F1000Prime REPORTS

Published: 03 December 2013

New and alternative approaches to tackling antibiotic resistance

Glenn S. Tillotson* and Nicolette Theriault

Address: TranScrip Partners LLC, 2941 Honeymead Road, Downington, PA 19335, USA

* Corresponding author: Glenn S. Tillotson (glenn.tillotson@transcrip-partners.com)

F1000Prime Reports 2013, **5**:51 (doi:10.12703/P5-51)

Abstract

Multidrug-resistant bacteria are becoming more common and due to their multiplicity of mechanisms, they are frequently resistant to many if not all of the current antibiotics. This daunting spectre has been the target of many research efforts into conventional antibiotics and alternative approaches. This review focuses on the more recent advances in these fields with an overview on peptidomimetics, nanoparticles and their derivatives, FimH inhibitors, quorum sensing inhibition molecules, neoglycosides and phage therapies. These various approaches are at different stages of development, some are closer to the clinic than others, but recent regulatory guidance and re-awakened interest from the pharmaceutical companies gives us some optimism for the future.

Introduction

It has been almost fifty years since an august group of internationally renowned infectious disease experts and microbiologists were gathered to discuss the question: "are new antibiotics needed?" [1]. This group, chaired by Maxwell Finland, included such luminaries as William Kirby, Ernest Chain, L. P. Garrod and others and all agreed that new antibiotics were needed due to the emergence of resistant Gram-positive infections, including staphylococci and pneumococcus. However, they were also unanimous in their concern regarding Gram-negative pathogens, including Pseudomonas, Aerobacter (Enterobacter) and other genera in the Enterobacteriaceae. They were relatively content with the progress being made at the time with *Mycobacterium tuberculosis*, but they did acknowledge that improvements in therapeutic regimens were needed. They were also concerned about fungal infections for which, at this time, there were few options. It is disconcerting that fifty years later, we are still grappling with similar problems.

So what are the bacterial challenges facing us today?

In the intervening period, the advent of the fluoroquinolones, carbapenems and oxyimino-cephalosporins appear to have given us the upper hand against Gram-negative species, including *Pseudomonas aeruginosa*. Unsurprisingly, these organisms have responded, assisted by both their membrane organization, which enables the exclusion and efflux of antibiotics, and by a remarkable propensity to transfer, recruit and modify the expression of resistance genes. These genes can encode extended-spectrum beta-lactamases (ESBLs), carbapenemases, aminoglycoside-blocking 16S ribosomal RNA (rRNA) methylases and an aminoglycoside-modifying enzyme which modifies quinolones. Some of the ESBLs and carbapenemases have been linked with strains that have demonstrated significant epidemic potential, spreading across countries and continents, for example *Escherichia coli* ST131 with CTX-M-15 ESBL and *Klebsiella pneumoniae* ST258 with *K. pneumoniae* carbapenemase (KPC) [2]. Perhaps one of the most worrying recent developments is the New Delhi metallo-carbapenemase, which is readily transferred among strains and species by highly promiscuous plasmids [3].

Although Finland *et al.* felt they had the measure of the Gram-positive species, Professor Chabbert recognized the evolving nature of *Staphylococcus aureus* and that further resistance developments were inevitable. Most beta-lactams are inactive against methicillin-resistant *S. aureus* (MRSA), the exceptions being ceftobiprole

(recently approved in Europe for community- and hospital- acquired pneumonia) and ceftaroline, and these MRSA strains are being identified in both community and hospital settings. Indeed, a recent survey of *S. aureus* in the United States showed that >65% of community-acquired isolates were methicillin resistant [4]. Among the recent strains of *Streptococcus pneumoniae* isolated in the US, resistance to macrolides, tetracyclines and penicillins were such that empiric therapy was moving towards the fluoroquinolones and older drugs like trimethoprim-sulphamethoxazole. Despite the changes to penicillin breakpoints, there is still a growing proportion of strains which are non-susceptible to beta-lactams. An interesting effect of the recent introduction of multivalent pneumococcal vaccines (such as the seven- and thirteen-valent vaccines) has been the selection of strains not covered by these combinations, and the initial impact this serovar shifting had on antibiotic susceptibility among the pneumococci. Serovars, such as 19A, became more prevalent in human disease and with that came a different set of bacterial challenges. The bêtes noires of the Gram-positive species are the enterococci which, although opportunistic pathogens, cause almost untreatable infections when they do occur, with last resort agents like daptomycin and linezolid being used sparingly.

