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Review

Antibiotics in late clinical development

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ABSTRACT

Most pharmaceutical companies have stopped or have severely limited investments to discover and develop new antibiotics to treat the increasing prevalence of infections caused by multi-drug resistant bacteria, because the return on investment has been mostly negative for antibiotics that received marketing approved in the last few decades. In contrast, a few small companies have taken on this challenge and are developing new antibiotics. This review describes those antibiotics in late-stage clinical development. Most of them belong to existing antibiotic classes and a few with a narrow spectrum of activity are novel compounds directed against novel targets. The reasons for some of the past failures to find new molecules and a path forward to help attract investments to fund discovery of new antibiotics are described.

Contents

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1. Introduction

Antibiotics are the first successful class of drugs that can cure disease and have been effective in treating many infections. Most of the antibiotics that are commonly used today were discovered in what is known as the "Golden Age" of antibiotics [1], have lost patent protection, and as with most generic drugs, are low priced. Low cost and easy access have led to their overuse and misuse. Bacteria evolve when subjected to selective pressure and multi-antibiotic resistance in organisms as ubiquitous as *Escherichia coli* and *Staphylococcus aureus* has been observed. Antibiotic resistance has drawn attention from infectious diseases specialists, the Centers for Disease Control and Prevention, the World Health Organization, and U.S. and European governments [2–4]. Large pharmaceutical companies have re-directed their resources to develop drugs for chronic use, and for other areas, such as cancer, where the drugs can be priced high, leaving antibiotic discovery and development to small companies and start-up biotechnology companies.

2. Why is it difficult to obtain investments for new antibiotics?

Many companies have been founded based on early-stage molecules or on screening methods against new targets. Novel compounds directed against novel targets have been reported against specific pathogens [5]. Pharmaceutical companies have invested in such programs over the past few decades but were not successful in finding new products. Some reasons for the lack of success of these programs are described here: i) Simple, small molecules directed against enzyme targets selected for resistance rapidly, even during treatment [1,5]. It should be noted that previous antibiotics were generally complex natural products with multiple binding sites at the target, making it less likely for resistance selection. ii) Many novel targets are genus, species or even strain specific. Clinical trials for new antibiotics are expected to cover disease indications that could have more than one bacterial species involved in the infection and not just a single pathogen. Recently approved, highly effective antibiotics, such as fidaxomicin that is pathogen-specific for *Clostridium difficile*, has not been able to compete in the market with older generics, such as vancomycin, which has uses in infections caused by other Gram-positive bacteria and therefore broader commercial use (such drugs are generally effective and sold at a lower price). iii) Antibiotic dosages are often in the range of hundreds of milligrams per day, so they should have exquisite selectivity for bacteria to avoid toxic effects on mammalian cells. However, many molecules that are active against bacteria are not selective and can cause collateral damage in the host. Exceptions are certain antibiotics, such as the polymyxins that have unacceptable renal toxicities that are used as the last resort to treat infections caused by multi-drug resistant pathogens. Rapid, ideally bedside, diagnostic tools using modern technology would be helpful to select a narrow spectrum, targeted therapy. Although a few have been approved for clinical use, the cost of these tools has impeded wide use. As a consequence of the above, new antibiotics are frequently reserved as a last resort for infections caused by multi-drug resistant pathogens that have failed treatment with currently available antibiotics. For all the reasons described above, investments made by large pharmaceutical companies did not lead to profitable products and the majority of pharmaceutical companies have exited this therapeutic area.

If large pharmaceutical companies have failed, why do startup companies believe they can find new antibiotics? Small start-up companies believe they can succeed where large companies do not because they take novel approaches to drug development, and have smaller commercialization needs to cover infrastructure costs. For example, novel natural product screening methods are being tested [6]; total synthesis of tetracyclines and macrolides which are of natural product origin have been conducted to allow modifications in the structure, that were hitherto not accessible to semi-synthesis [7,8]; novel chemistries [8]; expression of polyketide genes in heterologous hosts to synthesize new, complex products [9,10]. In addition, start-up companies have a razor-sharp focus and persistence to overcome barriers to development such as in chemistry or bioavailability. These approaches are expected to be helpful in finding new molecules for the drug discovery and development in start-up small companies that have the advantage of a singular focus with lower cost programs.

3. Antibiotics that have been approved in recent years

Three antibiotics, telithromycin, temafloxacin and trovofloxacin that were approved in the late 1990s and in the early 2000s were reported to have serious adverse events and subsequent product discontinuations [11–13]. These product failures and broad use of antibiotics, especially for simple infections, which amplified less frequent adverse events led to major changes in U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) guidance documents for conducting clinical trials. Efficacy endpoints were set with greater definition and clinical trials using non-inferiority margins were for complicated infections only. Stewardship and the movement to limit antibiotic usage are at odds with antibiotic use in simple infections, such as sinusitis and otitis media. The FDA guidance for clinical trials in simple infections requires that superiority be demonstrated [14]. Such trials are difficult to enroll and would need to be extraordinarily large to achieve statistical superiority, since most of these infections can resolve without therapy. Thus, new antibiotic approval is generally limited to marketing for complicated or more serious infections. Once approved, stewardship is likely to be practiced because of limited label indications and higher cost. According to the Association for Professionals in Infection Control and Epidemiology (APIC), antibiotic stewardship "promotes the appropriate use of antibiotics, improves patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms" [15]. The Infectious Diseases Society of America (IDSA) further emphasizes that selecting the optimal drug regimen, dose, duration, and route of administration are critical to the practice of stewardship. Furthermore, stewardship seeks to minimize toxicity and other adverse events as well as reduce the costs of healthcare infections [16].

During the last 6 years the following small molecule antibiotics have received marketing authorization: ceftaroline for acute bacterial skin and skin structure infection (ABSSSI) and community-acquired bacterial pneumonia (CABP); oritavancin for ABSSSI and CABP; telavancin for ABSSSI and CABP; dalbavancin for ABSSSI; tedizolid for ABSSSI; fidaxomicin for *C. difficile* colitis; ceftazidime-avibactam for complicated urinary tract infection

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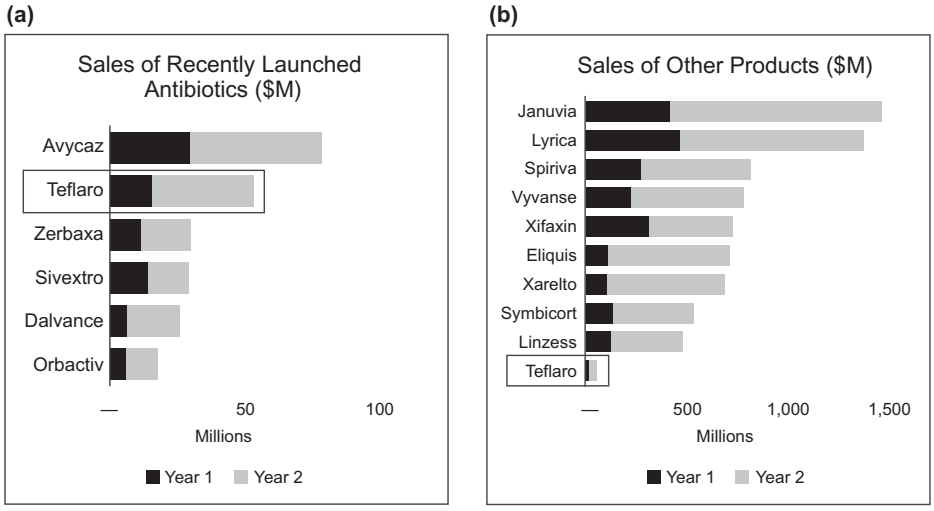

Fig. 1. (a and b) Product launches: new antibiotics vs. other brands: disappointing sales of recently launched antibiotics (~20 M–80 M) two years post launch; Teflaro (ceftaroline fosamil), an IV antibiotic indicated for CABP had sales ~$50 M 2 years post launch, while other classes of drugs had sales between $500 million – over $1B. *Projected Sales (year 2). Source: NSP $ Sales, IMS 2016.

