levofloxacin (**60**), lomefloxacin, moxifloxacin, norfloxacin, ofloxacin, sparfloxacin
Streptogramins	50S ribosomal subunit	Quinupristin (**69**) / dalfopristin (**70**), pristinamycin (**67** / **68**)
Oxazolidinones	50S ribosomal subunit	Linezolid (**74**)
Lipopeptides	Cell membrane	Daptomycin (**79**)

low affinity PBPs.[42] Efflux by RND and ABC efflux pumps,[233] and outer membrane impermeability[234] can also cause resistance to β-lactams.

Progressive generations of β-lactams have largely advanced through semi-synthetic modification. Within the penicillin subclass (Fig. 4) some early modifications were focused on increasing stability to early penicillinases through the attachment of bulky side chains as in the cases of methicillin (**5**) and oxacillin (**6**). Other modifications were made to increase spectrum activity, from penicillin G (**4**), which is comparatively narrow spectrum, especially against gram-negatives, as compared to other β-lactams. Examples of this include the aminopenicillins such as ampicillin (**7**) and amoxicillin (**8**), and ureidopenicillins like piperacillin (**9**). Despite dramatic proliferation of resistance to this class many penicillins remain important first line antibiotics.[42]

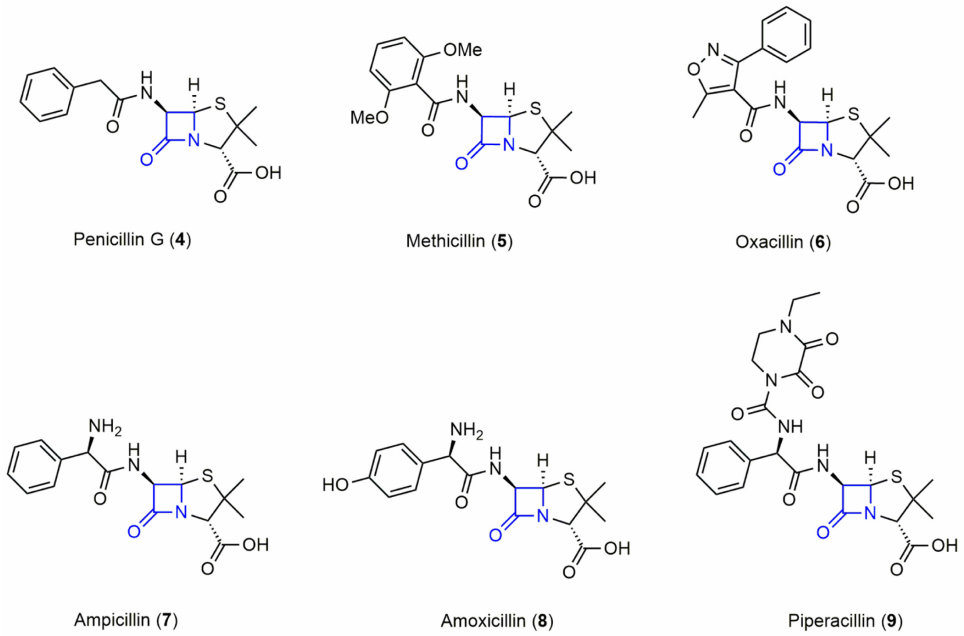


Figure 4. Penicillin subclass β-lactams.
Note: The β-lactam moiety is highlighted in blue.

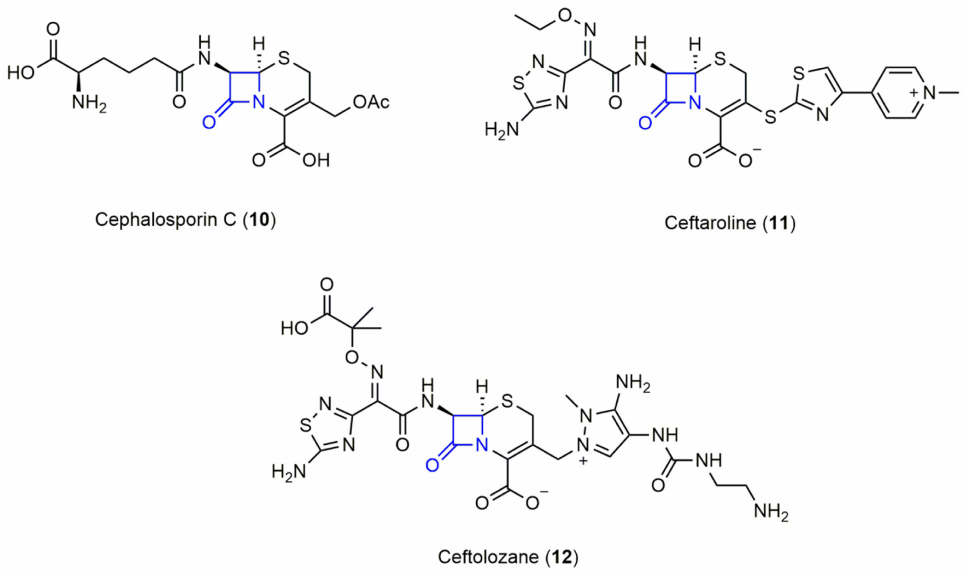


Figure 5. Cephalosporin subclass β-lactams.
Note: The β-lactam moiety is highlighted in blue.

The first cephalosporin was cephalosporin C (**10**), which was discovered in 1948 (Fig. 5). Early generations of semi-synthetic cephalosporins largely sought to improve pharmacokinetics and increase spectrum of activity against gram-negative pathogens primarily through increased cellular penetration. Later generations have become increasingly focused on combating β-lactam resistance.[42] Now in their fifth generation, excellent safety profiles and increased spectrum of activity have made modern cephalosporins some of the most highly utilized first line antibiotics.

Fifth-generation cephalosporin, ceftaroline (**11**), approved by the FDA in 2010, has shown increased activity against MRSA, but is no more potent against MDR gram-negatives, likely because it is still susceptible to most ESBLs.[235,236] However, it has shown promise in combination with the β-lactamase inhibitor, tazobactam (**21**), against many resistant strains though this doesn't prevent inactivation via MBLs.[221] Cubist's ceftolozane (**12**), in phase III trials, has shown complimentary activity. It has low activity against MRSA, but is active against many MDR gram-negatives including *E. coli* and *K. pneumoniae* strains, and superior activity against *P. aeruginosa* including strains with AmpC β-lactamases and upregulated efflux.[237] It will also likely be used in combination with tazobactam (**21**), which broadens their range of activity against ESBL producers.[238]

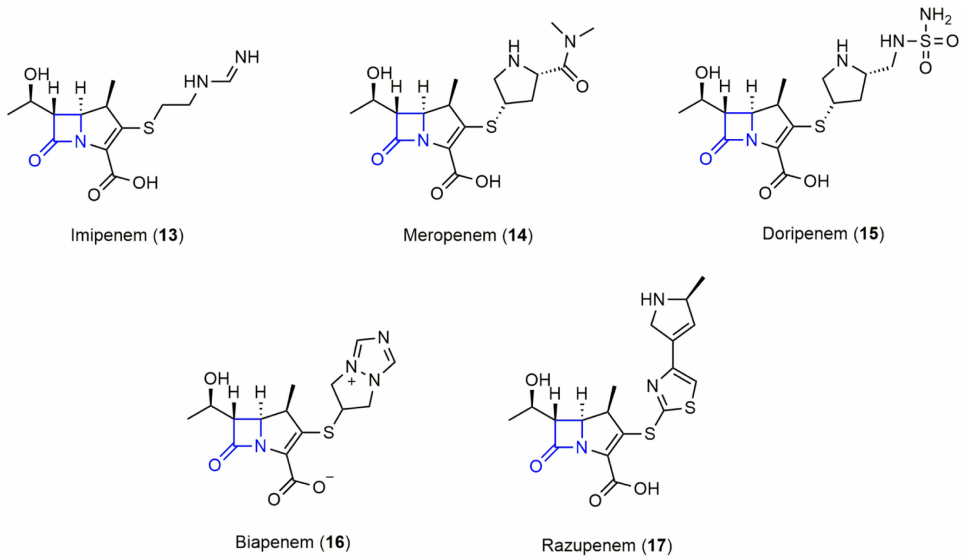


Figure 6. Carbapenem subclass β-lactams.
Note: The β-lactam moiety is highlighted in blue.

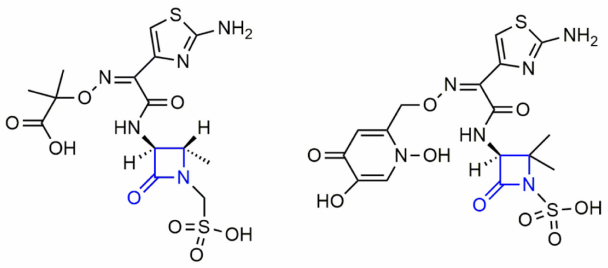


Figure 7. Monobactam subclass β-lactams.
Note: The β-lactam is moiety highlighted in blue.

Imipenem (**13**) was the first carbapenem to be identified in 1976 (Fig. 6). Carbapenems show enhanced activity against many anaerobic and gram-negative bacteria as compared to other β-lactams mainly because of their resistance to most ESBLs. Many are susceptible to the various carbapenemases that have more recently evolved though. Imipenem (**13**) and meropenem (**14**) resistance has also evolved in *P. aeruginosa* with loss of OprD porins and MexAB-OprM efflux upregulation.[222] Doripenem (**15**), approved in Japan in 2005 and the US in 2007, is impervious to certain KPCs and OXAs, but it is still susceptible to all MBLs.[239] It shows similar activity against most bacteria as imipenem (**13**) and is superior to other carbapenems against *P. aeruginosa*, but it notably lacks activity against MRSA.[58,240] Meropenem (**14**) and biapenem (**16**) (approved in Japan) have shown activity against some imipenem resistant, MBL producing *P. aeruginosa*.[241] Razupenem (**17**), a carbapenem in phase II trials, has shown promising activity against ampicillin resistant *E. faecium*.[242]

The first, and currently only FDA approved monobactam, aztreonam (**18**), was identified in 1981 (Fig. 7). Though it is useful against only gram-negative pathogens, it has the distinction of being the only β-lactam impervious to some of the most dreaded class B β-lactamases.[42, 224] Though only in phase I clinical trials, Basilea's BAL30072 (**19**) is a very promising monobactam, which shows superior activity against MBL producing *P. aeruginosa* and *Acinetobacter*, as well as many KPC producing *Enterobacteriaceae*.[243] It has also showed promising synergistic activity with meropenem (**14**) against *Acinetobacter*.[244]

Clavulanic acid (**20**), discovered in 1976, was the first identified β-lactamase inhibitor (Fig. 8). Augmentin, a combination therapy of clavulanic acid (**20**) and amoxicillin (**8**), is still one of the most successful antibiotics on the market. Piperacillin (**9**) and tazobactam (**21**) are also a popular combination therapy particularly against some *P. aeruginosa* infections including those producing ESBLs.[222] There has also been some renewed interest in sulbactam (**22**), which has been used successfully in the past with ampicillin (**7**) and has recently shown good synergistic activity in combination with meropenem (**14**) against a wide range of *A. baumannii* strains. Unfortunately this combination isn't yet clinically approved.[245] Avibactam (**23**) is a newer β-lactamase inhibitor with broad spectrum activity against class A, C, and D β-lactamases.[246] There are quite a few β-lactam inhibitors, both with and without β-lactam structures, that are clinically approved or in clinical trials in combination with β-lactam antibiotics. Many of these have activity against KPC, AmpC, and OXA β-lactamases.[247] There are very few with activity against MBLs, however, and none that are currently clinically approved.[248] Some combinations of inhibitors including ones that have siderophore activity have shown some promise against MBLs though.[221] Tricyclic competitive inhibitors of certain MBLs have also been isolated.[249]

Aminoglycosides

Aminoglycosides consist of amino-sugars connected through glycosidic bonds typically to a 2-deoxystreptamine (2-DOS) core (Fig. 9). The first aminoglycoside to be discovered was streptomycin (**24**) in 1943 and it was subsequently used clinically in 1946.[163] They target the 30S ribosomal subunit, most commonly the A-site within the 16S rRNA, leading to mistranslation of proteins. Some aminoglycosides are broad spectrum antibiotics with good activity against some aerobic gram-positive and most gram-negative species as well as *M. tuberculosis*. Their uptake is severely limited under certain anaerobic conditions, so their efficacy can be severely diminished for certain facultative or obligate anaerobes. They are notably the only class of translation inhibiting antibiotics that is broadly bactericidal. The precise mechanism of their bactericidal activity isn't fully understood.[215] Insertion of flawed membrane proteins has been implicated though and this is known to promote further aminoglycoside uptake.[250]

The aminoglycosides suffer from issues of nephrotoxicity and ototoxicity, which in most cases consigns them to the role of antibiotics of last resort rather than first line treatments. Resistance, particularly common in gram-negatives, has also developed through mechanisms including increased efflux by MexXY and ABC transporters, especially in *P. aeruginosa*, and

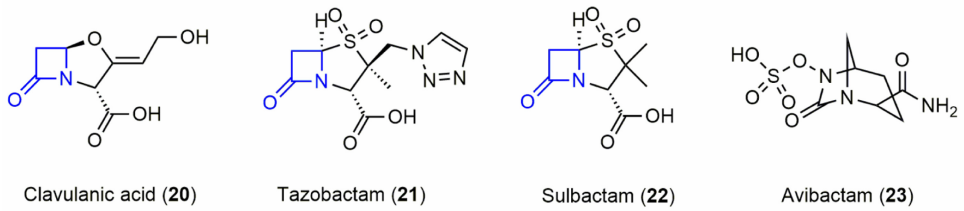


Figure 8. β-lactamase inhibitors.
Note: The β-lactam moiety is highlighted in blue.