It is beyond the scope of this paper to discuss the various hurdles and challenges involved in bringing a candidate antibacterial to the clinic, but it is reasonable to state that a good deal of resources and time are being put into this field. The regulatory hurdles, as well as commercial challenges, require another deeper discussion, but there are a plethora of new and "old" revisited approaches being studied which may move us from the end of the antibiotic era towards a new dawn of antibacterial agents. This review will discuss some of the more advanced drugs as well as several conceptual approaches. The topics to be covered include peptidomimetic antimicrobials, new aminoglycosides, FimH inhibitors, metal oxide nanoparticles and quorum sensing agents.

Peptidomimetic antimicrobials

Evidence suggests that naturally occurring or synthetic antimicrobial peptides (AMPs) could be a model for the design and creation of new functional classes of antibiotics [5,6]. Basically, AMPs are selective agents based on their activity against the prokaryotic membrane [7]. These agents induce bacterial membrane modifications ranging from minor lipid bending to complete membrane dissolution, this last event resembling a detergent-induced micelle formation that results in total membrane disintegration [8,9]. It has also been hypothesized that these agents may interfere with DNA, but this has yet to be confirmed. To date, there have been three main reasons which limit the AMP group's clinical utility: high susceptibility to proteolytic degradation by endogenous or microbial enzymes, possible toxicity due to large amounts of drug needed for treatment, and manufacturing costs [10]. Other possible restricting characteristics that may also limit the utility of these agents include high protein binding and high metabolic clearance, leading to a relatively short half-life.

Efforts to overcome these hurdles have centered mainly on the synthesis of proteolytically resistant versions of natural peptides by either complete or partial substitution of L-residues with non-natural D- or B-residues. McGrath *et al*. synthesized a Lys-Leu or klotho (KL) peptide known as (KLAKLAK)2 which had low toxicity towards mammalian cells [11]. A variant of this molecule—$_{D}$(KLAKLAK)2—was studied with regard to antimicrobial activity. They demonstrated complete growth inhibition of several Gram-negative species, including *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *Acinetobacter baumannii* with a median minimum inhibitory concentration (MIC) of 150 µg/ml. Conversely, none of the Gram-positive species tested were affected by the compound. Time-kill analyses showed that $_{D}$(KLAKLAK)2 is bactericidal against *E. coli* and *A. baumannii* and showed time-dependent growth inhibition. Comparative analysis of the activity of $_{D}$(KLAKLAK)2 against a range of multidrug-resistant organisms showed no correlation of pre-existing resistance with peptidomimetic susceptibility. This was particularly illustrated with a strain of *K. pneumonia*, which was resistant to all antibiotics and yet susceptible to $_{D}$(KLAKLAK)2 with a MIC of 75 µg/ml. Typically, antibiotics are tested for their activity during the exponential phase of growth using a strain of *P. aeruginosa* tested in both the stationary and exponential phases, $_{D}$(KLAKLAK)2 was shown to be active in dormant bacterial cells.

An elaborate series of *in vitro* studies were undertaken with $_{D}$(KLAKLAK)2 which demonstrated the following characteristics: it causes dose-dependent membrane morphology damage, it disrupts the bacterial bilayer, it specifically disrupts anionic phospholipid-containing membranes, it has synergistic activity in combination therapy with piperacillin/tazobactam, and it eliminates biofilms. McGrath *et al*. conclude that although these data are very encouraging, it is time for clinical trials to be undertaken. This same group investigated the activity of this molecule against mucor, a fungus causing an increasing number of mucormycosis infections among the immunocompromised. $_{D}$(KLAKLAK)2 was shown to induce apoptosis in mucor as well as inhibiting germination and reducing hyphal activity. These mechanisms yielded a marked fungicidal activity [12].

Table 1. Clinical response of brilacidin (PMX-30063) and daptomycin in acute bacterial skin and skin structure infections: a Phase II study

Clinical response	Low dose N = 52	Medium dose N = 54	High dose N = 54	Daptomycin N = 55
Day 3	94.4%	90.7%	81.5%	90.6%
Day 7	87.0%	92.6%	83.3%	96.2%
Sustained response				
Day 10	90.2%	91.8%	95.5%	97.9%
Day 28	95.7%	89.6%	95.6%	98.0%