(cUTI) and intraabdominal infection (IAI); and ceftolozane-avibactam for IAI and cUTI. Although the regulatory path is now clear, the revenues generated from each of these products is still small and the return on investment is low or negative compared with those of other therapeutic areas. (Fig. 1a, b). It is important to note that dalbavancin, telavancin and oritavancin compete with generic vancomycin and daptomycin. These new antibiotics were approved based on non-inferiority to lower cost antibiotics, and although the newer antibiotics may offer a more convenient dosing schedule, this alone has not convinced payers and patients that new antibiotics offer higher value. Fidaxomicin, as noted previously, must compete with generic vancomycin and metronidazole. Ceftaroline must compete with generic ceftriaxone, and tedizolid must compete with generic linezolid. Except for tedizolid, all recently approved antibiotics are administered intravenously. Generic antibiotics continue to be used in the outpatient sector despite treatment failures and increased hospitalizations [17].

This review will discuss those small molecules that are in late clinical development, i.e., those in Phase 3 clinical trials or with published Phase 2 clinical trial data. Where possible, antibiotics under development are grouped by class and similar mechanism of action. This review will focus on small molecules only and not vaccines, phage or antibody therapies.

4. Beta-lactamases, cephalosporinase, and carbapenemase inhibitors in combination with old and new beta-lactams

The success of ampicillin-clavulanic acid and ampicillin-sulbactam combinations that were widely used in outpatients, especially for urinary tract infections has led to the selection of new beta-lactamases and more treatment failures. Piperacillin-tazobactam, a next-generation product, was developed in the 1990s to treat serious Gram-negative infections, such as intra-abdominal infections and complicated urinary tract infections. The increasing incidence of new cephalosporinase and carbapenemases since introduction of piperacillin-tazobactam increased the interest in finding new beta-lactam plus new beta-lactamase inhibitor combinations [18,19]. Ceftazidime-avibactam (Avycaz) (**1**), a fixed-combination drug containing ceftazidime, a generic third-generation cephalosporin, and avibactam, a new beta-lactamase inhibitor, received regulatory approval to treat adults with complicated intra-abdominal infections (cIAI), and complicated urinary tract infections (cUTI) who have limited or no alternative treatment options. Interestingly, this approval was supported, in part, by the efficacy and safety of ceftazidime for the treatment of cIAI and cUTI. The contribution of avibactam to Avycaz was based on data from in vitro studies and animal models of infection. Two Phase 3 trials for Avycaz, one each in cIAI and cUTI were conducted (NCT01726023, NCT01599806) (Fig. 2).

Another cephalosporin–beta-lactamase inhibitor combination that was recently approved (December 2014) by the U.S. FDA was ceftolozane/tazobactam (Zerbaxa) (**2**), also used to treat adults with cIAI and cUTI. In this combination, the cephalosporin is new and the beta-lactam inhibitor was known, being the same beta-lactamase inhibitor as in piperacillin-tazobactam. Like the efficacy of the ceftazidime-avibactam combination, ceftolozane/tazobactam's efficacy to treat cIAI in combination with metronidazole was established in a clinical trial (NCT01147640) with a total of 979 adults. The results showed that Zerbaxa plus metronidazole was effective for the treatment of cIAI. In addition, the efficacy of Zerbaxa to treat cUTI was established in a clinical trial of 1068 patients where it was shown to be non-inferior to levofloxacin (NCT01345929).

Klebsiella pneumoniae strains resistant to carbapenem (carbapenem-resistant Enterobacteriaceae or CRE), have been reported. Effort is underway to find new combinations to treat these infections. Carbavance (Rempex, a subsidiary of Medicines Company), is a meropenem and a novel boronic beta-lactamase inhibitor RPX7009 (**3**) (aka vaborbactam) [20]. It is in Phase 3 development and will target Gram-negative bacteria that produce new beta-lactamase enzymes that have spread in the U.S. and Europe, including strains producing the *Klebsiella pneumoniae* carbapenemase (KPC) enzyme. The first Phase 3 trial (TANGO 1) (NCT02166476) was a randomized, comparative study against piperacillin/tazobactam for the treatment of cUTI. Carbavance was statistically superior to piperacillin/tazobactam, with overall success in 98.4% of treated patients. Safety of carbavance was comparable to that of piperacillin/tazobactam. A second Phase 3 trial (TANGO 2) (NCT02168946) is a multi-center, randomized, open-label study of carbavance versus best available therapy in subjects with selected serious infections due to CRE. Approximately 150 study subjects with cUTI, nosocomial pneumonia and/or bacteremia are expected to be randomly assigned (2:1) to carbavance or "best available therapy" for up to 14 days. This study is ongoing and will provide supportive data for the regulatory marketing authorization submission. When approved, carbavance will target

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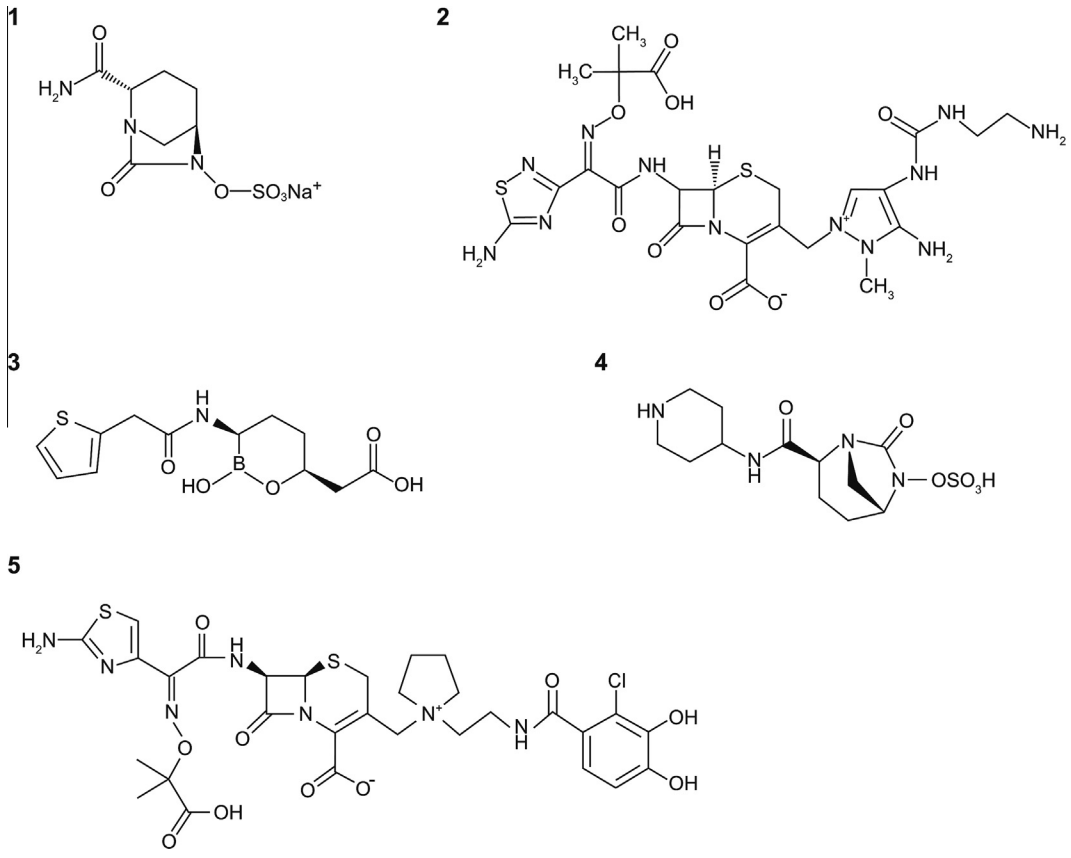