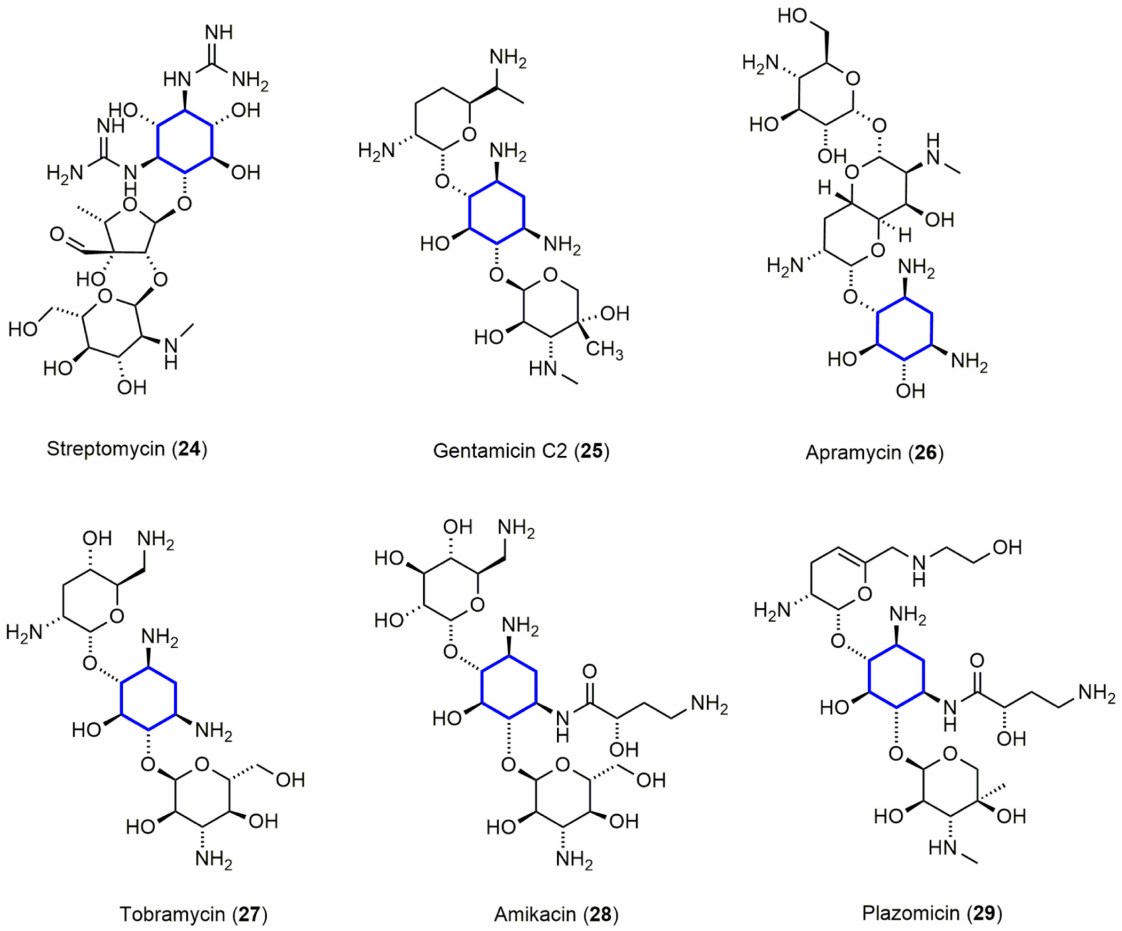


Figure 9. Aminoglycosides.
Note: The 2-DOS ring is highlighted in blue.

methylated ribosomal A-sites, which decrease target affinity. Rmt and Arm methylases methylate the N7 position of rRNA G1405 conferring resistance to gentamicin (**25**) and kanamycin subclasses of aminoglycosides. The less common NpmA enzymes methylate N1 of A1408 causing broad resistance to the aforementioned subclasses as well as the neomycin subclass and apramycin (**26**).[251–252] The most common resistance mechanism is AMEs, however, consisting of N-acetyltransferases (AAC), O-nucleotidyltransferases (ANT) and O-phosphotransferases (APH). These are often encoded on mobile genetic elements and are readily disseminated between bacterial species via lateral gene transfer.[225]

Streptomycin (**24**) was successfully used as a first line therapy for TB for many years until a mutation to the 30S ribosomal protein RpsL became commonplace, but it is still sometimes used as a second line therapy for MDR-TB.[253] Gentamicin (**25**), a natural product of *Micromonospora*, is the most widely used aminoglycoside. It is approved infections caused by *Enterococci*, *Streptococci*, and *P. aeruginosa*. Tobramycin (**27**) has activity against many gram-negative pathogens, but is primarily used for the treatment of cystic fibrosis and resultant *P. aeruginosa* lung infections.[254] A large number of aminoglycosides are natural products, but several of the more recently developed members of this class are semi-synthetic. Amikacin (**28**) is a semi-synthetic designer derivative of kanamycin A clinically introduced in 1976. The L-hydroxyaminobutyramide (HABA) side chain of amikacin (**28**) blocks many AAC and APH enzymes, which increases its spectrum of activity considerably.[255,256] It is currently used mainly for the treatment of highly drug resistant gram-negative organisms including MBL producers and for MDR-TB.[217,218]

For the last several decades and until recently very little was done to advance new aminoglycosides into the clinic. Plazomicin (**29**), a very promising new semisynthetic sisomicin derivative from Achaogen, is currently in phase II clinical trials. It was designed with several modifications,

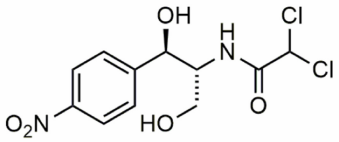


Figure 10. Chloramphenicol (**30**).

including a HABA side chain, to make it resistant to almost all AMEs and have lower toxicity than other aminoglycosides. It exhibits activity against fluroquinolone and aminoglycoside resistant pathogens as well as many β-lactamase producers.[11] It also shows impressive synergy with daptomycin (**79**) and several β-lactam antibiotics.[257,258] It has good activity against many MRSA strains and carbapenemase producing *Enterobacteriaceae*, including KPC producing *K. pneumoniae*, but most NDM-1 producing isolates show resistance to it along with all other aminoglycosides. This is because these strains also typically produce ArmA and RmtC 16S rRNA methylases.[11,259] It also has lower activity against some *A. baumannii* and *P. aeruginosa* strains than currently used aminoglycosides.[260,261]

Amphenicols

Amphenicols are a class of phenylpropanoid antibiotics. Chloramphenicol (**30**) was discovered in 1946 and introduced to the clinic in 1948 (Fig. 10).[163] It is the only member of this class FDA approved for human consumption although there are other amphenicols that have been approved for use in other countries and for veterinary purposes. These antibiotics bind the peptidyl transferase center (PTC) of the 50S ribosomal subunit to inhibit the elongation step of translation. Chloramphenicol (**30**) has fairly broad spectrum bacteriostatic activity against some gram-positive and negative species including anaerobes.[215] They are bactericidal against *H. influenzae*, *N. meningitidis*, and *S. pneumoniae*, however.[262]

Despite their broad spectrum, amphenicols have never been popular first line antibiotics in the developed world largely because of concerns regarding their safety. They are widely used in the developing world because they are inexpensive and readily available, though.[263] Resistance can occur through target modification by the *cfr* gene encodes a methylase that methylates the C8 position of A2503 of the 23S rRNA causing resistance to amphenicols as well as many other PTC targeting antibiotics.[264] Acetyltransferases are also a common resistance mechanism.[265] Efflux of amphenicols is also common with members in all of the major efflux pump families that recognize them.[42, 266]

Macrolides

Macrolides are macrocyclic lactones with deoxy-sugars, usually cladinose or desosamine, appended through glycosidic bonds (Fig. 11). The first macrolide to be discovered was erythromycin (**31**) in 1949 and it was introduced clinically in 1951.[163] Macrolides bind the 50S ribosomal subunit blocking the peptide exit tunnel, inhibiting elongation of translation by causing premature disassociation of peptidyl tRNA from the

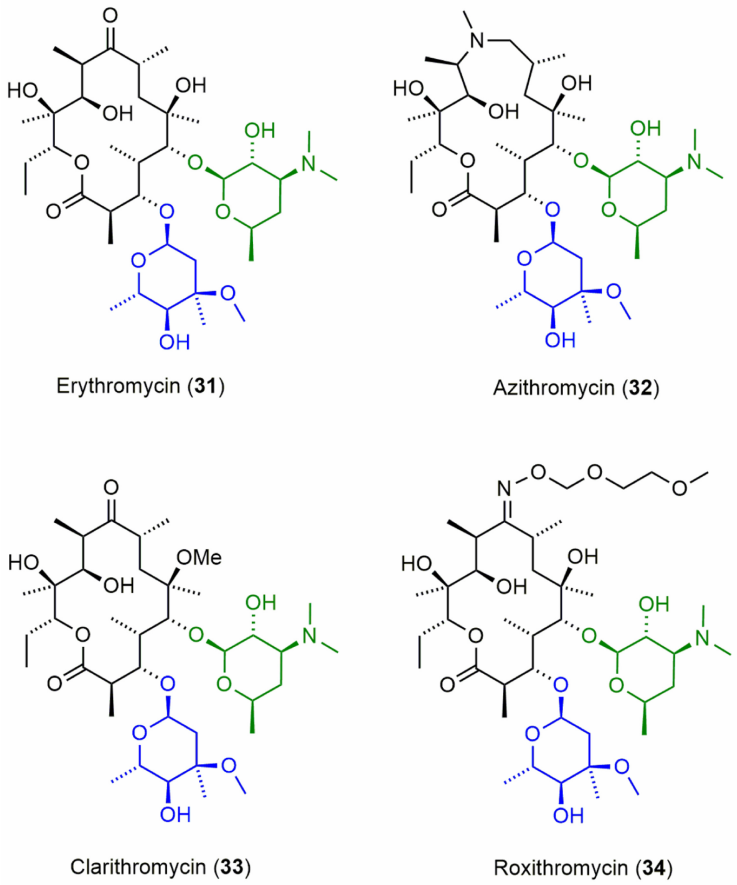


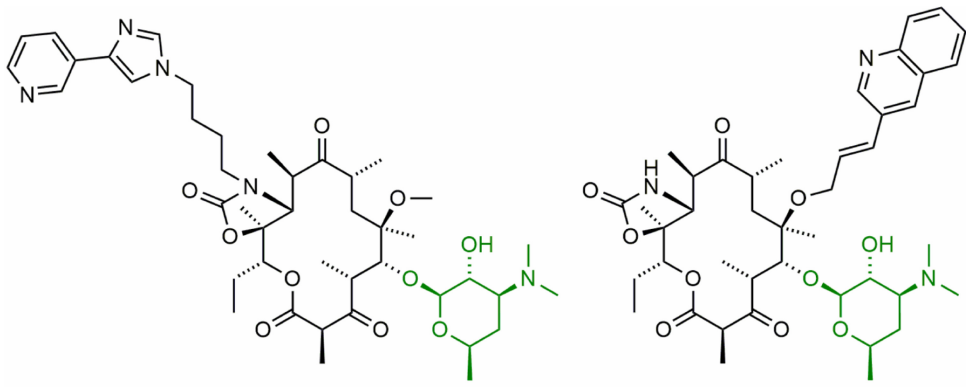
Figure 11. Macrolides.
Note: The cladinose ring is highlighted in blue, deosamine in green.

ribosome.[267] They have fairly broad spectrum antibacterial activity against aerobic and anaerobic gram-positive and some gram-negative bacteria. They are bacteriostatic except at very high concentrations and in select situations, such as azithromycin (**32**), which is bactericidal against *H. influenzae*.[268]

Resistance to macrolides occurs via a variety of target modifications. Methylases encoded by the *erm* gene cause resistance. Mono-methylation of rRNA A2058 and occasionally A2509 in the N6 positions confers resistance many macrolides, but has no effect on the newer ketolide subclass. Di-methylation confers resistance to both macrolides and ketolides, however.[269] A2058G and A2059G mutations cause resistance although the effect is much diminished in ketolides. A2062C confers resistance selectively to 16-membered ring macrolides.[270] Mutations in the ribosomal protein L22 and L4 can also result in resistance.[271,272] Macrolide modifying enzymes also exist.[273] As with many antibiotics, efflux is the main source of resistance, particularly in 14-membered ring macrolides.[42,274,275]

The semi-synthetic members of this class, azithromycin (**32**) and clarithromycin (**33**), showed expanded spectrums of activity, better acid stability, and improved pharmacokinetics as compared to erythromycin (**31**) making them popular first line antibiotics.[42] Azithromycin (**32**) was the most commonly prescribed outpatient antibiotic in the US in 2010.[276] Roxithromycin (**34**) another semi-synthetic macrolide is currently the only other member of this subclass on the US market.