A related compound—a defensin-mimetic, brilacidin (PMX-30063)—was under development by PolyMedix, these assets have been acquired by Cellceutix, Beverly, MA, USA in September 2013. This molecule was shown to have activity against *S. aureus*, *Enterococcus faecium* as well as Gram-negative species, for example *E. coli*. The drug was shown to be bactericidal and selective against bacterial cells by specifically disrupting the microbial membrane. A phase II study enrolled 215 patients with acute skin and skin structure infections who were randomized into four different arms, with three different doses of brilacidin and daptomycin. Patients were evaluated at days 3, 7, 10 and 28 (as per Food and Drug Administration [FDA] guidance) to look for a clinical response and sustained response. The trial achieved its objectives of meeting efficacy in all evaluated doses of brilacidin (see Table 1). The safety profile of brilacidin was notable in that there was reporting of numbness and tingling in 65-87% of patients, but the event was insufficient to cause discontinuation. Excluding this numbness, the treatment-related events were 9.5%, 5.6%, and 7.4% for the low, medium and high-dose groups respectively, compared with 10.9% using daptomycin. Eight patients discontinued therapy due to treatment-related events, two due to hypertension [13].

Another company pursuing this line of investigation is Polyphor, (Allschwill, Switzerland) with POL7080 a new agent which is specific for *P. aeruginosa*. This compound has a broad spectrum of activity. Biochemical and genetic studies showed that the peptidomimetics had a non–membrane-lytic mechanism of action and identified a homolog of the β-barrel protein LptD (Imp/OstA), which functions in outer-membrane biogenesis, as a cellular target. The peptidomimetic showed potent antimicrobial activity in a mouse septicemia infection model. POL7080 has completed Phase I studies in healthy volunteers (please see http://www.polyphor.com/products/pol7080).

Aminoglycosides and derivatives

Most protein synthesis inhibitors (e.g. macrolides, tetracyclines etc.) are bacteriostatic, whereas aminoglycosides are bactericidal antibiotics that affect translational fidelity and have been used to treat mainly Gram-negative infections for over 40 years, with kanamycin, gentamicin, tobramycin and amikacin being most popular in the clinic. When combined with a Beta-lactam or vancomycin, the aminoglycosides have been used to treat staphylococcal infections and enterococcal endocarditis. Molecularly, aminoglycosides work by interacting with 16S RNA, which results in a conformational change in the decoding site A, yielding a site that resembles the closed state induced by the interaction between cognate transfer RNA (tRNA) and messenger RNA (mRNA), leading to mistranslation in protein synthesis. Bacteria have developed various methods to resist the action of aminoglycosides and cells can possess several different genetic determinants, thus making it difficult to overcome all of them. Enzymatic inactivation by acetylation, adenylation, or phosphorylation at different locations on the aminoglycoside molecule is among the most potent mechanisms employed by bacteria to overcome these antibiotics [14,15]. These are known as aminoglycoside-modifying enzymes. Other mechanisms exist, notably mutation of the 16S rRNA, methylation of 16S rRNA and reduced permeability by modification of the outer membrane, export by efflux pump and other rarer methods. The aminoglycoside N-acetyltransferase belong to a large superfamily containing about 10,000 proteins which share the ability to acetylate a primary amine in a variety of acceptor molecules. AAC(6′)-1b (aminoglycoside 6'-N-acetyltransferase type Ib) is the most prevalent of the known enzymes, being found in 70% of AAC- producing Gram-negative clinical isolates [16].

Clearly, the development of new AAC-tolerant aminoglycosides is the best way to circumvent this emerging problem. Plazomicin (ACHN-490)—a novel neoglycoside—is currently in clinical trials. Plazomicin was derived from sisomicin that carries a hydroxymethyl group at position 6′, and plazomicin has been shown to have enhanced activity against multidrug-resistant (MDR) Gram-negatives [17].

Plazomicin is a bactericidal aminoglycoside with enhanced activity against MDR Gram-negative bacteria and *S. aureus*, including methicillin-resistant strains. Plazomicin is not affected by any of the currently known aminoglycoside-modifying enzymes except AAC(2′)-Ia, -Ib, -Ic, which are only found in the *Providencia* species. Methylation of 16S rRNA confers MICs >8 μg/ml for

plazomicin, and indeed high-level resistance to all other aminoglycosides. In an "acid-test" of the activity of this agent against the most problematic MDR Gram-negative pathogens, Galani *et al.* [18] evaluated the compound against 300 MDR isolates from four hospitals in Athens, Greece. These isolates possessed a range of resistance mechanisms, including carbapenemases and/or ESBL-producing strains. Additionally, most of the isolates were resistant to the currently approved aminoglycosides (tobramycin, gentamicin and amikacin). Plazomicin demonstrated activity against all isolates of *K. pneumoniae*, *E. coli* and *Enterobacter* species with MIC_{50} and MIC_{90} of 1 and 2 µg/ml respectively.