Fig. 2. Structures of compounds targeting new beta-lactamase producing enteric pathogens: i) beta-lactamases: Beta-lactam–beta-lactamase inhibitors. Only the chemical structures of new members of each combination are shown: 1) Avibactam; 2) Ceftolozane; 3) Vaborbactam (RPX7009); 4) Relebactam; ii) Siderophore-containing beta lactams: 5) S649266.

the urgent and growing global threat of deadly Gram-negative "superbugs", including the CRE.

In an effort to meet the growing need for antibiotics to treat CRE, a new monobactam antibiotic, relebactam (**4**), in combination with the old carbapenem, imipenem, and the carbapenemase inhibitor, cilastatin (MK-7655) is being developed (Merck & Co. Inc.) [21]. The challenge is to optimize the pharmacokinetics and safety for three drugs while gaining efficacy against CRE and other serious infections caused by Gram-negative bacteria. The target indications are cUTI, acute pyelonephritis, cIAI, and hospital-acquired bacterial pneumonia (HAP)/ventilator-associated bacterial pneumonia (VAP). In a phase 2 blinded, comparative study of patients with cUTI or acute pyelonephritis (NCT01505634), relebactam plus imipenem/cilastatin was non-inferior to imipenem/cilastatin alone in the proportion of microbiologically evaluable patients with a favorable microbiological outcome at the end of IV infusion. A phase 3 trial of relebactam with imipenem/cilastatin has been initiated in which imipenem/cilastatin/relebactam is being compared to colistin in combination with imipenem/cilastatin for the treatment of resistant bacterial infections, including those caused by *Pseudomonas aeruginosa* and KPC-producing organisms (NCT02452047). A second phase 3 study is also enrolling patients, in which a fixed-dose combination of imipenem/relebactam/cilastatin is being compared to piperacillin/tazobactam in patients with HAP/VAP (NCT02493764).

Another combination, avibactam with aztreonam (AstraZeneca plc. and its U.S. partner Allergan plc., formerly Actavis), another generic antibiotic, is being developed for the treatment of IAI along with metallo-β-lactamase-producing Gram-negative infections [22]. It is currently in Phase 2 clinical development but with the precedence of avibactam and aztreonam both being approved drugs, the combinations may receive regulatory approval without additional trials. (A phase 2 trial to determine the PK, safety, and tolerability of aztreonam-avibactam (ATM-AVI) in the treatment of hospitalized patients with cIAI is currently recruiting patients; NCT02655419). This could be similar to an "Intel inside" model, where avibactam could be used in combination with other penicillins, monobactams, cephems and cephalosporins giving new life to old beta-lactams.

Other beta-lactamase inhibitors are in development but no other combinations are in late clinical development capable of addressing new beta-lactamases that are likely to evolve but entering a crowded antibiotic market.

5. New cephalosporins and beta-lactams that have activity against beta-lactamase and carbapenemase-producing bacteria

A new cephalosporin, which is not being used in combination with a beta-lactamase inhibitor but does have activity against beta-lactamase and carbapenemase-producing pathogens, is S-649266 [23]. S649266 (Shionogi Inc.) is a novel, siderophore cephalosporin (**5**). It is an injectable cephalosporin in Phase 3 development, which is highly active against Gram-negative pathogens including multidrug-resistant Gram-negative bacteria known to cause bloodstream infections, HAP, VAP, and cUTI. It was shown to be effective in a Phase 2 trial in cUTI (NCT02321800) and is currently being evaluated in a Phase 3 study (CREDIBLE) for treatment of severe infections caused by carbapenem-resistant Gram-negative pathogens (NCT02714595).

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Although BAL30072 [24] (Basilea Pharmaceutica Ltd.) is in earlier clinical development, it is mentioned here as it is a new siderophore monosulfactam antibiotic (**6**) with activity against multidrug-resistant Gram-negative bacteria. It also utilizes natural iron uptake systems to gain access to its target. The siderophore side chain also contributes directly to its antibacterial activity against clinically increasingly problematic multidrug-resistant Gram-negative bacteria such as *Pseudomonas* spp. and *Klebsiella* spp. Furthermore, there is new evidence that an accelerated uptake of BAL30072, facilitated by the compound's siderophore side chain, potentiates its in vitro activity against *Acinetobacter baumannii*, a pathogen that can cause severe pneumonia, infections of the urinary tract, and bacteremia.

6. New aminoglycosides

Streptomycin was the first aminoglycoside discovered in 1943 and is known for being one of the early drugs used to treat tuberculosis. Gentamicin, also a natural product is an aminoglycoside that was introduced in the 1960s to treat *P. aeruginosa* infections. This was followed by newer aminoglycosides, the most successful being tobramycin and in the 1980s, amikacin. Resistance to the older aminoglycosides, such as gentamicin and tobramycin is now widespread. There are three main mechanisms of aminoglycoside resistance that are known: decreased cell permeability; alterations at the ribosomal binding sites; and production of aminoglycoside modifying enzymes. Permeability mutants have low-level resistance. While resistance caused by ribosomal mutations that interfere with binding to the 30S subunit is rare and occurs primarily with streptomycin, the newer aminoglycosides have multiple binding sites and therefore resistance cannot be selected by a single step mutation. There are over 50 different bacterial antibiotic resistance-modifying enzymes, with some isolates carrying more than one enzyme, making this the most common type of aminoglycoside resistance. Enzymatic modification results in high-level resistance. There are three types of aminoglycoside modifying enzymes: N-Acetyltransferases, O-Adenyltransferases and O-Phosphotransferases. Amikacin is currently the most potent aminoglycoside, but resistance to this drug has been observed. Aminoglycosides were introduced as intravenous therapies for serious Gram-negative infections but their use is secondary to the cephalosporins and carbapenems due to the need to monitor aminoglycoside blood concentration to avoid aminoglycoside-associated ototoxicity and nephrotoxicity. Newer aminoglycosides share the same narrow therapeutic window with the older aminoglycosides. More recently, the site of interaction that results in ototoxicity has been characterized [25] and may provide a tool for screening these compounds in the future.

Plazomicin (**7**) (Achaogen, Inc.) is a new aminoglycoside that is in Phase 3 development for the treatment of serious bacterial infections due to MDR *Enterobacteriaceae*, including CRE [26]. Two Phase 3 trials are currently underway. The first trial (EPIC), with a planned enrollment of 530 patients, is a pivotal marketing registrational study to evaluate plazomicin treatment of cUTI, including acute pyelonephritis (NCT02486627). The second trial (CARE), with a planned enrollment of 100 patients, is a supportive study to evaluate plazomicin treatment of serious CRE infections (NCT01970371).