Telithromycin (**35**), a semi-synthetic erythromycin derivative, was the first ketolide identified in 1997 and it is currently the only FDA approved member of this subclass (Fig. 12). Telithromycin (**35**) shows improved activity against many strains with upregulated macrolide efflux and erm methylases including *Streptococci* and *S. Aureus* strains.[277] It was partially withdrawn on the US market after rare but serious side effects including blurred vision and liver failure were observed.[278] SAR studies have likely pinpointed the structural origin of this toxicity, however, and ketolides currently in development including Advanced Life Science's



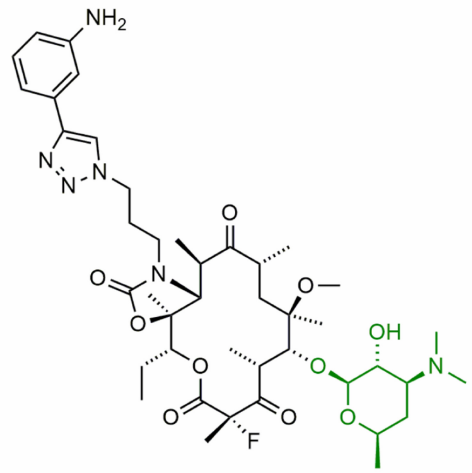


Figure 12. Ketolides.
Note: The deosamine ring is highlighted in green.

cethromycin (**36**), in post-phase III trials, and Cempra's solithromycin (**37**), awaiting phase III trials, have sought to remedy this. Cethromycin (**36**) has improved activity against *S. pneumoniae*, but is less effective against erm methylases producers.[279] Solithromycin (**37**) has good activity against many gram-positive and some gram-negative pathogens, including some with erm methylase resistances, but reduced activity against some *S. aureus* strains.[280]

Tetracyclines

Tetracyclines are an antibiotics class that shares a common octahydrotetracene skeleton (Fig. 13). The first tetracycline was discovered in 1945 and was named chlortetracycline (**38**). It was introduced clinically in 1952. These antibiotics are broad spectrum, bacteriostatic agents used against aerobic gram-positive and negative bacteria that bind the 30S ribosomal subunit and block aminoacyl tRNA access to the ribosome. Their typically low incidence of severe side effects has made them a first line therapy. Tetracycline resistance is most often due to efflux by SMR, RND, or ABC efflux pumps or by ribosomal modification. A tetracycline inactivating enzyme, TetX, has also been reported.[42,281]

Early members of this class were natural products (tetracycline **39**, oxytetracycline **40**, and demeclocycline **41**), but later members (doxycycline **42** and minocycline **43**) were semi-synthetic.[42] The semisynthetic derivatives have better pharmacokinetic features.[282] Tetraphase has a fluorocycline, eravacycline (**44**), in phase III trials with broad spectrum activity against many MDR pathogens including MRSA, VRE, *C. difficile*, and KPC producing gram-negatives, but it has low activity against *P. aeruginosa* and some *Acinetobacter* strains.[11,283] It circumvents several tetracycline resistance mechanisms including tetracycline specific efflux, tetracycline inactivating enzymes, and ribosomal modification.[11,284] Also, Paratek's omadacycline (**45**), a derivative of minocycline (**43**), has passed phase II clinical trials. It has similar activity in many cases to the glycylcycline, tigecycline (**46**). Like both tigecycline (**46**) and eravacycline (**44**) it has little activity against *P. aeruginosa* though, despite its advantages against many other species including highly resistant *N. gonnorhoeae*.[142, 279]

The only FDA approved glycylcycline, a new tetracycline subclass, is tigecycline (**46**). It is a derivative of minocycline (**43**), which was first identified in 1998 making it the first new tetracycline introduced in 30 years. It overcomes previous

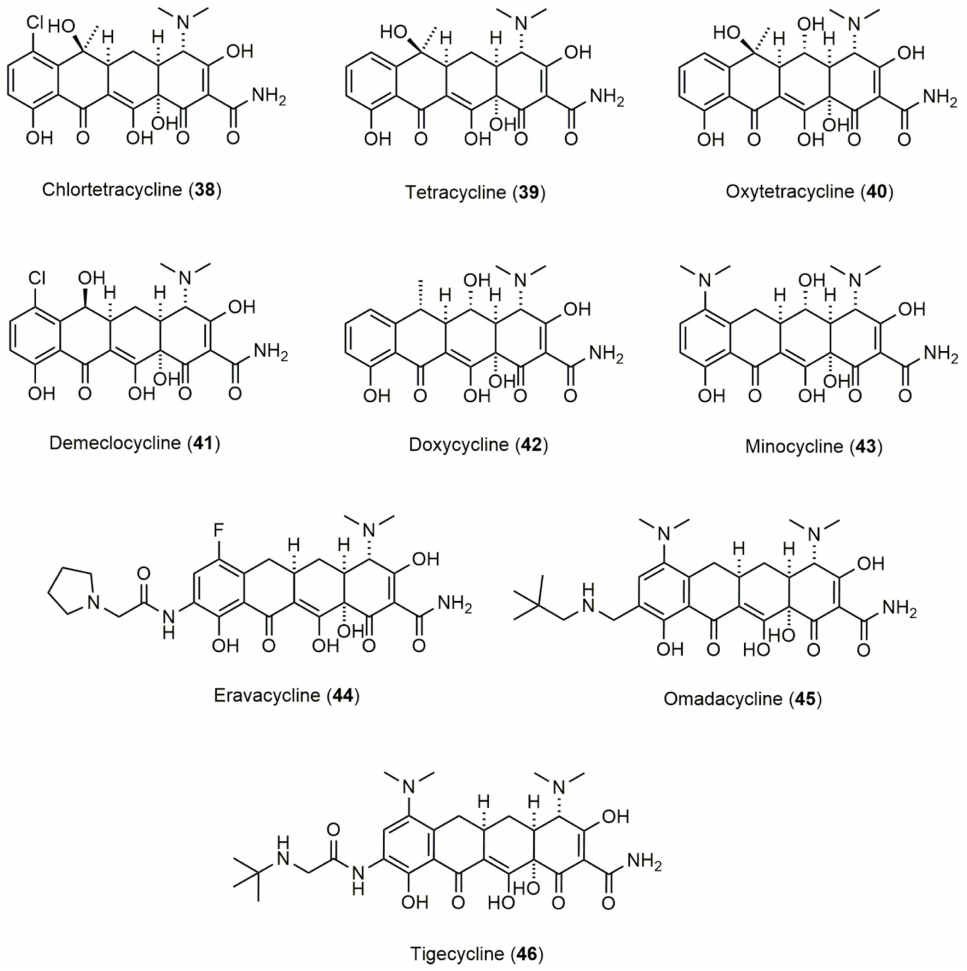


Figure 13. Tetracyclines and the glycylcycline, tigecycline (**46**).

tetracycline resistance mechanisms of ribosomal modification and efflux.[285] It exhibits broad spectrum activity, but most importantly it has good activity against MRSA, VRE, many MDR gram-negatives, including *A. baumannii* and ESBL producing *Enterobacteriaceae*. It notably has low activity against *P. aeruginosa* though. Low blood serum levels and some toxicity are reported disadvantages. Also, efflux pumps have quickly evolved to recognize it, particularly *A. baumannii* AdeABC multi-drug efflux pumps. [286–291]

Rifamycins

Rifamycins are ansamycin antibiotics possessing macrocyclic structures bridging an aromatic moiety. Rifampicin (**47**), the first rifamycin, was made as a semi-synthetic derivative of the *Nocardia* natural product rifamycin B (**48**) in 1957 (Fig. 14).[163] It quickly thereafter introduced to the clinic in 1958. These compounds exert antibacterial activity by binding the β-subunit of RNA polymerase inhibiting transcription. They are bactericidal against gram-positive bacteria and *M. tuberculosis*. They are bacteriostatic against some gram-negative bacteria, which has been attributed to their relatively lower cellular permeability.[292] Mutations to the β-subunit, most often on the side chains of residues 406 and 411, cause resistance. Efflux by VceB and Acr efflux pumps can also occur.[42]

Rifampicin (**47**) administered as a combination therapy with isoniazid and pyrazinamide is still a first line treatment for TB infections. Though less commonly used rifabutin (**49**) and rifapentine (**50**) are also primarily used for treating TB. These three compounds are all designated as critically important by the WHO largely because of their efficacy and common usage against TB.[219] The emergence of MDR- and more recently XDR-TB strains, which are resistant to these treatments has necessitated an interest in the development of next generation TB therapies, however.[293] Rifaximin (**51**) is a newer rifamycin approved by the FDA in 2004. Its only antibiotic indication is

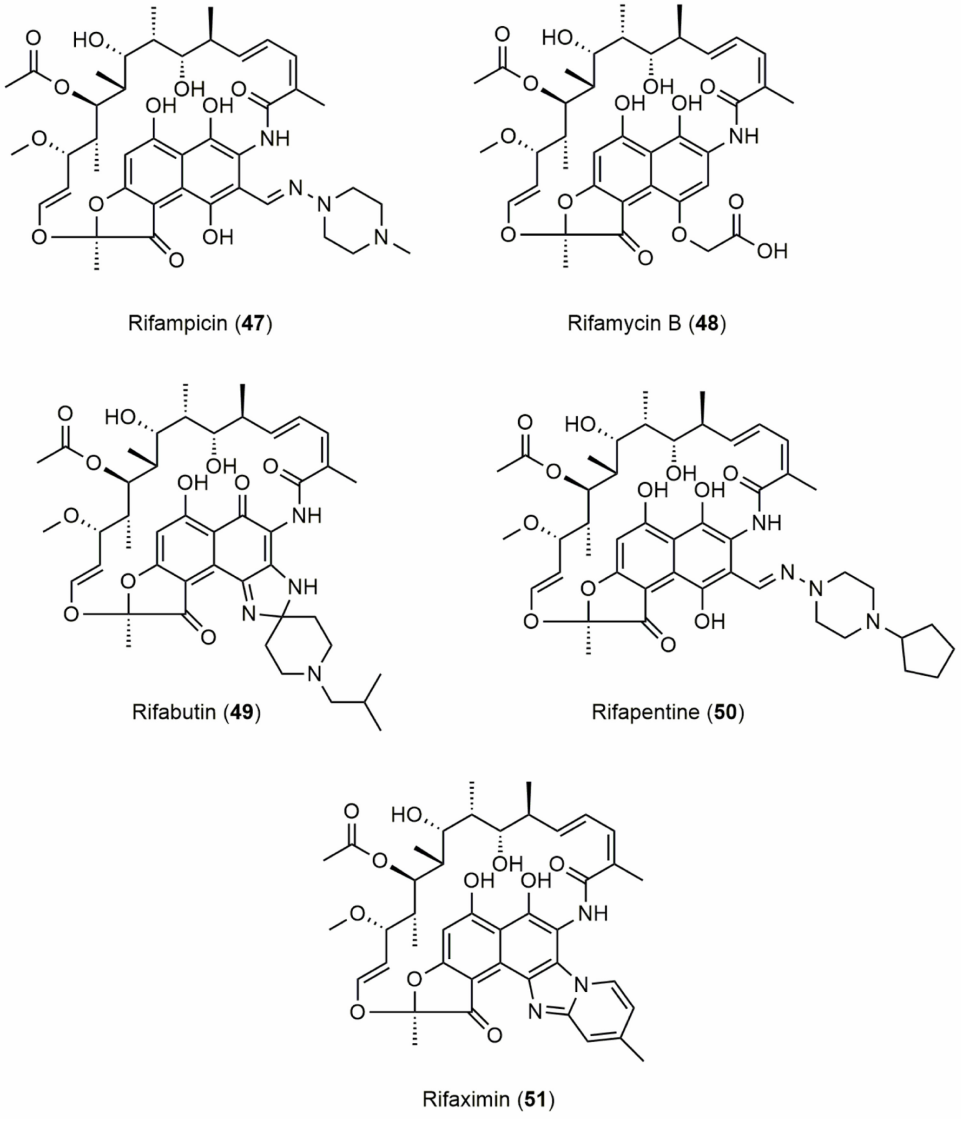


Figure 14. Rifamycins, including the newly approved rifaximin (**51**).