Intravenous dosing of plazomicin of 15 mg/kg yielded a maximum concentration of 113 µg/ml, and the half-life was 3.0 hours and the steady-state volume of distribution was 0.24 l/kg. Human phase I and II studies have shown no nephrotoxicity or ototoxicity. Moreover, a lack of ototoxicity was reported in the guinea pig model [19]. Plazomicin completed phase II clinical trials in complicated urinary tract infections when compared with levofloxacin. All clinical endpoints were achieved. Subsequently, Achaogen recently agreed a phase II study design evaluating plazomicin compared with colistin in carbapenem-resistant Enterobacteriaceae using a superiority outcome (please see http://tinyurl.com/psrfm2d).

FimH inhibitors

Urinary tract infections (UTIs) affect more than 15 million women in the United States annually, costing an estimated $2.5 billion [20]. Uropathogenic *E. coli* (UPEC), the causative organism of more than 85% of all UTIs have become progressively more resistant to antimicrobials, and not just the community agents but broad spectrum Beta-lactams and fluoroquinolones, thus creating a therapeutic conundrum [21]. UPEC can colonize all parts of the urinary tract, including the urethra, ureter, kidney, bladder and urine. Additionally, UPEC can cause acute, chronic, persistent and recurrent infections. Acute infections commence when UPEC is introduced into the urinary tract using type 1 pili tipped with the FimH adhesin to bind specifically to mannosylated receptors on the luminal surface of the mammalian bladder epithelial cells. This facilitates the process of colonization and invasion of bacteria into the uroepithelial cells. Bladder cells can expel UPEC from the epithelial cells as part of an innate defense mechanism, but a single bacterial cell can replicate to 10^4–10^5 which can then aggregate in a type 1 pilus-dependent manner to form an intracellular bacterial community (IBC) inside the epithelial cell. An IBC is transient in nature—after they mature, bacteria disperse from the IBC and spread to neighbouring cells to form further IBCs. FimH is essential for invasion, IBC formation and the ability of bacteria to colonise the bladder. Thus, therapeutic agents that target FimH are in development. The mannose-binding pocket of FimH is composed of amino acid residues that are consistent in all strains of *E. coli* Mutations in these residues disrupt mannose binding and attenuate virulence [22]. FimH inhibitors were derived from mannosides, which are composed of a-D-mannose residues. These inhibitors have excellent cellular potency and low molecular weight. Cusumano *et al.* [23] optimized a series of six candidate compounds with the plan to create an orally available agent which could treat and prevent chronic UTI.

Cusumano *et al.* [24] used a haemagglutination inhibition assay to determine the functional activity of the six lead compounds and showed that compound 6 was able to block pilus-dependent biofilm formation *in vitro*, displayed oral bioavailability, treated an established UTI and prevented infection and, most interestingly, potentiated antibiotic activity (TMP = SMX). Based on these promising observations, the same workers developed a further four compounds (using compound 6 as the basis) seeking to improve the half-life and volume of distribution. Using the chronic mouse infection model, improvements in both pharmacokinetics and efficacy were noted with compounds 8 and 10. Extending the dosing of compound 8 to a three times a day regimen improved the efficacy. If these observations in mice can be translated into humans, the threat of MDR Gram-negative pathogens causing UTIs may be manageable [25].

Nanoparticles

Nanomaterials possess unique properties compared to their larger bulk counterparts. Metal oxide nanomaterials, such as zinc oxide (ZnO) and copper oxide (CuO), have been exploited in paints, cosmetics, textiles and plastics [24]. It is, however, the antibacterial activity, particularly against an array of pathogens, which has led to recent research in this field [24]. Nanoparticles have received attention in several fields due to their unique physical, chemical and effective biological properties. The properties of nanoparticles can be altered by changing their size, especially when at the nanometer scale. Indeed, the smaller particles have been shown to have activity against *E. coli* and *S. aureus*. Interestingly, these species seem to be highly susceptible to ZnO and CuO nanoparticles. The bactericidal activity of these nanoparticles depends on size, stability and concentration in the growth medium. Bacterial cells are typically in the micrometer range in size with cell wall peptidoglycan pores being a nanometer in dimension, thus

nanoparticles possess a unique ability to cross into the bacterial cell.