7. New pleuromutilins

Pleuromutilin is a natural product of the fungi *Pleurotus mutilus* (now called *Clitopilus scyphoides*) that inhibits bacterial protein synthesis by binding to the peptidyl transferase site on 23S RNA of the 50S ribosome. Many analogs have been made to enhance activity and bioavailability as this molecule is metabolized extensively. The structure consists of a common tricyclic mutilin core, a C21 keto group, essential for antimicrobial activity, and various diverse chemical extensions at C14. During the early 1980s, extensive effort was devoted to formulate azamulin [27] but because of potent inhibition of cytochrome P450, its development was subsequently discontinued. Another member of this class, retapamulin, was approved by the FDA in 2006 for topical use to treat impetigo. Another pleuoromutilin, tiamulin, has been used as a veterinary drug in Europe and Canada.

Lefamulin (BC-3781) (**8**) (Nabriva Therapeutics AG), which was discovered at Roche, is a semi-synthetic compound that inhibits bacterial protein synthesis [28,29]. However, during a brief period of out-license in the US to Forest laboratories, it was tested in a Phase 2 trial for ABSSSI against vancomycin (NCT01119105). Lefamulin has a spectrum of activity that includes multi-drug resistant Gram-positive strains. In the completed Phase 2 trial in ABSSSI that was conducted by Forest Laboratories (NCT01119105), intravenous lefamulin achieved a high cure rate against multi-drug resistant Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). It also achieves high drug concentrations in the lung tissue and is expected to be available in both IV and oral formulations. A Phase 3 trial in CABP using intravenous and oral lefamulin (NCT02559310) was initiated in September 2015 and a second Phase 3 trial in CABP (NCT02813694) using oral lefamulin was initiated in April 2016. If these trials are successful and lefamulin is approved, it would be the first pleuromutilin antibiotic available for systemic human infections.

8. New tetracyclines

The tetracyclines are another old class of natural product antibiotics, discovered in the 1940s. Initially, they were used topically but after the development of doxycycline, they were used to treat a variety of infections including respiratory tract and skin infections. Tetracyclines also inhibit protein synthesis by inhibiting acyl-tRNA transfer on the 30S ribosome. Tetracyclines have a fused linear tetracyclic structure and form chelation complexes with divalent cations. Analogs that reduce chelation also have lower activity. Tetracyclines have broad-spectrum activity, including against Gram-positive and Gram-negative bacteria as well as anaerobic bacteria. Their use has been limited to adults because of their calcium complexing properties, but even in adults, their use has been limited by gastrointestinal side effects, especially nausea. They are a useful antibiotic class for patients who are intolerant of macrolides and for treating infections due to macrolide-resistant pathogens. Resistance to tetracyclines results from small proteins that block the tetracycline-binding site on the ribosome or by the expression of efflux proteins [30]. Tigecycline is a semi-synthetic tetracycline, called a glycylcycline, that is administered intravenously for treating serious infections caused by resistant *S. aureus*, *A. baumannii*, and *E. coli*. The major side effects are nausea, vomiting, and diarrhea. There is also a small but significant (0.6%, with a corresponding 95% confidence interval (0.0%, 1.2%) unexplained increase in mortality that resulted in an FDA required black box warning. Omadacycline (**9**) (Paratek Pharmaceuticals Inc.) was developed with the goal of finding potent tetracycline molecules with activity against resistant pathogens and that could be administered intravenously and orally. It is semi-synthetically derived from tetracycline in a subclass called the aminomethylcyclines. It has a broad-spectrum of activity, for Gram-positive, Gram-negative, aerobes, anaerobes, and atypical bacteria. It is being developed for the treatment of ABSSSI and CABP, and is expected to be available in intravenous and oral formulations for use in the hospital and outpatient settings [31]. In the first Phase 3 trial (NCT02378480), a

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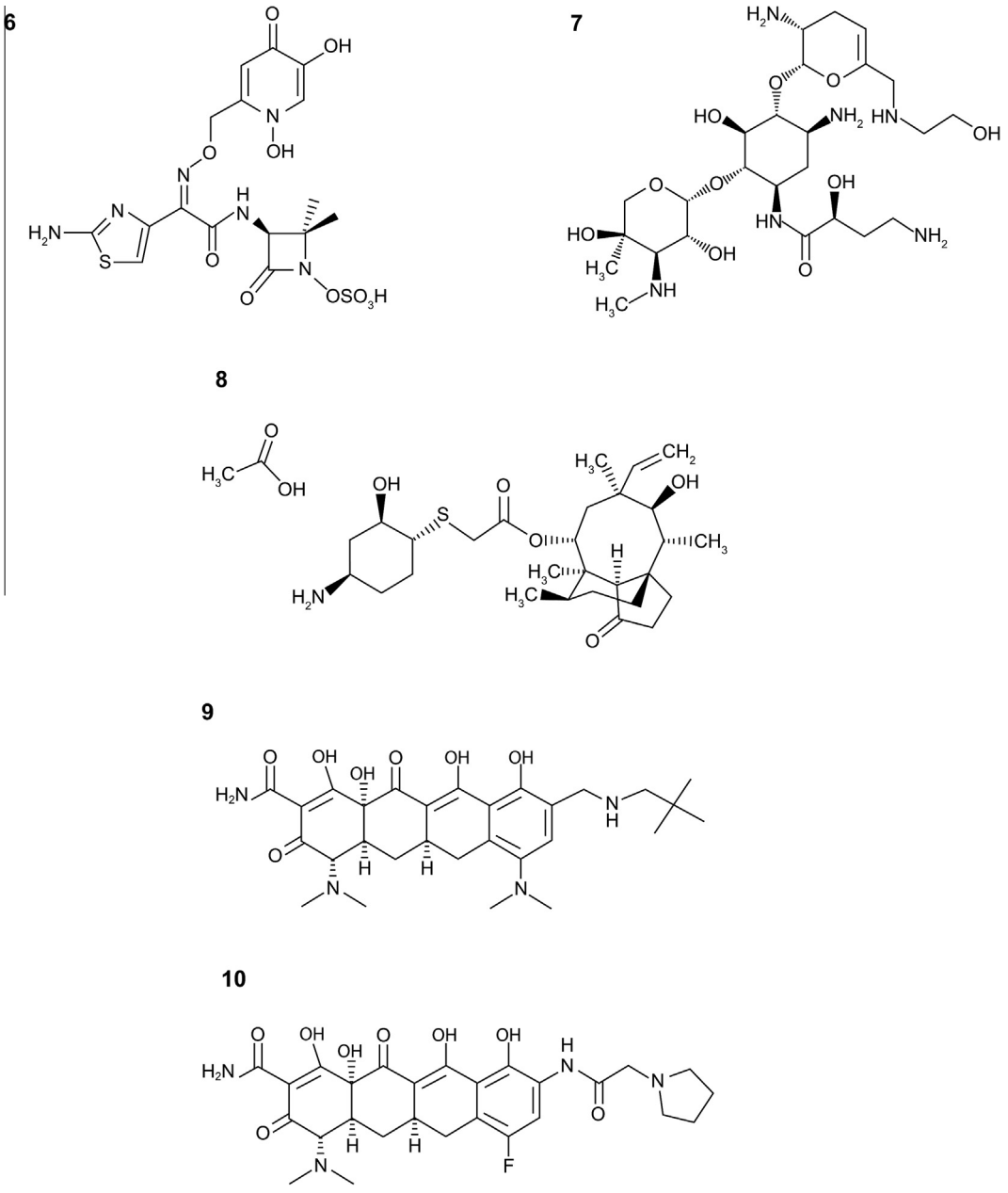

Fig. 3. Chemical structures of the following molecules are shown: 6) BAL30072; 7) Plazomicin; 8) Lefamulin; 9) Omadacycline; 10) Eravacycline.

comparison of intravenous and oral omadacycline to linezolid for treatment of ABSSSI (OASIS), the primary and secondary endpoints were achieved for both oral and IV omadacycline. A second Phase 3 trial comparing omadacycline to moxifloxacin for CABP is currently enrolling (NCT02531438) (Fig. 3).