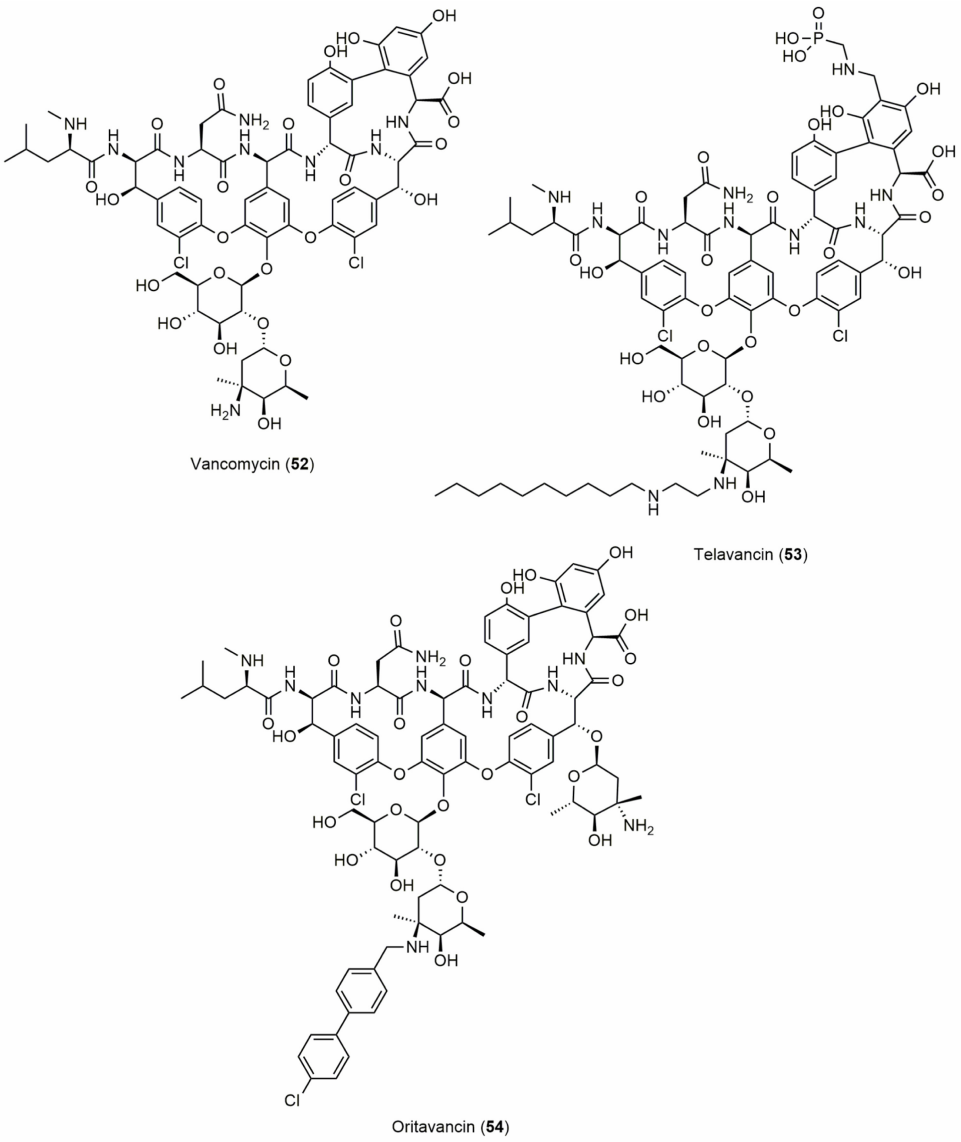


Figure 15. Vancomycin (**52**) glycopeptide and derivatives.

for treatment of *E. coli* associated traveler's diarrhea, although its spectrum of activity is considerably broader.[294]

Glycopeptides

Glycopeptides are macrocyclic peptides with interspersed bridged aromatic moieties and saccharide side chains linked through glycosidic bonds. The first glycopeptide to be discovered was vancomycin (**52**) in 1952 (Fig. 15). It was then introduced clinically in 1958.[163] In contrast to β-lactams, glycopeptides inhibit cell wall biosynthesis in gram-positive bacteria by binding the terminal D-Ala-D-Ala dipeptide of peptidoglycan units sterically inhibiting their use as substrates for PBPs and transglycosylases. Five vancomycin resistant phenotypes (VanA-E), originating in VRE, have altered peptidoglycan termini with lower affinities for vancomycin (**52**).[295] VanH, VanR, VanS, and VanX are also proteins involved in the regulation and reprograming of vancomycin resistance. Efflux mediated resistance is rare for glycopeptides, but AcrF efflux pumps have been known to cause resistance.[42]

Vancomycin (**52**) stays free in the periplasm while teicoplanin (**55**) is membrane anchored by a lipophilic side chain (Fig. 16).[296] Their spectrum of activity and efficacy is generally similar, but its side chain allows teicoplanin (**55**) to overcome *vanB* encoded resistance that vancomycin (**52**) is susceptible to.[297] In 2009 telavancin (**53**), a derivative of vancomycin (**52**), became the first glycopeptide approved for use in the US since vancomycin (**52**). It has a secondary mechanism of action, membrane depolarization, similar to daptomycin (**79**). It has shown particularly good activity against MRSA, resistant *Enterococci*, and activity against biofilm forming bacteria that vancomycin (**52**) lacks.[298] Oritavancin (**54**), a derivative of a vancomycin precursor, has had a complicated development, but is currently undergoing

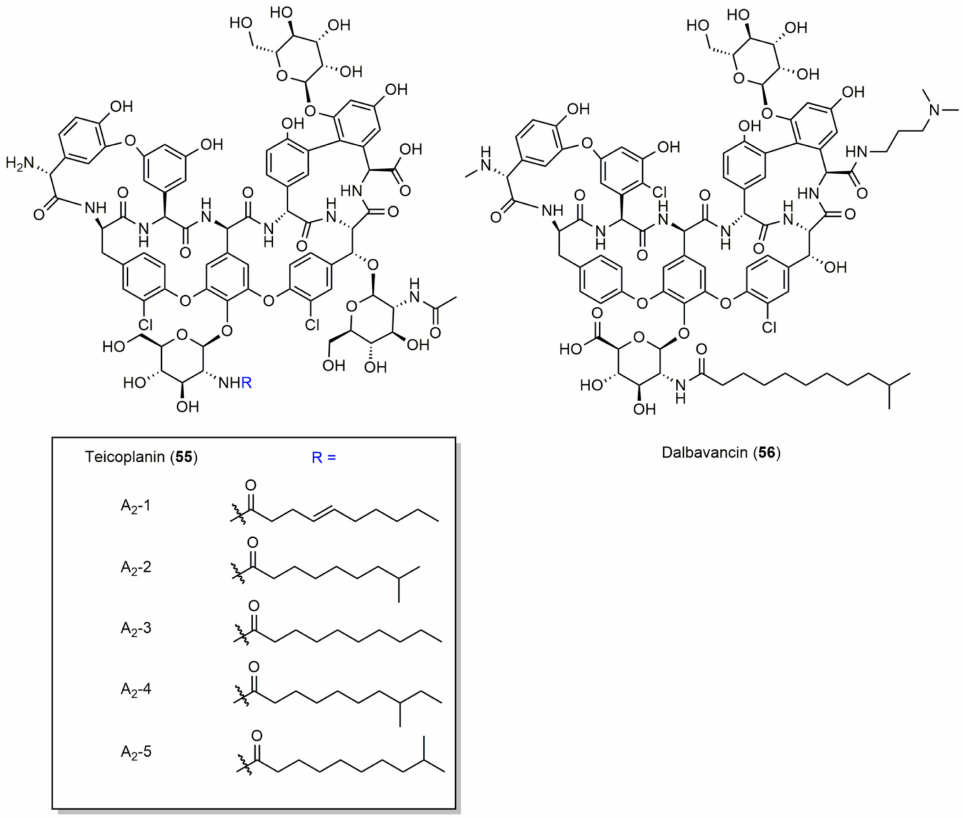


Figure 16. Teicoplanin (**55**) glycopeptide and derivative.

phase III trials under the management of The Medicines Co. It appears to have a higher affinity for peptidoglycan and has a wider range of activity than vancomycin (**52**) including activity against VRSA, *S. pneumoniae*, and an impressive 1000 fold greater activity against VRE.[299–301] Durata's dalbavancin (**56**), a teicoplanin analog, is undergoing phase III clinical trials. It has a significantly higher activity than vancomycin (**52**) against *Staphylococci*, but is not active against VRE. Its main advantage isn't its increased activity though, but once weekly dosing.[299, 302]

Nanotherapeutics' ramoplanin (**57**), a structurally similar lipoglycodepsipeptide, isolated from *Actinoplanes*, has also generated a lot of interest and is currently in phase III trials (Fig. 17). Ramoplanin (**57**) inhibits cell wall biosynthesis, but through a different mechanism than glycopeptides. It inhibits the transglycosylation step by stopping lipid I transformation into lipid II in gram-positive bacteria. It would primarily be used in the treatment of MDR *C. difficle* if approved.[303]

Quinolones

Quinolone antibiotics possess a quinolone core that typically has a N linked cyclic moiety and various substituents at the C(6) and/or C(7) positions (Fig. 18). Nalidixic acid (**58**), the first quinolone, was discovered in 1962. It wasn't until 1968 that a quinolone, ciprofloxacin (**59**), which is a fully synthetic analog of nalidixic acid, was introduced clinically.[163] Quinolones inhibit topoisomerases II (DNA gyrase) and IV trapping the enzymes in the DNA cleavage stage, ultimately inhibiting DNA synthesis among other things. Modern quinolones are bactericidal and have broad spectrum activity that covers most aerobic gram-positive and negative bacteria, some anaerobic gram-negatives, and *M. tuberculosis*. Most quinolones preferentially target DNA gyrase or topoisomerase IV, though some, particularly later generation compounds, target both equally.[42]

Resistance by target modification commonly occurs by mutations to genes *gyrA* and *parC* in both *P. aeruginosa* and *A. baumannii* as well as the *grl* gene in *S. aureus*.[225, 304,305] The plasmid mediated *qnrA* gene, which produces a protein that protects DNA from quinolone binding, has also been found primarily in *Enterobacter* and *Klebsiella*.[306,307] A number of other Qnr proteins have also been identified in gram-negative bacteria.[308] Fluoroquinolone efflux pumps, which can be intrinsic or acquired, commonly show broad activity against multiple antibiotic classes.[42,309]

First generation quinolones are rarely used today because of poor biodistribution and spectrum of activity compared to more modern members of this class. Second generation drugs were characterized by expanded activity particularly against aerobic gram-negative bacteria, but were not broadly active against gram-positive bacteria.[310] Ciprofloxacin (**59**), a standout second generation fluoroquinolone, is still one of the most active quinolones against *P. aeruginosa* and has also garnered a lot of attention for its