Azam *et al*. [26] synthesized ZnO, CuO and iron (III) oxide (Fe_2O_3) nanoparticles using a gel-combustion method. These particles were tested against *S. aureus*, *Bacillus subtilis*, *P. aeruginosa* and *E. coli* using well-diffusion methods. The three nanoparticle agents were tested against the four species and ZnO and CuO were shown to be more potent than Fe_2O_3 nanoparticles. The largest zone of inhibition was seen with *B. subtilis* and ZnO (25 mm) compared with 21 mm and 15 mm for CuO and Fe_2O_3 respectively. Smaller and similar zones were seen with the three other tested species. Corroboration of these observations using liquid susceptibility-testing methods is needed. Azam *et al*. [26] showed that the antibacterial activity of the nanoparticles increased with a larger surface-to-volume ratio due to the decrease in the size of the nanoparticles. Indeed, previous studies [27] have shown that the smaller the ZnO particles the greater the antibacterial activity. Notably, previous reports have shown ZnO to be both bactericidal [28] and bacteriostatic agents [29]; these conflicting reports may have limited their study. In another experiment, Azam *et al*. [26] analyzed the minimum bactericidal concentration of the three metal oxide nanoparticles and showed that ZnO was found to be the most bactericidal compound compared to the two other nanoparticles. The bactericidal activity of the synthesized nanoparticles was $ZnO > CuO > Fe_2O_3$. These data are supported by Wang *et al*. [30] and Baek and An [31].

The nano-sized particles of pure ZnO, CuO and Fe_2O_3 were shown to have antibacterial activity and that ZnO was the most potent. It is important, however, to assess the size, shape, morphology and electronic properties of nanoparticles on cytotoxicity if this approach is going to have broad medical applicability. Nevertheless, in the battle against MDR pathogens, this route is worth exploring further with other antibacterial agents possibly in combination with these other molecules.

Lin *et al*. [32] studied a lipid nanocarrier as a treatment for acne using tetracycline and tretinoin as a topical therapy. Two different nanocarriers, nanoemulsions and nanostructured lipid carriers were prepared. The various characteristics of the two carriers were compared: their average size, zeta potential, drug encapsulation percentage and drug permeation via the skin. The antibacterial properties of the nanosystems were assessed against *S. aureus*, *P. aeruginosa* and *Propionibacterium* acnes using an agar diffusion assay. Tetracycline mainly resided in the aqueous phase and an *in vitro* skin permeation model showed that the nanostructured lipid carriers enhanced tetracycline flux by twofold over the control solution. Conversely, tretinoin permeation was not affected by the non-carriers. Overall, nanoparticulate loading mostly maintained the antibacterial activity of tetracycline. Negatively charged nanocarriers potentially strengthened the antibacterial activity of tetracycline against *S. aureus*. This series of studies is the first to show the permeation and antibacterial activities of dual-drug nanocarriers for acne treatment. Although this approach has not yet been studied in parenterally administered formulations, lipid carriers (such as lipid forms of amphotericin) have been in use for some time and contribute to the improved pharmacokinetics and tolerability of the parent compound. As our therapeutic options against MDR pathogens diminish, we may be able to extend the viability of some of the current compounds by the use of nanocarriers by enabling existing antibacterial agents to enter cells such as phagocytes and other cells and achieve bactericidal action in settings otherwise unobtainable by existing methods.

Quorum sensing approaches

P. aeruginosa infections in burn wounds can be devastating and potentially fatal. Management of *P. aeruginosa* infections can involve complex fluid and ventilator management, definitive surgical debridement and advanced technologies. Additionally, the use of antimicrobial agents, such as colisitin, are being used to combat the antibiotic resistant strains of *P. aeruginosa*. Research in the area of *P. aeruginosa* pathogenesis has revealed a complex regulatory communication system, known as quorum sensing (QS), which controls about 10% of *P. aeruginosa* genes [33]. QS is a cell-density-dependent phenomenon based on the release of low-molecular weight molecules that co-ordinate gene expression in a given cell population [34]. In response to high population density, *P. aeruginosa* releases molecules which act as specific chemical signals that control the production of virulence factors that mediate acute infection. The QS regulator known as multiple virulence factor regulator (MvfR) controls the expression of most of *P. aeruginosa*'s acute virulence factors in addition to more than 60 excreted anthranilic acid derivatives, most of which are members of the 4-hydroxy-2-akylquinolines (HAQs) family. These HAQs, in tandem with MvfR, positively regulate the transcription of two operons which encode biosynthesis of the virulence molecules. Que *et al*. [35] demonstrated the presence of HAQs in necrotic burn tissue, fat, pus and liquefied fat and drainage liquid to a varying extent. They hypothesized that targeting QS by the use of anti-QS inhibitors may diminish infections caused by MDR bacteria. Thus QS could be a