Natural products are complex molecules. Semi-synthetic analogs can be made but access using synthetic chemistry to some of the sites on the core molecule is limited. Myers et al. [7,8] were able to synthesize tetracyclines synthetically and this led to further exploration of the structure to make analogs. One analog that was derived from this new chemistry is eravacycline (Tetraphase Pharmaceuticals) (**10**), a broad-spectrum, potent tetracycline against multidrug-resistant (MDR) Gram-negative bacteria, being developed for treatment of IAI and cUTI [32]. In the first Phase 3 trial (NCT01844856), a comparative study in cIAI (IGNITE1), eravacycline met the primary endpoint of non-inferiority of the clinical response rate. In the second Phase 3 trial (NCT01978938), a study of intravenous to oral eravacycline compared to levofloxacin in cUTI (IGNITE2), eravacycline did not achieve the primary endpoint. Eravacycline's path to regulatory approval is likely through a second IAI trial but its commercial potential has been decreased with the failure of the oral formulation.

9. New macrolides

Erythromycin was the first member of the macrolide class that was developed for clinical use in the 1950s. In the 1980s, two acid-stable analogs, clarithromycin and azithromycin, were developed. Macrolides are frequently used for respiratory tract infections as they have targeted activity against respiratory pathogens. Macrolide antibiotics achieve high tissue and intracellular concentrations, which helps to address bacteria that are intracellular. In addition, they have strong anti-inflammatory properties. Macrolides inhibit protein synthesis by binding to the 23S RNA of the 50S ribosomal subunit at the exit of the peptide synthesis tunnel. Resistance to the approved macrolides is caused by methylases that

di-methylate the erythromycin binding site on the 23S RNA. Low-level resistance is a result of expression of efflux proteins and both methylases and efflux proteins can be expressed in the same strain. In 2001, a new macrolide, telithromycin, belonging to a new subclass called ketolide, received marketing approval. Ketolides are macrolides in which a keto group replaces the cladinose sugar in the older macrolides. In addition, there is an alkyl-aryl side chain at the 11–12 position. The side chain allows telithromycin to interact at additional sites on the 23S RNA of the bacterial ribosome and this confers activity against strains that are resistant to the older macrolides [33,34]. Following telithromycin's approval in the US in 2003, a series of serious adverse events that included hepatotoxicity, visual disturbance, syncope and exacerbation of myasthenia gravis were reported, which led to changes in the labeled indication. Although still approved for the treatment of CABP, it is no longer used because of the serious adverse events. Cethromycin, another ketolide, was developed for respiratory tract infections but not approved based on lack of efficacy. Solithromycin is a fluoroketolide, with a fluorine at the 2-position of the macrolactone ring (**11**) and an alkyl-aryl side chain at the 11–12 position of the macrolactone ring. It is the next generation macrolide/ketolide as it has activity against telithromycin-resistant bacteria [34]. Working with experts in the nicotinic acetylcholine receptor area, Cempra Inc., conducted extensive work to show that the pyridine on the side chain of telithromycin inhibits the nACh receptors in the eye, neuromuscular junction, liver and brain. Inhibition of these receptors is known to cause the same effects as was observed with telithromycin. Having determined the reasons for the adverse events of telithromycin, Cempra was able to continue the development of solithromycin. Cempra has completed two Phase 3 trials (Solitaire trials) in CABP where solithromycin was shown to be non-inferior to the comparator fluoroquinolone, moxifloxacin. The first Phase 3 trial (NCT01756339) tested oral capsules of solithromycin while the second Phase 3 trial (NCT01968733) tested the intravenous formulation, allowing a switch to the oral capsules of solithromycin. The results of these two trials have been published [35,36]. Solithromycin has good oral bioavailability, is stable in solution, does not prolong cardiac QT interval, and has demonstrated activity against azithromycin-resistant and telithromycin-resistant strains both in vivo and in vitro. New drug applications have been submitted to the FDA and an MAA has been submitted in the European Union. Toyama/Fujifilm, who have licensed solithromycin for use in Japan, have completed a successful Phase 2 trial in CABP where solithromycin was comparable to levofloxacin. Marketing authorization in the US is expected at the end of 2016. Following successful treatment of urogenital gonorrhea in a comparative Phase 2 trial (NCT01591447) [37], solithromycin is now being tested in a Phase 3 trial (NCT02210325). Solithromycin is also being tested in pediatrics and a Phase 2/3 pivotal trial (NCT02605122) in pediatric patients (2 months to 17 years of age) with CABP has been initiated. In addition to the oral capsules and intravenous formulation, a suspension formulation has been developed to provide dosing flexibility for treating children. Finally, non-clinical studies have demonstrated the potential utility of solithromycin for the treatment of infections in pregnancy [38].

10. New fluoroquinolones and DNA gyrase inhibitors

Nalidixic acid was the first quinolone antibacterial agent, which although not of microbial origin also originated indirectly from a natural product, the by-product of chloroquine synthesis, which itself is an analog of quinine extracted from the bark of the *Cinchona* tree. Nalidixic acid was named Negram, because its activity was limited to Gram-negative bacteria. It had high plasma protein binding and was used in simple urinary tract infections. The pharmacokinetics and spectrum of activity were improved by the addition of a fluorine in mefloquine and later by the additional aryl rings to make ciprofloxacin followed by levofloxacin, moxifloxacin, and a host of other fluoroquinolones with potent broad-spectrum activity [39]. Resistance to ciprofloxacin and levofloxacin are now a concern and newer fluoroquinolones that inhibit bacterial DNA gyrase and topoisomerase IV (*gyr*A and *par*C) have been developed. Delafloxacin (Melinta Therapeutics Inc.) (**12**), also known as Baxdela, was initially synthesized at Abbott Laboratories. Delafloxacin has potent coverage of key ABSSSI bacterial pathogens, including MRSA, and is formulated for intravenous and oral administration. It has completed two Phase 3 trials (known as PROCEED), in which the active comparator was vancomycin + aztreonam. The first trial was IV only (NCT01811732) while the second ABSSSI trial was IV/oral (NCT01984684). Both trials met the primary endpoints demonstrating delafloxacin was non-inferior to the comparator. Diarrhea and nausea were the most frequent treatment-related adverse events reported. Melinta has also initiated a Phase 3 program in hospital-treated CABP (NCT02679573), and plans to develop additional indications such as treatment of cUTI.