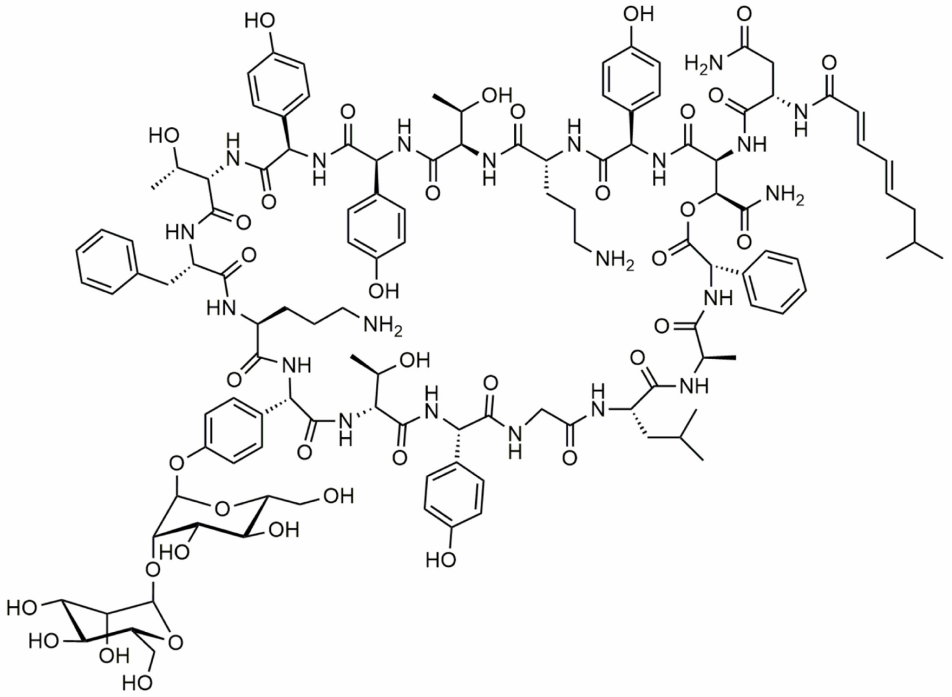


Figure 17. The lipoglycodepsipeptide ramoplanin (**57**).

activity against extremely virulent bacteria such as *Bacillius anthracis* (anthrax) and *Yersinia pestis* (plague). Some third generation compounds showed improved activity against gram-positives. Levofloxacin (**60**), for example, showed marked improvements against *Streptococcus*.

The fourth generation of quinolones expanded activity even further, especially in their coverage of anaerobic bacteria and bacteria that had developed resistances against this class. Some have also had more issues with toxicity than most second and third generation compounds though.[311] Fourth generation fluoroquinolones, sitafloxacin (**61**) (approved in Japan) and clinafloxacin (**62**), overcome individual target modification resistances because they simultaneous target both DNA gyrase and topoisomerase IV. In some cases they are even active against double mutants in relevant organisms including *S. pneumoniae*, *E. faecium*, and *N. gonorrhoeae*.[312]

Three promising quinolones in development are Rib-X's delafloxacin (**63**), TaiGen's nemonoxacin (**64**), and Furiex's avarofloxacin (**65**), which are all in phase III trials. All have enhanced activity against gram-positive bacteria including *S. pneumoniae* and MRSA strains including some that are ciprofloxacin resistant.[313–316] MerLion's finafloxacin (**66**), also in phase III clinical trials, shows enhanced activity over other quinolones at low pH and has particularly good activity against CA-MRSA and *A. baumannii* including strains with ciprofloxacin resistance.[317,318]

Streptogramins

Streptogramins are divided into class A and class B based on their structures, which also correlates with their mechanism of action. Class A streptogramins are 23-membered unsaturated macrocycles containing peptide and lactone bonds. Class B sreptogramins are 19-membered depsipeptides (Fig. 11). Streptogramin B was discovered in 1963, but it wasn't until 1999 that members of this class would be used clinically.[163] They are typically administered clinically as pairs of molecules from each class. Pristinamycin (**67 / 68**) itself is a combination of class A and B molecules.

Group A streptogramins bind the 50S ribosomal subunit at the PTC to inhibit initiation and translocation, whereas group B antibiotics bind the peptide exit tunnel to inhibit the elongation stage of translation. They have activity against gram-positive and in select cases gram-negative bacteria, but their overall narrow activity combined with poor aqueous solubility have limited the clinical use of many members of this class. They are usually bacteriostatic when administered alone. When they are administered as combinations of group A and B streptogramins they exhibit bactericidal activity.[215]

Quinupristin (**69**) / dalfopristin (**70**) and pristinamycin (**67 / 68**) both show good bactericidal activity against MRSA, and the former also shows very high activity against vancomycin resistant *E. faecium*, but their activity becomes bacteriostatic in strains that exhibit erm methylases.[303] Erm methylases, which also produce resistance to macrolides, cause resistance in group B streptogramins.[319] Cfr methylases create resistance specifically to group A streptogramins.[320] There are now strains that have an *mlr* operon, which has both *erm* and *cfr* genes. These strains are resistant to all PTC targeting antibiotics.[321]

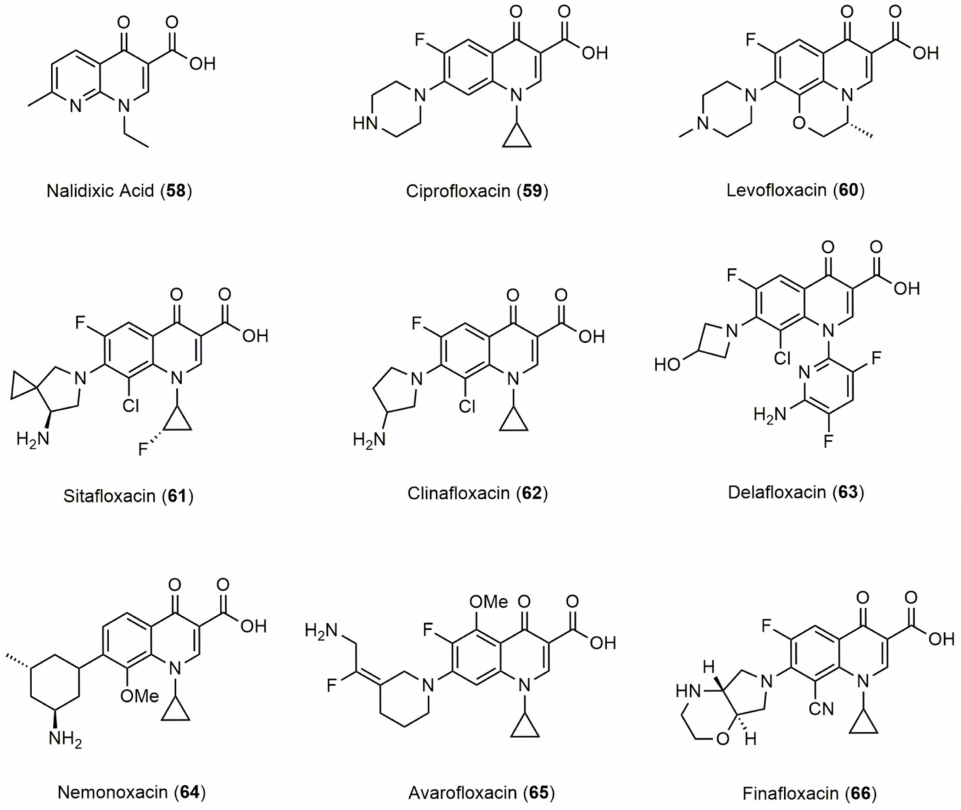


Figure 18. Select first through fourth generation quinolones.

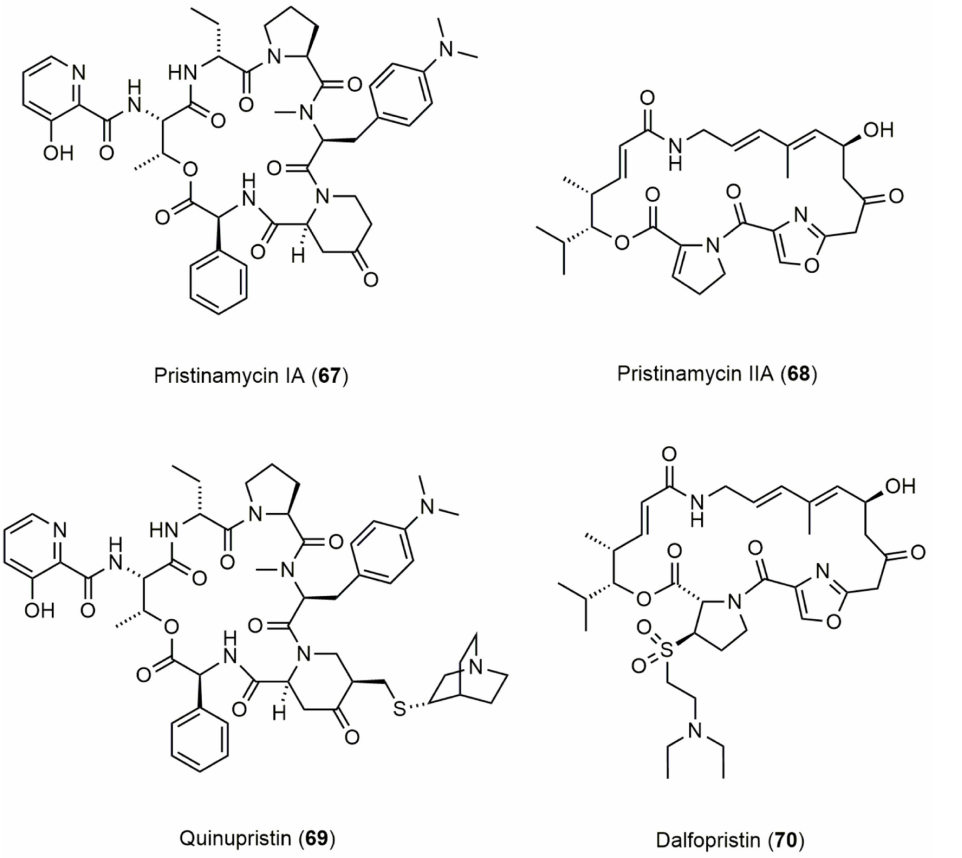


Figure 19. Class A and B streptogramins.

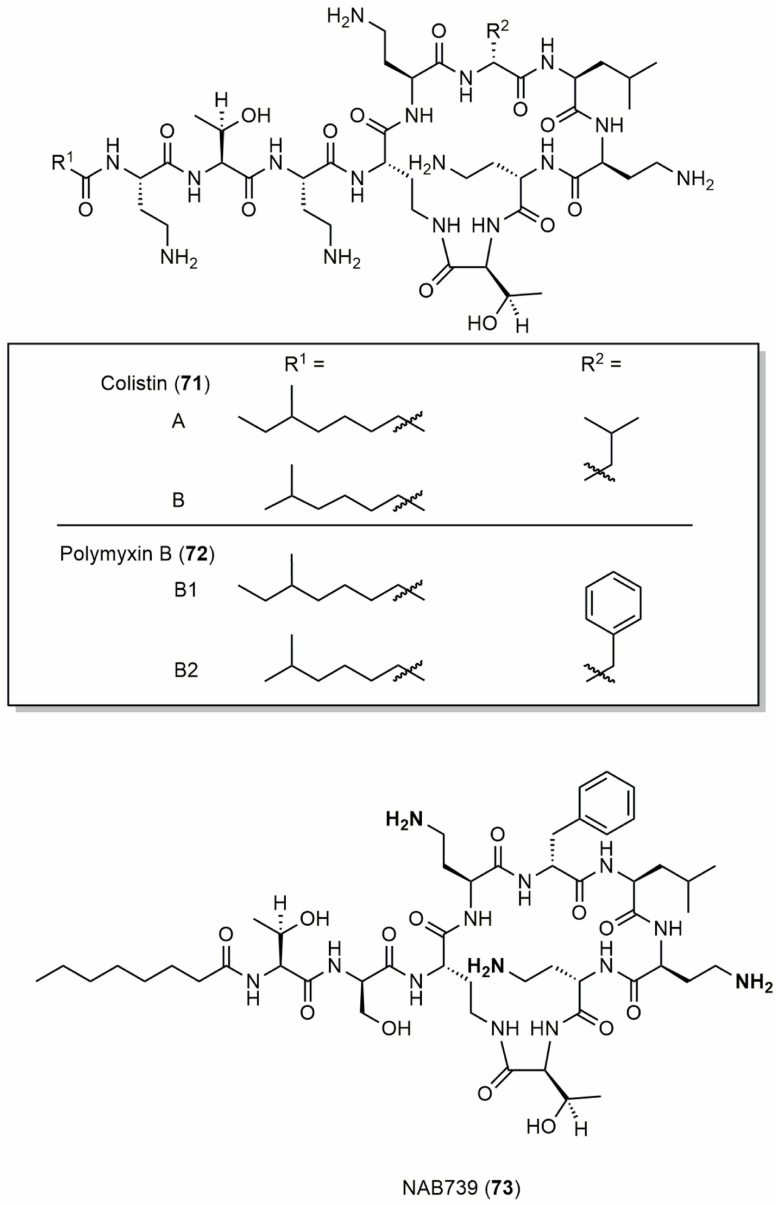


Figure 20. Polymyxins including preclinical polymyxin B analog, NAB739.