suitable drug target for new antimicrobials because it controls pathogenesis and is evolutionarily conserved among pathogenic bacteria. Moreover, the pharmacoogical targeting of non-essential functions, such as virulence factors, may decrease the development of resistance, by reducing the need for the use of current agents, which, in and of themselves, select for resistance. Lesic *et al*. showed that pharmacological inhibition of MvfR regulon significantly reduced *P. aeruginosa* virulence in mice [36]. The applicability of QS agents to infection may not just reside in being a therapy but also as a prophylactic approach to preventing colonization of ventilators, catheters etc., where the nidus of infection can be formed.

Where are we currently?

This review is intended to raise awareness of several novel approaches to combating the emergence of MDR bacteria which are becoming more commonplace in our hospitals and even in our community settings. The broad spread of beta-lactamase-producing Enterobacteriaceae and other Gram-negative species has become an international crisis with few options near to the clinic. It is fair to say that there are several expansions of known classes in clinical development such as the various beta-lactam/beta-lactamase inhibitor combinations that may dramatically improve the clinical picture (see Table 2 and [37-39]).

To improve our fight against MRSA, a pathogen which we have not spent much time on in this article, there are new oxazolidinones under investigation (with tedizolid being submitted to the FDA in October, 2013) for acute bacterial skin and skin structure infections, as well as the extended spectrum cephalosporins, ceftobiprole (approved in Europe) and ceftaroline (approved in the United States and Europe). Seven new agents which target protein synthesis are in development for the treatment of moderate to severe community-acquired bacterial pneumonia and/or skin and skin structure infections: solithromycin (CEMPRA), cethromycin (Advanced Life Sciences), omadacycline (Paratek), fusidic acid (Taksta, Cempra), GSK1322322 (GSK), radezolid (Rib-X) and tedizolid S (Trius/Cubist/Bayer). Eravacycline, a fluoro-tetracycline (Tetraphase) is being developed for MDR Gram–negative infections including complicated UTIs and intra-abdominal infections which increasingly involve Carbapenem-resistant Enterobacteriaceae (CRE) [40].

Other novel approaches being investigated for the treatment of MDR infections include control of efflux pumps (EPs) using EP-inhibitors. Systemic use appears to lead to some toxicity issues, but topical use may be promising [41]. Indeed, control of EPs has been suggested as a possible approach to managing isoniazid resistance in *Mycobacterium tuberculosis* in which multi-drug resistance is becoming a global problem [42].

Finally, there has been a resurgent interest in the use of lytic bacteriophages to manage bacterial infections. However, just as bacteria can become resistant to antibiotics, they can also develop resistance to phages that attack them. Equally, phages can evolve resistance and adapt to resistant bacteria resulting in a co-evolutionary race between bacteria and phages. Phico Therapeutics is presently developing a unique technology which utilises an antibacterial protein, SASP, to inactivate bacterial DNA and cause rapid destruction of target bacteria. This technology, known as SASPject™, can be targeted to any selected bacteria with potential for administration as a topical nasal, oral or intravenous treatment. *In vitro* testing shows that SASPject™ can destroy targeted bacteria more rapidly than any other licensed antibiotic whilst leaving the remaining bacterial flora unharmed: SASPject™ can destroy up to 99.9% of target bacteria in 2 minutes. Equally important, laboratory studies have not revealed any mechanism by which bacteria can become resistant to SASP and its unique mode of action. Clinical trials are in progress which evaluate its potential against *S. aureus* and in pre-clinical development are therapies targeting *P. aeruginosa* and other Gram-negative species (please see http://www.phicotherapeutics.co.uk/).

Table 2. Comparison of developmental Beta-lactam/Beta-lactamase inhibitor combinations

Beta-lactam	Beta-lactamase inhibitor	Ratio	Company	Comment
Ceftolozane	tazobactam	2:1	Cubist	Active vs *Pseudomonas aeruginosa*
Ceftazidime	avibactam	4:1	A-Z	Active vs ESBLs
Ceftaroline	avibactam	1:1	Forest/Cerexa	Active vs ESBLs
Imipenem-cilastatin	MK 7655	2:2:1	Merck	Active vs carbapenemase
Imipenem-cilastatin	MK 7655	4:4:1	Merck	Active vs carbapenemase
Meropenem	RPX7009	1:1	Rempex	Active vs class A serine carbapenemases, + *Pseudomonas* species

ESBLs, extended-spectrum beta-lactamases.