Another fluoroquinolone in Phase 3 clinical development is Zabofloxacin (**13**) (Dong Wha Pharmaceutical Co. Ltd.) Like delafloxacin, it is a potent inhibitor of the essential bacterial DNA gyrase and topoisomerase IV. It is highly active against respiratory tract pathogens (*S. pneumoniae, S. aureus, Streptococcus pyogenes, and Moraxella catarrhalis*) and is being developed in both intravenous and oral formulations to treat CABP. Finafloxacin (MerLion Pharmaceuticals Pte Ltd.) is another fluoroquinolone that is currently in development. It is approved in the US for treatment of acute otitis externa (swimmer's ear) caused by *P. aeruginosa* and *S. aureus*. It is being developed for cUTI, acute pyelonephritis, cIAI, ABSSSI, diabetic foot infection, and tuberculosis. Other potential applications of finafloxacin are treatment of infections in chronic obstructive pulmonary disease (COPD) patients and *Helicobacter pylori*-related gastritis.

Nemonoxacin (TaiGen Biotechnology Co. Ltd.) is a non-fluorinated quinolone (**14**) that is being developed as once-a-day dosing in both oral and intravenous formulations. It has a broad-spectrum of activity against Gram-positive, Gram-negative, anaerobic, and atypical pathogens and is also active against drug-resistant pathogens, such as MRSA and quinolone-resistant MRSA. Unlike the fluoroquinolones, it has reduced propensity for resistance development, requiring mutations in three different bacterial genes. It has completed Phase 2 clinical trials for treating CABP and diabetic foot infections (NCT01944774, NCT00685698).

The fluoroquinolones were discussed at an Advisory committee meeting convened by the US FDA in November 2015 when a number of serious side effects such as tendonitis, including Achilles tendon rupture, peripheral neuritis and *C. difficile* colitis were discussed. These adverse events led to changes, including black box warnings, which were made to the labeled indications for the approved fluoroquinolones including ciprofloxacin, levofloxacin and moxifloxacin. The impact of label changes for the older fluoroquinolones on new antibacterials in this class will not be known until their regulatory approval.

DNA gyrase is a proven, selective target for antibacterial product development as shown by the fluoroquinolones. Coumermycin is an old natural product antibiotic that inhibits the B subunit of DNA gyrase unlike the fluoroquinolones, that inhibit the A subunit of DNA gyrase. It is active against Gram-positive bacteria but had unacceptable toxicities and is therefore not used clinically. The B subunit of DNA gyrase has also been the target of more recent drug discovery efforts [40]. Gepotidacin (GSK2140944; GlaxoSmithKline plc.) (**15**) is a triazaacenaphthylene DNA gyrase B subunit inhibitor that inhibits bacterial DNA replication [41]. It is being developed for the treatment of cUTI, uncomplicated urogenital gonorrhea, and CABP. Zoliflodacin (ETX0914/AZD0914; Entasis/Astra Zeneca plc.) (**16**) is

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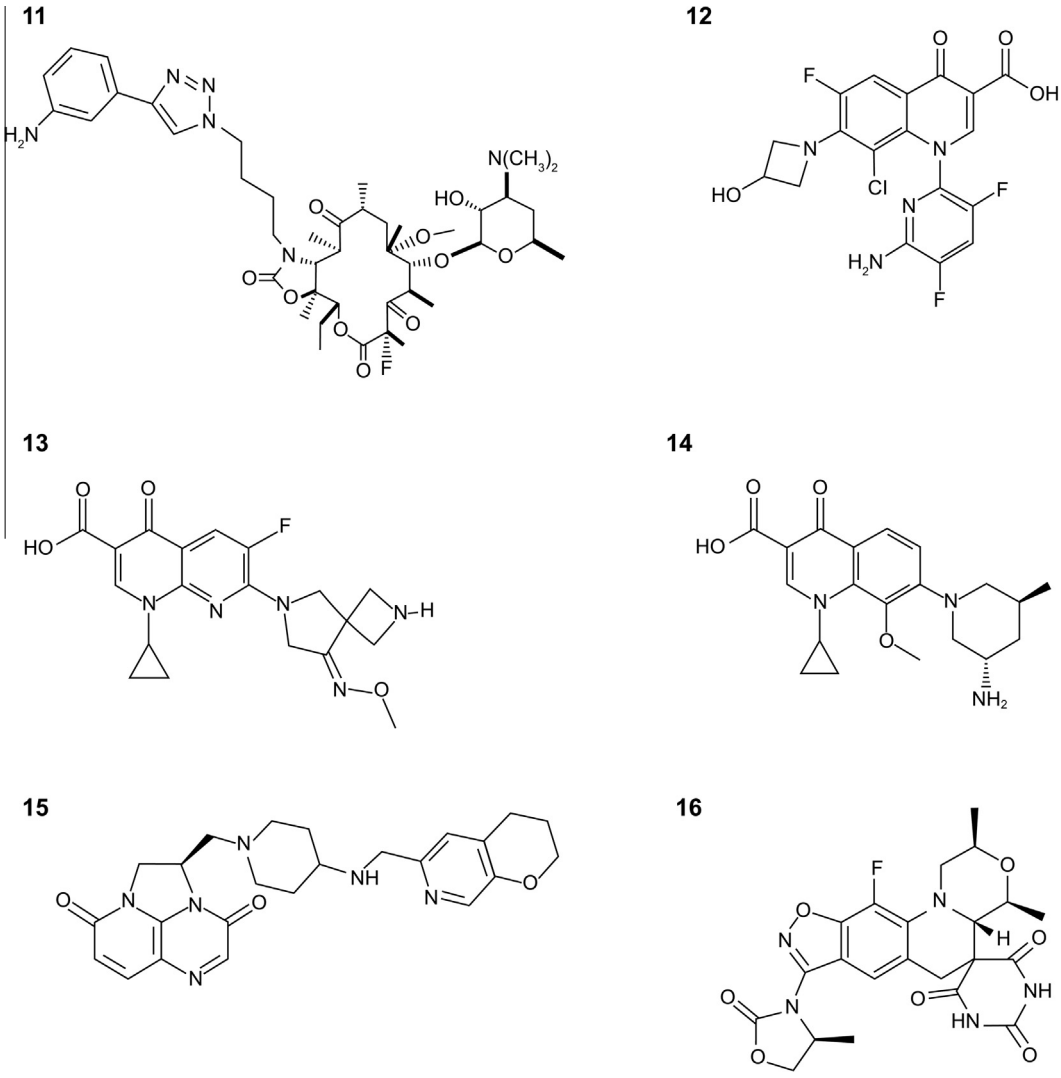

Fig. 4. Chemical structures of the following molecules are shown: 11) Solithromycin; 12) Delafloxacin; 13) Zabofloxacin; 14) Nemonoxacin; 15) Gepotidacin; 16) Zoliflodacin.

a spiropyrimidinetrione [42] that inhibits DNA gyrase (B subunit). It is being tested in a Phase 2, randomized, open-label study in gonorrhea [43] (NCT02257918) (Fig. 4).

11. New oxazolidinones

The oxazolidinones, unlike the previous classes, have no origin in natural products. This class was first discovered at DuPont but because of unacceptable toxicities, was not further developed. The first approved member of this class is linezolid, which was made at Pharmacia and later marketed by Pfizer after its acquisition of Pharmacia. It is a bacterial protein synthesis inhibitor through its binding to the peptidyl transferase site. The peptidyl transferase site in bacteria is similar to the peptidyl transferase site in mitochondrial RNA and some of the limitations of dosing with linezolid, such as myelosuppression, could be related to the effect on mitochondrial protein synthesis. Linezolid is also a monoamine oxidase inhibitor and should not be used with serotonergic drugs. Tedizolid is a newer oxazolidinone, which is more potent and is effective at a lower dose than linezolid. It has additional binding sites at the peptidyl transferase site and thus can overcome linezolid resistance.