Polymyxins

Polymyxins are cyclic peptides with peptidyl side chains capped with a hydrophobic, saturated alkyl tail (Fig. 20). Polymyxins A-E, natural products of *Bacillus*, were discovered in 1947. Colistin (polymyxin E) (**71**) has been on the market since 1950. Primarily because of significant nephrotoxicity and neurotoxicity[322] it was infrequently used until recently, when interest was renewed in it and polymyxin B (**72**), as drugs of last resort. Recent studies have shown that colistin nephrotoxicity may have been overstated possibly because of improper dosing or inferior formulation.[323,324] They have potent broad spectrum activity against most gram-negative bacteria although some strains of *E. Coli*, *Klebsiella*, *Enterobacter*, *M. tuberculosis*, and others have developed resistances.[325–327]

Polymyxins, which are polycationic, displace stabilizing magnesium and calcium ions to electrostatically interact with the anionic lipopolysaccharide (LPS) outer layer of gram-negative cell membranes. This disrupting interaction leads to increased cell membrane permeability, cell leakage, and rapid cell death.[328] Colistin (**71**) also has the added benefit of having potent anti-endotoxin activity also.[329] Resistance to polymyxins is fairly uncommon, although its frequency varies significantly by bacterial species and by geographic region.[330] Some gram-negative bacteria including *E. coli* and *P. aeruginosa* can exhibit resistance through expression of lower affinity modified LPS. *P. aeruginosa* can also upregulate membrane protein H1, which replaces divalent cations in the LPS, decreasing polymyxin affinity. *K. pneumoniae* increases production of its capsule polysaccharide,

which limits polymyxin penetration to the LPS layer. Also, though not yet known to spread to pathogenic bacteria, strains of *B. polymyxa* are known to produce a degrading colistinase.[284]

Colistin (**71**) is now used in the treatment of MDR gram-negative pathogens with few other treatment options, particularly MDR *Pseudomonas*, *Klebsiella*, and *Acinetobacter* strains including NDM-1 producers.[124] Cubist's CB-182,804 is a polymyxin B analog currently in phase I trials that shows activity against many MDR gram-negative bacteria and even some colistin resistant strains.[331] It was developed to not only have greater activity, but to have a more favorable toxicity profile though no evidence has been shown that this was indeed successful.[332] Polymyxin B analogs with reduced overall positive charge have recently been shown to retain good antibacterial activity, while showing much improved in vitro toxicity profiles. One of these molecules, NAB739 (**73**), is being actively developed in preclinical studies by Northern Antibiotics Ltd.[333–337]

Oxazolidinones

Oxazolidinone antibiotics have a shared oxazolidinone core with various N-linked aryl and heterocyclic rings and short C(5) side chains (Fig. 21). The first and currently only clinically approved oxazolidinone, linezolid (**74**), was first identified in 1995 and approved by the FDA in 2000, though the roots of this class go back to the 1970s.[338] Oxazolidinones bind the PTC on the 50S ribosomal subunit blocking peptide bond formation to elicit bacteriostatic activity against gram-positive bacteria and *M. tuberculosis*. Resistance mechanisms to oxazolidinones are still somewhat rare. Target modification of the PTC by the G2576U mutation confers, however, resistance in some *Enterococci* and *S. Aureus* strains.[339] U2500A and U2571C rRNA mutations, mutations to ribosomal proteins L3 and L4, and *cfr* encoded methylation of A2503 are also known to result in resistance.[279] Although still rare, cfr methylases have spread to many countries and recently caused serious outbreaks of linezolid resistant *Staphylococci.* [340-343]

Linezolid (**74**) is useful against hard to treat gram-positive infections including those caused by MRSA and VRE.[344,345] Since linezolid's approval, many oxazolidinones in development have been plagued by issues including poor solubility and pharmacokinetics, toxicity, and few improvements to activity. As a result most have failed in early developmental stages.[346] Trius's tedizolid (**75**), in phase III trials, and Rib-X's radezolid (**76**), in phase II trials have shown promise, however. They have both shown broadly improved activity and even activity against a wide range of linezolid resistant *Staphylococci* including MRSA strains.[347] Tedizolid (**75**) was found to have significantly greater activity against a variety of strains known to have point mutations or methylations that normally result in linezolid resistance.[348] The series of compounds that include radezolid (**76**) were designed based on computational models that were created using atomic level structures of linezolid (**74**). It was successfully designed to extend activity to include gram-negatives *H. influenzae* and *Moraxella catarrhalis*.[349,350]

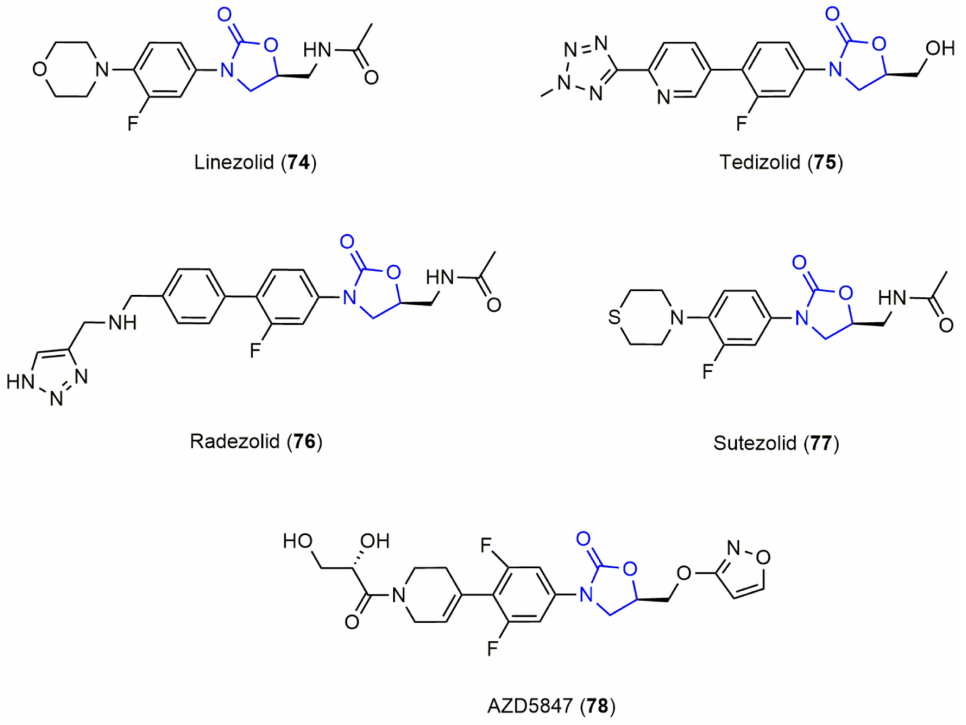


Figure 21. Oxazolidinones.
Note: The oxazolidinone rings are highlighted in blue.

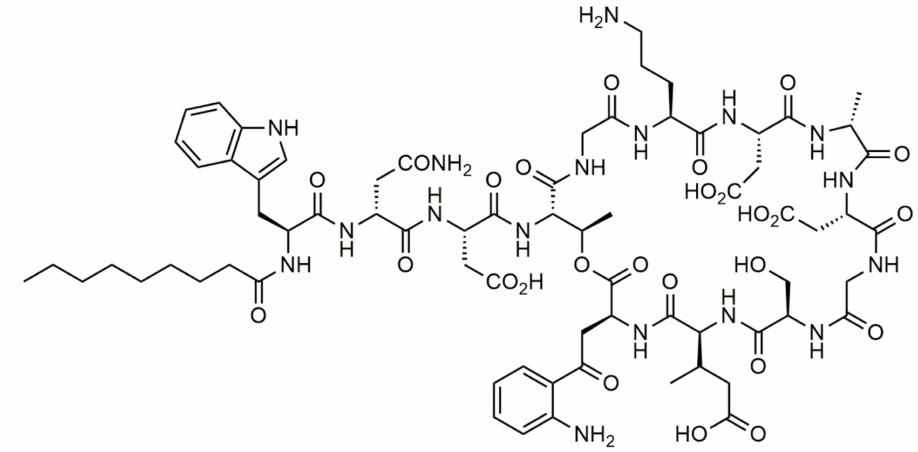
It also circumvents resistance mutations to L3 and L4, but it doesn't do as well as tedizolid (**75**) against rRNA point mutation resistances, or against cfr methylase producers.[299,349,350] Pfizer's sutezolid (**77**) and AstraZeneca's AZD5847 (**78**), both in phase II trials, are being developed for use against MDR- and XDR-TB. Sutezolid (**77**) was designed to be potentially less toxic than linezolid (**74**) and was found to have broadly superior activity against *M. tuberculosis* isolates including those with resistance to isoniazid, rifampicin (**47**), ethambutol, and streptomycin (**24**).[351]

Lipopeptides

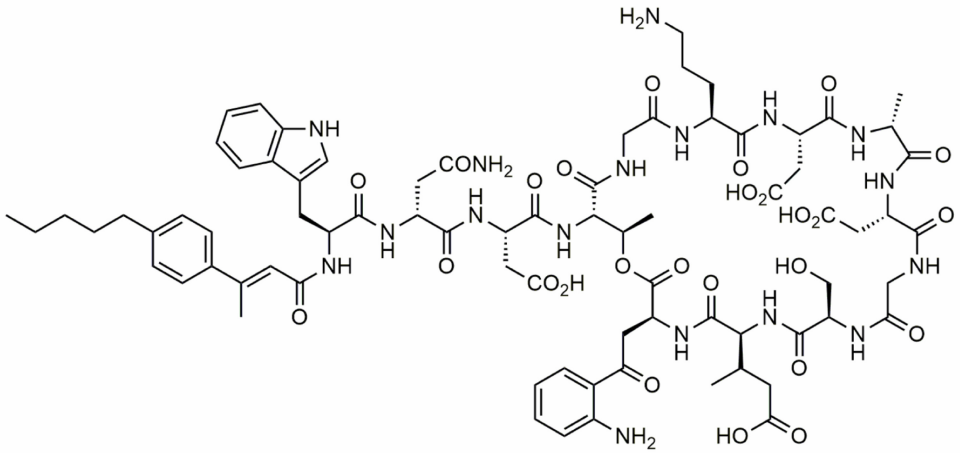
Lipopeptides are cyclic depsipeptides with a peptidyl side chain capped with a saturated alkyl tail. Daptomycin (**79**), discovered in 1985, was the first lipopeptide antibiotic to be identified though it wasn't used clinically until 2003 (Fig. 22).[163] It is currently the only clinically approved member of the lipopeptide class. Lipopeptides work by inserting their lipid tails into the cytoplasmic membrane of gram-positive bacteria, which depolarizes the membrane leading to potassium efflux. This disrupts the structural integrity of the membrane resulting in cell lysis.

Daptomycin resistance is still rare and the mechanisms of its occurrence are not fully understood. Resistance in *Enterococci* has been linked to genes that alter cell envelope stress response and upregulation of cardiolipin synthase, an enzyme involved in cell membrane homeostasis.[352] *S. aureus* strains with thickened cell walls caused by increased production and D-alanylation of cell wall teichoic acids show daptomycin resistance.[353] Single nucleotide polymorphisms in *S. aureus mprF* and *dltA-D* genes resulting in increased cell wall positive charge are also resistant.[354,355]

Daptomycin (**79**) displays good activity against many drug resistant gram-positive pathogens including MRSA and VRE.[349] Cubist's surotomycin (**80**) is a lipopeptide in phase III trials for the treatment of *C. difficile*. So far it has been found to have a similar cure rate and a lower rate of relapse than vancomycin (**52**), the current standard treatment for this bacterium.[356]



Daptomycin (**79**)



Surotomycin (**80**)

Figure 22. Daptomycin (**79**) and surotomycin (**80**), a lipopeptide in clinical development.