Parallel use of phages and antibiotics could help to reduce the chance of resistance evolution for the same reasons as using combination antibiotic therapy. Several studies have shown that combined therapy is better or equally good at decreasing bacterial populations compared with antibiotics or phages alone [43]. This new approach to the use of lytic phages is one we watch with keen interest.

Conclusions

As Patel and Bonomo [44] forecast in their recent perspective, clinicians are confronted with increasing changes and they predict "stormy waters" ahead. We suggest that a more appropriate meteorological metaphor would be the "perfect storm", consisting of rapidly increasing antibiotic resistance across many species and genera, regulatory hurdles of uncertain drug development pathways, and the lack of commercial incentives. With the recent efforts of the United States GAIN Act and European moves towards a simplified guidance procedure helping to lower the regulatory hurdles and put in place some commercial incentives (such as extended patent life) added to the continued innovation of biotech companies in examining novel approaches to combating resistant bacteria, there may be a path forward and calmer times ahead. We still need to move the commercial "value" needle away from a "generic cost approach" to antibiotics, to regard them as life-saving medicines and, as such, pay a fair market price for these endangered agents. The concept of a premium price for an antibiotic is still an anathema but one sees thousands of dollars/pounds paid for cancer drugs which often only prolong life as opposed to saving a life in an acute infection.

We have presented some of the exciting and noteworthy ongoing developments in the field of antibacterials. It will be interesting to see if they pass the regulatory hurdles and reach the clinic in the foreseeable future.

Abbreviations

AAC(6')-1b, Aminoglycoside 6'-N-acetyltransferase type Ib; AMP, antimicrobial peptide; *B. subtilis*, *Bacillus subtilis*; CPE, carbapenemase producing enterobacteriaceae; CuO, copper oxide; EP, efflux pump; ESBL, extended-spectrum beta-lactamase; *E. coli*, *Escherichia coli*; Fe_20_3, Iron (III) Oxide; HAQ, 4-hydroxy-2-akylquinoline; IBC, intracellular bacterial community; *K. pneumonia*, *Klebsiella pneumonia*; *KPC*, *K. pneumoniae carbapenemase*; MIC, minimal inhibitory concentration; MRSA, methilin-resistant *Staphylococcus aureus*; MDR, multidrug-resistant; MvfR, multiple virulence factor regulation; QS, quorum sensing; *S. aureus*, *Staphylococcus aureus*; *S. pneumonia Streptococcus pneumonia*; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection; ZnO, Zinc Oxide.

Disclosures

Glenn S. Tillotson is an employee of TranScrip Partners and is a consultant to Summit Incorporated, Atlas Ventures, Vivonyx and Astellas.

References

1. Finland M, Kirby WM, Chabbert YA, Chain EB, Dowling HF, Garrod LP, Pettinga CW, Todd AC: **Round table: are new antibiotics needed?** *Antimicrob Agents Chemother (Bethesda)* 1965, **5:**1107-14.
2. Livermore DM: **Current epidemiology and growing resistance of gram-negative pathogens.** *Korean J Intern Med* 2012, **27:**128-42.

   F1000Prime RECOMMENDED
3. Rasheed JK, Kitchel B, Zhu W, Anderson KF, Clark NC, Ferraro MJ, Savard P, Humphries RM, Kallen AJ, Limbago BM: **New Delhi metallo-β-lactamase-producing Enterobacteriaceae, United States.** *Emerging Infect Dis* 2013, **19:**870-8.
4. Tillotson GS, Draghi DC, Sahm DF, Tomfohrde KM, Del Fabro T, Critchley IA: **Susceptibility of Staphylococcus aureus isolated from skin and wound infections in the United States 2005-07: laboratory-based surveillance study.** *J Antimicrob Chemother* 2008, **62:**109-15.
5. Marr AK, Gooderham WJ, Hancock RE: **Antibacterial peptides for therapeutic use: obstacles and realistic outlook.** *Curr Opin Pharmacol* 2006, **6:**468-72.
6. Hancock RE, Lehrer R: **Cationic peptides: a new source of antibiotics.** *Trends Biotechnol* 1998, **16:**82-8.