Cadazolid (Actelion Pharmaceuticals Ltd.), a quinolonyl-oxazolidinone (**17**) is in Phase 3 development for the treatment of *C. difficile*-associated diarrhea (CDAD) [44]. Two Phase 3 trials (IMPACT) are enrolling, and will compare the efficacy and safety of cadazolid versus vancomycin (NCT01987895, NCT01983683).

MRX-1 (MicuRx Pharmaceuticals Inc.), another novel oxazolidinone, is active against multi-drug resistant (MDR) Gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). It is being developed for the treatment of ABSSSI. It had comparable activity to linezolid in a Phase 2 ABSSSI trial conducted in China. A second Phase 2 study evaluating patients with ABSSSI is being conducted in the U.S. (NCT02269319). In these clinical trials, MRX-I has not caused myelosuppression, the key limiting side effect seen with linezolid.

Sutezolid (PNU-100480) (Sequella, Inc.) is an oxazolidinone active against multi-drug resistant (MDR) Gram-positive pathogens and is being developed for the treatment of extensively drug-resistant tuberculosis. The drug was initially developed by Pfizer and successfully completed a Phase 2 study in tuberculosis (NCT01225640), but was then licensed by Sequella, Inc. in 2013.

12. New fatty acid biosynthesis inhibitors

Enzymes involved in fatty acid synthesis have been targets of antibiotic drug discovery. Triclosan is a chlorinated aromatic compound that is used in soaps and is a known FabI inhibitor (fatty

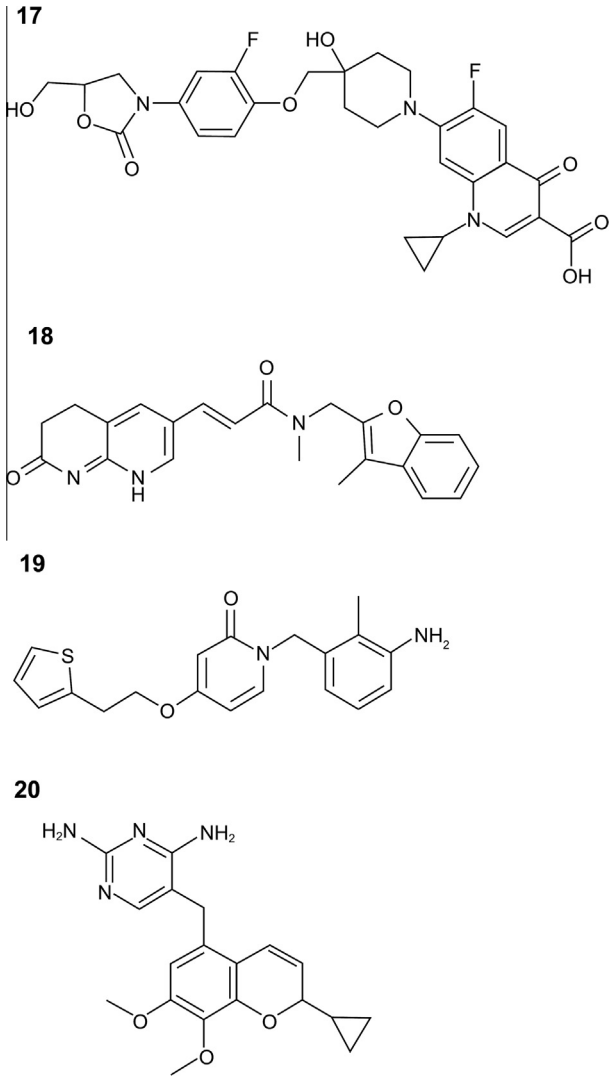

Fig. 5. Chemical structures of the following molecules are shown: 17) Cadazolid; 18) Debio 1452; 19) CG400549; 20) Iclaprim.

acid biosynthesis type 1 inhibitor with the actual step in biosynthesis being Enoyl-acyl carrier protein reductase). Its use in soaps was shown to be problematic due to the fact that it induces resistance to other antibiotics by activating efflux pumps [45]. Debio 1452 (Debiopharm Group) (**18**), which was licensed from Affinium and is active against all staphylococcal-resistant strains tested to date, is currently in Phase 2 development. This FabI inhibitor is a staphylococcus-specific antibiotic designed from the crystal structure of the active site of the enzyme [46]. It is being developed for treatment of ABSSSI and osteomyelitis. A Phase 2 study in ABSSSI (NCT02426918) was initiated in 2015 to compare efficacy of intravenous Debio 1450, a Debio 1452 prodrug, with a switch to oral Debio 1450 compared to intravenous vancomycin with a switch to oral linezolid. CG400549 (CrystalGenomics Inc.) is another FabI inhibitor (**19**) that is being developed [47]. As with the Debio FabI inhibitors, CG400549 is also a staphylococcus-specific antibiotic, which is currently in a Phase 2a study in the U.S for the treatment of ABSSSI caused by MRSA (NCT01593761).

13. New folate biosynthesis inhibitors

Trimethoprim binds to dihydrofolate reductase and inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF) and has selectivity for the bacterial enzyme. It is used in combination with sulfamethoxazole, which inhibits dihydropteroate synthase, an enzyme involved further upstream in the same pathway. Sulfa drugs have been known even before the discovery of penicillin. Serious adverse events including photosensitivity and severe skin rash have been observed with these drugs. Theoretically, inhibition of two enzymes in the same pathway is expected to show synergism and also decrease resistance development. Therefore, the combination of trimethoprim with sulfamethoxazole was developed and is commonly used to treat urinary tract and skin infections. Trimethoprim is also known to cause thrombocytopenia by lowering folic acid levels. In the 1990s, pharmaceutical companies like Roche, were looking for ways to eliminate the use of sulfa drugs in combination with trimethoprim while improving potency for the oral treatment of resistant bacterial infections such as MRSA.

Iclaprim (Motif Bio, plc.) is a diaminopyrimidine (**20**) and a next-generation dihydrofolate reductase (DHFR) inhibitor that was first developed by Roche and licensed by Arpida. It is being developed to treat ABSSSI and HAP [48] and is now in Phase 3 development (NCT02600611, NCT02607618). It exhibits potent, bactericidal activity against staphylococci, including MSSA and MRSA and has a low propensity for the development of resistance (Fig. 5).

14. Defensin-mimetic peptides

Antibacterial peptides are innate antibacterial compounds found in human and animal tissues and cells, especially white cells. These peptides help in defending the body against bacterial infections. The first molecule that entered clinical development was magainin, which was derived from frog skin. However, this molecule dropped out of development due to manufacturing, stability, and potency issues. Many antibacterial peptides have been described since the discovery of the magainin peptide. Brilacidin (Cellceutix Corp/Polymedix/U.Penn) is a defensin-mimetic, non-peptidic molecule that was designed by chemists to mimic the amphiphilic properties of antimicrobial peptides and is being developed to treat ABSSSI. Brilacidin is bactericidal for Gram-positive and Gram-negative bacteria and is extraordinary in that it is also bactericidal even for non-replicating bacteria [49]. It targets the bacterial cell membrane and has been demonstrated to have low mammalian membrane disruptive properties along with low mammalian cell cytotoxicity. Two Phase 2 studies (NCT02052388) were completed successfully in 430 subjects with ABSSSI comparing three dosing regimens of brilacidin to daptomycin for the treatment of ABSSSI. All brilacidin treatment regimens were shown to be well tolerated.