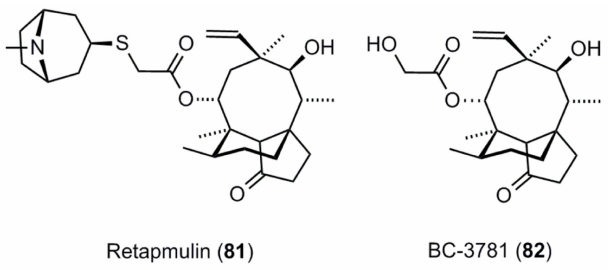


Figure 23. Retapmulin (**81**) and the BC-3781 (**82**), a pleuromutilin in clinical development.

Pleuromutilins

Pleuromutilins all have a common fused cyclo-octane / pentanone with a bridged cyclohexane ring system. They have a variety of ester linked side chains. Retapmulin (**81**) became the first clinically approved pleuromutilin in 2007 (Fig. 23), though they were used extensively for several decades in veterinary medicine before that and some were discovered as early as the 1950s. Like many other antibiotics they bind the PTC on the 50S ribosomal subunit and thus inhibit translation. Upregulated *vga* genes that code for ABC efflux transporters confer resistance to pleuromutilins.[357,358] Target modifications including *cfr* mediated methylations, mutations to ribosomal protein L3, and point mutation to the 23S rRNA also cause resistance.[320,359]

Retapmulin (**81**) has been approved for topical usage against gram-positive bacteria including MRSA, resistant *Streptococci*, and erm methylase producers.[320] Nabriva's BC-3781 (**82**) is currently in phase II trials for non-topical applications. It shows good activity against MRSA, VRE, and macrolide and quinolone resistant *S. pneumoniae*, and has compared favorably to vancomycin (**52**) in trials.[360,361]

Macrolactones

Antibacterial macrolactones have unsaturated lactone cores decorated with deoxysugars and aromatic motifs. Macrolactones with antibacterial activity were first discovered in the 1970s, but it wasn't until 2011 that the first and currently only macrolactone, fidaxomicin (**83**), was approved for clinical use (Fig. 24).[163] Fidaxomicin (**83**) is an actinomycete natural product that inhibits RNA polymerase in gram-positive and some gram-negative bacteria to elicit bactericidal activity. It has almost no systemic bioavailability though which makes it unsuitable for the treatment of many infections.[362]

Fidaxomicin (**83**) is a very narrow spectrum antibiotic with approval only for *C. difficile* infections. As *C. difficile* associated diarrhea is a gastrointestinal affliction it has been argued that it's narrow spectrum of activity is actually advantageous because of its low activity against beneficial commensal bacteria, which is thought to help prevent reoccurring infections.[362,363]

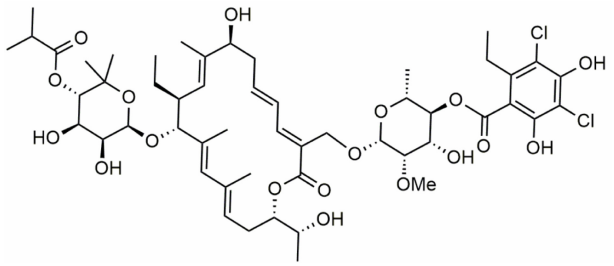


Figure 24. The macrolactone fidaxomicin (**83**).

Diarylquinolines

Antibacterial diarylquinolines consist of a quinoline core with two other aryl groups linked through the C(3) position of the quinoline. Diarylquinolines with antibacterial activity were first discovered in 1997 through whole cell high throughput screening of synthetic molecules for direct antibacterial activity against the *M. tuberculosis* surrogate *M. smegmatis*.[364] Bedaquiline (**84**), the only clinically approved member of this class was first used in 2012. It inhibits F_1F_0-ATPase, the proton pump for ATP synthase (Fig. 25).

Like fidaxomicin (**83**), bedaquiline (**84**) is a very narrow spectrum antibiotic. It has activity against *Mycobacteria*, and in particular *M. tuberculosis* making it the first new TB drug in more than forty years.[364] It will be used only for MDR-TB and XDR-TB. There was some controversy over its approval though as it was based on clearance of TB from sputum cultures rather than patient mortality.[365]

Combination Therapies

Taking a note from the drug cocktails used to combat HIV in the successful highly active anti-retroviral therapy (HAART) and the longtime strategy for combating *M. tuberculosis*, the use of antibiotics in combination therapies is becoming an increasingly attractive approach to combat resistance. Synergy can be such a powerful force that even molecules too weakly active on their own to be considered for monotherapies can be administered in combination therapies to great effect.[366] Evidence suggests that some antibiotic producing *Streptomyces* may also naturally employ combination approaches to eliminate

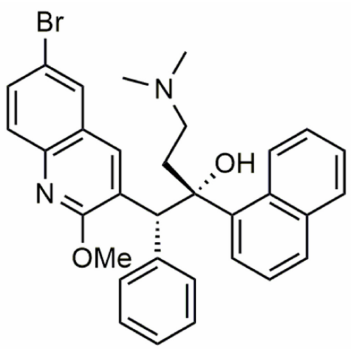


Figure 25. The diarylquinoline bedaquiline (**84**).

competition.[367] There are many combinations of antibiotics that are known to exhibit synergy. Some antibiotics are almost always administered as combination therapies even, such as streptogramins and rifamycins.

An extension of this strategy that is also being pursued is the production of covalently linked hybrids of two antibiotic classes. Actelion's cadazolid (**85**), in phase III trials, is structural hybrid of a fluoroquinolone and an oxazolidinone that primarily inhibits translation in gram-positive bacteria (Fig. 26).[332] It has activity against *C. difficile* strains that are resistant to linezolid (**74**) and moxifloxacin, so it is being developed primarily to combat that pathogen.[368] A hybrid of two cell wall biosynthesis inhibiting classes, Theravance's TD-1792 (**86**), in phase II trials, is a cephalosporin/glycopeptide hybrid.[332] This hybrid principally targets the D-Ala-D-Ala terminus of peptidoglycan units in gram-positive bacteria. It is being developed chiefly as a treatment for MRSA although it also has activity against *C. difficile*.[369,370] The obvious advantage to this approach is the potential added benefit of synergistic secondary antibiotic effects with the administration of a single molecule. One disadvantage that has been noted, however, is that gram-negative activity is commonly lost in hybrid molecules likely because of their bulk, which is prohibitive of uptake.[371]

New Antibacterial Targets

With the advancements of the last several decades have come the identification of numerous novel potential antibacterial targets. Though some of these have proven to be dead ends many remain promising. For the sake of brevity only a selection of the most promising targets, chosen based on interest generated in the field and by clinical advancement of compounds targeting them, are discussed in this review.

Symmetric bis-indoles have been identified that function as groove binders of double stranded nucleic acids to inhibit DNA and RNA synthesis and induce a SOS response. Though established antibiotic classes are known to inhibit both DNA and RNA synthesis, this particular mechanism is unprecedented for antibiotics. One compound, MBX-1162 (**87**), is currently in phase I clinical trials and has shown good, broad spectrum activity against gram positive and negative bacteria including problem pathogens such as MDR *A. baumannii*, *K. pneumoniae*, VRE, and MRSA.[372, 373]

Many of the most promising new targets are antibacterial enzymes and one of the most extensively discussed is undoubtedly FtsZ, a highly conserved, GTPase, tubulin homolog that assembles into dynamic contractile ring structures that act as a scaffold for the protein complexes that drive cell division. A host of antibacterial molecules have been recently discovered that target cell division by interfering with FtsZ. Taxanes, such as SB-RA-5001 (**88**), which display anti-cancer properties derived from their stabilization of tubulin structures, are also capable of stabilizing FtsZ ring structures and have shown promising anti-tuberculosis activities.[374] Several structurally unique classes of compounds including benzimidazoles and pteridines possess antibacterial activity based on inhibition of FtsZ polymerization.[375–378] Benzamides, such as PC190723 (**89**), cause FtsZ to polymerize

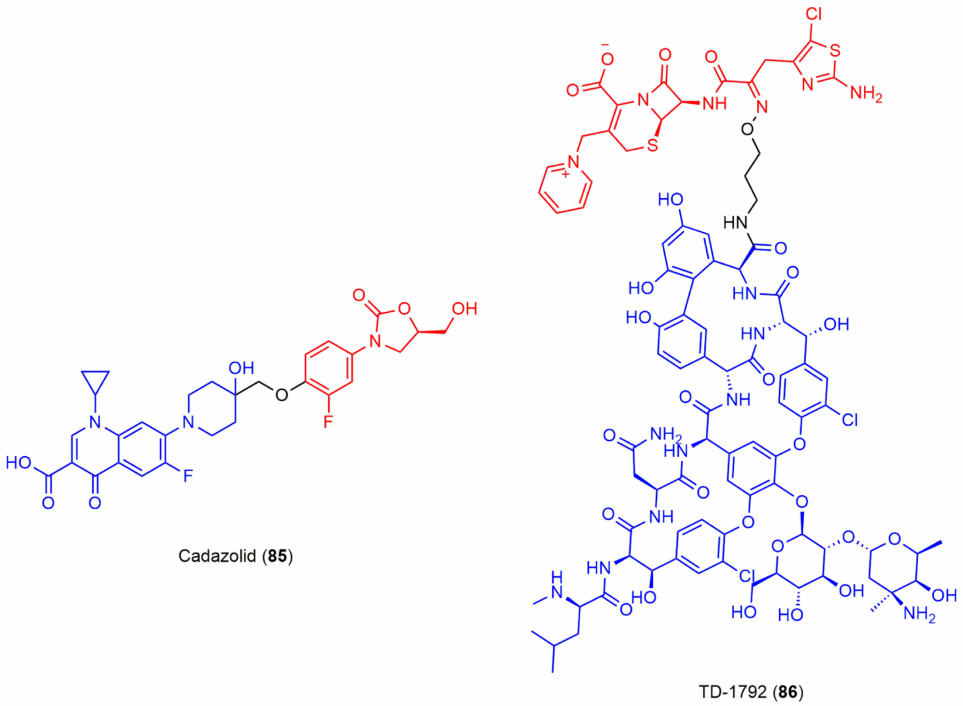


Figure 26. Potential combination therapies.
Notes: Cadazolid (**85**), a quinolone (blue) and oxazolidinone (red) combination. TD-1792 (**86**), a glycopeptide (blue) and β-lactam (red) combination.

into non-functional, hyperstable polymers in *Staphylococci*.[379,380] Several classes of aryl tricyclic heterocycles have also shown good activity against gram positive bacteria including MRSA and VRE also through a polymerization stimulating mechanism of action.[381–384] Many disparate classes of molecules have also been found, among other functions, to inhibit the GTPase activity of FtsZ, a function that is essential to the dynamic nature of FtsZ superstructures.[377,385–391] Acyldepsipeptides have been discovered that activate ClpP peptidase. The dysregulated peptidase causes uncontrolled degredation of FtsZ. These molecules show broad spectrum activity against a wide range of gram positive bacteria.[392–394]

Given the success of antibiotics that target protein synthesis it is unsurprising that other efforts have focused on