15. Other classes

Ridinilazole (SMT19969) (Summit Therapeutics) is a selective, non-absorbable novel compound for the treatment of *C. difficile* infection with reduced disease recurrence [50]. It is active against all strains of *C. difficile* and was shown to be superior to vancomycin in a Phase 2 study (NCT02092935).

16. Old antibiotics with new dosing regimens

16.1. Ramoplanin

Ramoplanin (NTI-851) (Nanotherapeutics Inc.) is an old glycolipodepsipeptide that inhibits cell wall biosynthesis and has a bactericidal effect. It is not absorbed orally and is proposed to enter a

Phase 2 trial to treat antibiotic associated diarrhea with *C. difficile* but no clinical trial is currently underway in the U.S.

16.2. Fusidane

Fusidic acid is a steroidal natural product belonging to the fusidane class of antibiotics that was discovered in the 1960s at Leo Laboratories. Fusidic acid is a protein synthesis inhibitor, and the only bacterial elongation factor inhibitor that has been developed for clinical use. Oral fusidic acid has a long history of safety and efficacy outside the U.S. against ABSSSI, including MRSA, and bone and joint infections. However, it was never granted regulatory approval in the United States. Cempra has a patented oral loading dose regimen of fusidic acid (sodium fusidate) to minimize development of bacterial resistance. This loading dose/maintenance dose regimen was tested in a Phase 2 ABSSSI trial against linezolid as a comparator (NCT00948142). It is now being tested in pivotal Phase 3 trials for treatment of ABSSSI as well as an exploratory trial for long-term oral treatment of refractory bone and joint infections [51] (NCT02570490, NCT02569541). There is no FDA guidance or approved treatment for the latter indication.

16.3. Fosfomycin

Fosfomycin (also called phosphonomycin) was originally isolated from a *Streptomyces* sp. but is now made synthetically. It has a broad-spectrum of activity and is bactericidal. It inhibits UDP-*N*-acetylglucosamine-3-enolpyruvyltransferase, also known as MurA, an enzyme that catalyzes the first committed step in cell wall biosynthesis. Fosfomycin is a phosphoenolpyruvate phosphate (PEP) analog that inhibits MurA by alkylating an active site cysteine residue in the enzyme. Fosfomycin enters the bacterial cell through the glycerophosphate transporter. Fosfomycin is available for oral use but not intravenous use in the US. However, it is available for intravenous use overseas [52]. It has good activity against CRE pathogens and a new intravenous dosing formulation (Zavante Therapeutics) is currently under development. A Phase 3 comparative trial has been initiated to evaluate intravenous fosfomycin (also known as ZTI-01) against piperacillin/tazobactam in the treatment of cUTI and acute pyelonephritis in hospitalized adults (NCT02753946).

17. Summary

The above sections summarize those antibiotics that are in late clinical development. The discussion of early-stage molecules, such as POL7080 (Polyphor Ltd.), a macrocycle protein epitope mimetic [53], teixobactin, a novel, cyclic depsipeptide, which inhibits cell wall synthesis by binding to lipid II and lipid III (isolated from *Eleftheria terrae*, a Gram-negative bacterium that had previously been uncultured) [54] and lugdunin, a novel thiazolidine-containing cyclic peptide (produced by *Staphylococcus lugdunensis*) [55] that inhibits Gram-positive bacteria, are beyond the scope of this review. Early-stage molecules have significantly higher risk of failure and those that inhibit unproven targets and unproven chemical classes have an even greater risk than known classes. Despite the higher risk, the search must go on, and drug developers, government organizations and investors must take calculated risks with new approaches in order to avoid returning to the pre-penicillin era.

Most of the new antibiotics in late-stage development belong to existing classes of antibiotics. So is there "nothing new under the sun" (Ecclesiastes 1:9)? New approaches that address drug resistant pathogens could save lives but it is not correct that there is lower risk for drug resistance with novel targets and novel classes as noted with PDF inhibitors that were developed by GlaxoSmithKline [5]. Analogs of existing classes are equally difficult to develop as novel compounds against novel targets, since every chemical change in old classes of molecules do pose safety and efficacy risks. Just as new patents recognize novel molecules, new antibiotics, even those that belong to old classes, deserve the acknowledgment that they are also difficult to develop and are also creative since many of these classes have been mined for decades. Most of the new antibiotics in late-stage development originated from natural products. The lesson learned is that complex molecules with multiple sites of interaction are a higher hurdle for mutations and resistance selection among bacteria.

18. Conclusion

Even the limited investments that are being made today are mostly made to develop intravenous antibiotics for use in hospitals. This choice has been primarily driven by less price sensitivity for hospital drugs, which is equated to higher revenue. With rising outpatient antibiotic resistance in respiratory and urinary tract infections, new oral antibiotics for outpatient use are urgently needed [17,56]. Solithromycin and omadacycline are two antibiotics being developed for both outpatient and hospital use. Another important point is that most antibiotics are developed for adults with the expectation that they will be used "off-label" in children. New legislation has required the development of new antibiotics for use in children. Since most new antibiotics only have intravenous formulations, new antibiotics for pediatric use will only be used in hospitalized children. Some antibiotic classes, such as the tetracyclines and fluoroquinolones, have unacceptable side effects in children and are therefore not approved for use in this special patient population. This leaves only a few classes of antibiotics for development for oral administration in pediatrics. The macrolide antibiotic class has been preferred for use in children. Solithromycin, the new fluoroketolide, belongs to the macrolide class and is being developed for both outpatient and inpatient pediatric use. As in the case of drug development exclusively for pediatric use, very few drugs are developed for infections that occur during pregnancy. Safety issues are believed to be a high hurdle in this special population. Development of solithromycin is expected to be pursued to treat infections in pregnancy and has shown desirable antibacterial activities and safety in pre-clinical studies. Notably, addressing all populations will become key as resistance rates rise.

Development of new antibiotics requires large investments. It is not feasible to obtain the approximately $200MM in investments to perform the required nonclinical work, clinical trials and manufacture of the product if an antibiotic is left on the shelf for use only in times of future need. Just as governments invest in ammunition to protect our countries, governments will also need to make investments to create new antibiotics to add to our armamentarium. Stockpiling these antibiotics will also be necessary to ensure that they are available in the event of immediate need, such as during bioterrorism events. There have been several models recently proposed to increase the incentives for developing new antibiotics in order to combat the growing problem of antimicrobial resistance. The first involves the concept of antibiotic reimbursement that is delinked from sales [57]. Specifically, this would mean that the link between rewards for R&D (and innovation) and revenues of sales (price and volume) would be broken [58]. In other words, the innovation could be rewarded through prizes and/or fixed series of milestone payments, thereby encouraging companies to develop new antibiotics [57]. A second approach for creating incentives for new antibiotics is the Options Market for Antibiotics (OMA) model [59]. This model would incentivize early development yet also

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share the risks between payers and developers [59]. The OMA model further emphasizes that companies would be given subsidies at different stages in the drug's life cycle as opposed to only at the time of marketing. The hope would be to stimulate the R&D of new antibiotics by pharmaceutical companies due to the fact that current polices have been largely inadequate. The arms race is, and will always be, an on-going battle with bacteria that are continuously evolving due to selective pressure to develop resistance to the antibiotic *du jour*. We can only hope to stay ahead with our pursuit of new antibiotics.

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Conflict of interest

The authors are employees of Cempra, Inc., that is developing solithromycin and fusidic acid and have no other conflicts of interest to declare.

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