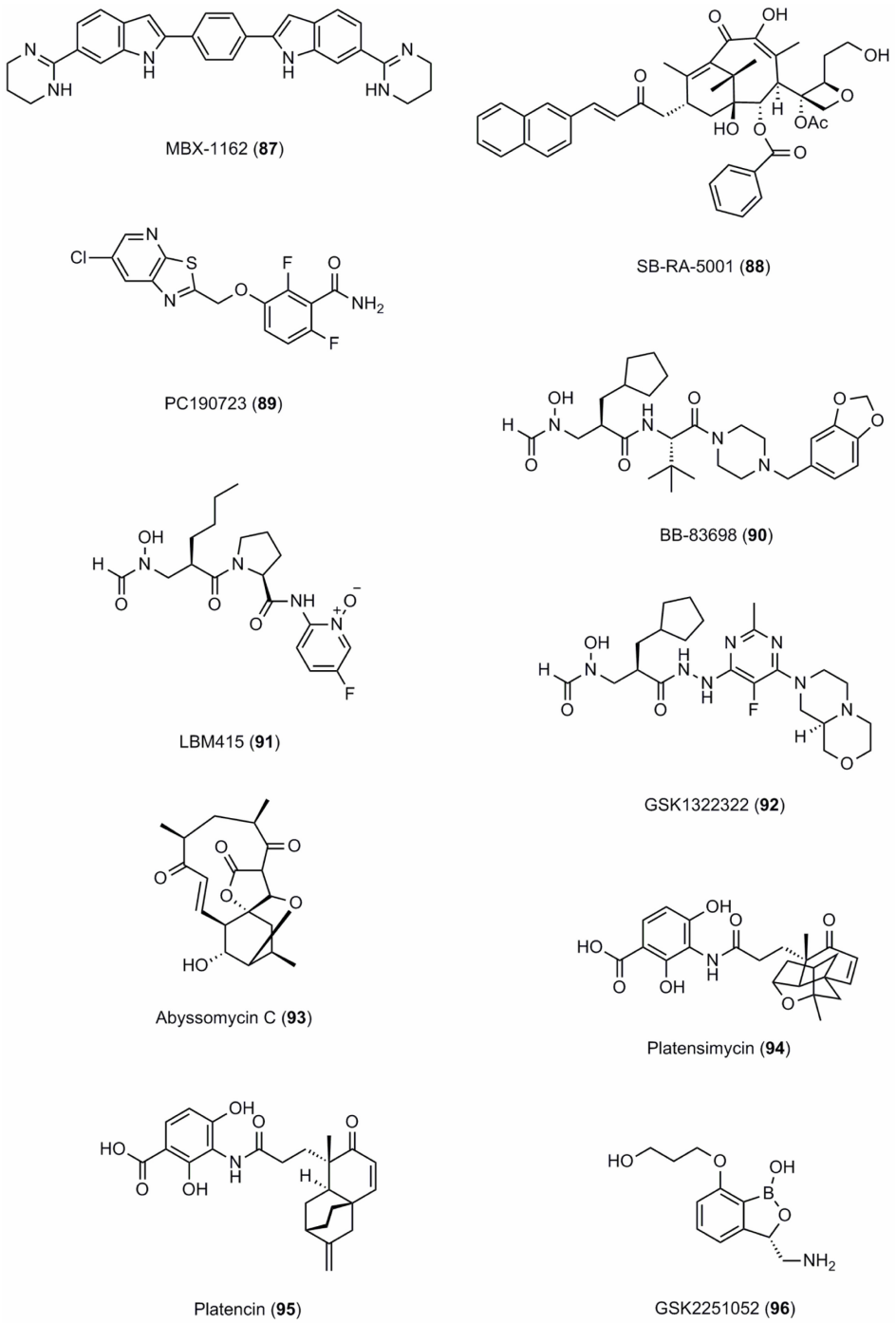


Figure 27. Antibiotic candidates with novel targets.
Notes: MBX-1162 (**87**) is a double stranded nucleic acid groove binder. SB-RA-5001 (**88**) and PC190723 (**89**) target FtsZ. BB-83698 (**90**), LBM415 (**91**), and GSK1322322 (**92**) target peptide deformylase. Abyssomycin C (**93**) blocks p-aminobenzoic acid formation in the tetrahydrofolate biosynthetic pathway. Platensimycin (**94**) targets FabF and platencin (**95**) is a dual FabF / FabH inhibitor. GSK2251052 (**96**) inhibits leucyl-tRNA synthetase.

enzymes involved in post-translational peptide modification. Peptide deformylase, a highly conserved metalloprotease that hydrolyzes the terminal *N*-formyl group present in all bacterial peptides, is a target that has garnered attention for many years now. Significant research efforts have led to many diverse inhibitors of this enzyme, but they have met with limited success for a variety of reasons.[395, 396] Disappointingly, two compounds, BB-83698 (**90**) and LBM415 (**91**), were even advanced to phase I trials before clinical development was discontinued.[397, 398] A hydrazide, GSK1322322 (**92**), has recently advanced to phase II clinical trials for the treatment of gram positive infections, however, keeping hope alive that this target may see a fully developed antibiotic.[399]

Numerous efforts at generating new antibiotics have been focused on biosynthetic enzymes. In 2004 the first of a family of spirotetronate-polyketides, abyssomycin C (**93**), was isolated from a marine strain of actinomycetes.[400] These molecules, like sulfonamides, are tetrahydrofolate synthesis inhibitors. Their antibacterial activity is derived, however, from blocking formation of *p*-aminobenzoic acid rather than its conversion to dihydropteroate, an unprecedented mechanism of action. The potency of these molecules against highly resistant *S. aureus* strains has made them attractive lead compounds, however their cytotoxicity has thus far stifled their advancement towards clinical usage.[401, 402]

Fatty acid biosynthesis inhibitors have also recently generated quite a bit of interest in the antibiotics field. Like inhibiting tetrahydrofolate synthesis, targeting fatty acid synthesis is not in itself novel with FabI [enoyl-acyl carrier protein (ACP) reductase] as an established target for the antibiotics isoniazid and triclosan. However, other enzymes in this biosynthetic pathway remain unexploited. FabF [β-ketoacyl-acyl carrier protein (ACP) synthase II] in particular has recently received significant attention[403–410] largely due to the 2006 discovery of platensimycin (**94**) and its subsequent identification as a FabF inhibitor through an RNA antisense based assay.[411412–413] This molecule has shown potent activity against broad spectrum of gram positive bacteria by blocking the malonyl-ACP binding site of FabF.[412] Even more promising is a synergistic dual inhibitor of FabF and FabH (β-ketoacyl-ACP synthase III), platencin (**95**).[414,415] Though these compounds are both promising, their pharmacokinetics is not optimal and both remain only in pre-clinical stages.[416]

The vast majority of antibiotic candidates with untried targets have shown promise only against gram positive bacteria. The 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo) biosynthetic pathway is one target that could have an impact against gram negative bacteria, however. This monosaccharide is an essential component of the LPS layer of the bacterial outer membrane, but intriguingly is not present among mammalian carbohydrates. Unfortunately, though *in vitro* inhibitors of many steps in this pathway have been synthesized, they have thus far all lacked *in vivo* activity. In some cases this is because of insufficient bioavailability, but in many others it is cellular permeability which is likely the challenge as it is often the case when targeting gram negative pathogens.[417–424]

The target currently closest to potentially achieving clinical validation is likely leucyl-tRNA synthetase. Benzoxaborole compounds have been found to inhibit protein synthesis by binding the terminal adenosine ribose of this enzyme. One of these compounds, GSK2251052 (AN3365) (**96**), has good activity against a broad spectrum of gram positive and negative bacteria. It advanced to phase II clinical trials where its development has currently stalled.[425, 426] Another benzoxaborole has advanced to phase III trials for the treatment of fungal infections and mupirocin, an isoleucyl-tRNA synthetase inhibitor, has been an exemplary topical antibiotic suggesting that it may only be a matter of time until an antibiotic with this target is approved.[427]

Conclusions and Outlook

Many years of stagnant development and the alarming rise of bacterial resistance fueled by irresponsible policies and practices has created an undeniably dangerous quandary for the field of antibiotics research. Recent efforts by diverse groups including scientists, medical doctors, and even in some cases politicians, have shed light on this predicament, however. The approval of five new classes of antibiotics since the turn of the century to combat the emergent resistant gram-positive pathogens of the 1990s was a step in the right direction. Advances in scientific technology have provided the tools necessary for the discovery of new antibiotic classes and the improvement of already established ones to combat the largely unchecked rise of resistant gram-negative pathogens. It remains to be seen whether these encouraging developments will flower with increases in funding and the backing of major pharmaceutical companies into an antibiotic renaissance or if they will wilt, paving the way for a dreaded "post-antibiotic" era.

Glossary

Actinomycetes: Soil bacteria that produce the majority of currently identified natural product antibiotics. In particular, the genus *Streptomyces* has historically been a prolific source of antibacterial agents.

Aerobic Bacteria: All aerobic bacteria require oxygen for growth. Microaerophiles require some oxygen for growth, however they are harmed by high concentrations of it.

Anaerobic Bacteria: Bacteria that do not require oxygen for growth. Obligate anaerobes are incapable of growing in oxygenated environments. Aerotolerant anaerobes can grow in oxygenated environments, but are incapable of utilizing oxygen. Facultative anaerobes are capable of utilizing oxygen for growth, but are also capable of surviving in oxygen free environments.

Bactericidal Agent: An agent that is capable of killing bacteria. These can be antiseptics, disinfectants, or antibiotics.

Bacteriostatic Agent: An agent that stops bacteria from reproducing while not harming them otherwise. Unlike bactericidal agents they are not capable of killing bacteria on their own.

Biofilm: A sessile community of microorganisms that adhere to a surface. Some biofilm forming bacteria produce

exopolysaccharide sheaths that make them dramatically less susceptible to antibiotics and other environmental toxins.

Center for Disease Control and Prevention (CDC): An agency of the United States Department of Health and Human Services that is in charge of monitoring and maintaining the health safety of its residents in regard to both non-communicable and communicable disease.

Commensal Bacteria: Bacteria that benefit from their host environment without causing harm to the host. These bacteria are non-pathogenic.

Cytotoxin: Substances that are toxic to cells. They can induce cell death through apoptosis or necrosis or they can simply reduce cell viability.

Efflux Pump: Protein or glycoprotein complexes located in the cell membrane that are responsible for energy-dependent, active transport of toxins out of cells. These structures play a major role in bacterial antibiotic resistance. Bacterial efflux pumps are categorized by five sub-families: Major facilitator superfamily (MFS), ATP-binding cassette superfamily (ABC), small multi-drug resistance family (SMR), resistance-nodulation cell-division superfamily (RND), and multi-antimicrobial extrusion protein family (MATE).

Endotoxin: Toxins that are not secreted by bacteria, but rather are a part of their cellular membrane and are released only upon its degradation. These toxins are most often lipopolysaccharides.

***Enterobacteriaceae*:** A family of gram-negative bacteria that includes many non-pathogenic species as well as many problem pathogens including *Klebsiella*, *Shiegella*, *Enterobacter*, *Salmonella*, *E. coli*, and *Y. pestis*.

Enterotoxin: Protein exotoxins that target the intestines.

Exotoxin: A broad term referring to any toxin that is secreted by the bacteria. Many exotoxins are highly potent and can be potentially lethal to humans.

Food and Drug Administration (FDA): An agency of the United States Department of Health and Human Services that regulates food, drugs, and cosmetic products. One of the duties of the FDA within the context of pharmaceuticals is the approval of new drugs for public consumption.

Gram-negative Bacteria: Bacteria that have a lipopolysaccharide / protein outer cell membrane and an inner cell membrane with a peptidoglycan layer sandwiched between the two. Their outer cell membrane does not retain Gram stain allowing them to be differentiated from gram-positive bacteria.

Gram-positive Bacteria: Bacteria that have a thick peptidoglycan cell wall surrounding their cell membrane which is capable of retaining Gram stain.

Infectious Diseases Society of America (IDSA): An association based in the United States that represents health care professionals and scientists from around the world that specialize in infectious diseases. The society promotes research, education, and initiatives related to this field.

Methylase: Otherwise known as methyltransferases, these enzymes are highly relevant in many aspects of biology and medicine. In the context of antibiotics they are a common bacterial resistance mechanism. Bacteria utilize them to modify drug targets with methyl groups thereby decreasing the affinity of the antibiotic.

Nosocomial Infection: Also referred to as hospital acquired infections (HAIs), these infections occur in hospital associated environments.

Opportunistic Pathogen: A microorganism that is normally commensal, but can become pathogenic in hosts with compromised immune systems.

Penicillin-binding Proteins: A large group of proteins essential for cell wall biogenesis that are all characterized by their ability to irreversibly bind β-lactam antibiotics.

Peptidoglycan: A polymeric saccharide and amino acid structure. In a cross linked form it is the primary constituent of the cell wall of bacteria. Gram positive bacteria have a thick peptidoglycan layer outside of their cell membrane. Gram negative bacteria have a much thinner peptidoglycan layer located between an inner and an outer cell membrane.

Porin: Beta-barrel, transmembrane, transport proteins that allow small to medium sized molecules to pass through cell membranes.

Structure-activity Relationship (SAR): The relationship between the chemical structure of a molecule and its biological activity. Medicinal chemists probe this relationship by manipulating functional groups or even larger portions of a molecule and then observing the changes to biological activity that result.

World Health Organization (WHO): An agency of the United Nations with a focus on international public health. The WHO monitors and advises on all aspects of public health including trends in communicable diseases.

Zoonotic Infection: A disease transmitted from animals to humans. These infections can occur via contact with living animals or through the consumption of foods that are either products of animals or have been contaminated by animals.

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Author Contributions

Wrote the first draft of the manuscript: RJF. Contributed to the writing of the manuscript: RJF, YT. Agree with manuscript results and conclusions: RJF, YT. Jointly developed the structure and arguments for the paper: RJF, YT. Made critical revisions and approved final version: RJF, YT. Both authors reviewed and approved of the final manuscript.

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