   F1000Prime RECOMMENDED
7. Brogden KA: **Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?** *Nat Rev Microbiol* 2005, **3:**238-50.
8. Shai Y: **Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides.** *Biochim Biophys Acta* 1999, **1462:**55-70.
9. Huang Y, Huang J, Chen Y: **Alpha-helical cationic antimicrobial peptides: relationships of structure and function.** *Protein Cell* 2010, **1:**143-52.
10. Peters BM, Shirtliff ME, Jabra-Rizk MA: **Antimicrobial peptides: primeval molecules or future drugs?** *PLoS Pathog* 2010, **6:**e1001067.

    F1000Prime RECOMMENDED
11. McGrath DM, Barbu EM, Driessen WHP, Lasco TM, Tarrand JJ, Okhuysen PC, Kontoyiannis DP, Sidman RL, Pasqualini R, Arap W: **Mechanism of action and initial evaluation of a membrane active all-D-enantiomer antimicrobial peptidomimetic.** *Proc Natl Acad Sci USA* 2013, **110:**3477-82.

    F1000Prime RECOMMENDED
12. Barbu EM, Shirazi F, McGrath DM, Albert N, Sidman RL, Pasqualini R, Arap W, Kontoyiannis DP: **An antimicrobial peptidomimetic induces mucorales cell death through mitochondria-mediated apoptosis.** *PLoS ONE* 2013, **8:**e76981.
13. **Brilacidin (PMX-30063) Antibiotic Fact Sheet.** [http://files.shareholder.com/downloads/ABEA-4ITCYZ/1997024712x0x611205/FEA66C67-D02F-4FF2-B230-AA711F46ED7F/PMX-30063_Antibiotic_Fact_Sheet_Nov_2012_.pdf]

14. Zaher HS, Green R: **Fidelity at the molecular level: lessons from protein synthesis.** *Cell* 2009, **136:**746-62.

15. Ramirez MS, Tolmasky ME: **Aminoglycoside modifying enzymes.** *Drug Resist Updat* 2010, **13:**151-71.

16. Ramirez MS, Nikolaidis N, Tolmasky ME: **Rise and dissemination of aminoglycoside resistance: the aac(6')-Ib paradigm.** *Front Microbiol* 2013, **4:**121.

F1000Prime RECOMMENDED

17. Livermore DM, Mushtaq S, Warner M, Zhang J, Maharjan S, Doumith M, Woodford N: **Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceae isolates.** *J Antimicrob Chemother* 2011, **66:**48-53.

F1000Prime RECOMMENDED

18. Galani I, Souli M, Daikos GL, Chrysouli Z, Poulakou G, Psichogiou M, Panagea T, Argyropoulou A, Stefanou I, Plakias G, Giamarellou H, Petrikkos G: **Activity of plazomicin (ACHN-490) against MDR clinical isolates of Klebsiella pneumoniae, Escherichia coli, and Enterobacter spp. from Athens, Greece.** *J Chemother* 2012, **24:**191-4.

F1000Prime RECOMMENDED

19. Cass RT, Brooks CD, Havrilla NA, Tack KJ, Borin MT, Young D, Bruss JB: **Pharmacokinetics and safety of single and multiple doses of ACHN-490 injection administered intravenously in healthy subjects.** *Antimicrob Agents Chemother* 2011, **55:** 5874-80.

F1000Prime RECOMMENDED

20. Griebling TL. **Urinary tract infection in women.** In: Litwin MS, Saigal CS, editors. Urologic Diseases in America. Washington, DC: U.S. Government Printing Office; 2007. pp. 587-620.

21. Gupta K, Hooton TM, Stamm WE: **Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections.** *Ann Intern Med* 2001, **135:**41-50.

22. Chen SL, Hung CS, Pinkner JS, Walker JN, Cusumano CK, Li Z, Bouckaert J, Gordon JI, Hultgren SJ: **Positive selection identifies an in vivo role for FimH during urinary tract infection in addition to mannose binding.** *Proc Natl Acad Sci USA* 2009, **106:**22439-44.

F1000Prime RECOMMENDED

23. Cusumano CK, Pinkner JS, Han Z, Greene SE, Ford BA, Crowley JR, Henderson JP, Janetka JW, Hultgren SJ: **Treatment and prevention of urinary tract infection with orally active FimH inhibitors.** *Sci Transl Med* 2011, **3:**109ra115.

F1000Prime RECOMMENDED

24. Han Z, Pinkner JS, Ford B, Chorell E, Crowley JM, Cusumano CK, Campbell S, Henderson JP, Hultgren SJ, Janetka JW: **Lead optimization studies on FimH antagonists: discovery of potent and orally bioavailable ortho-substituted biphenyl mannosides.** *J Med Chem* 2012, **55:**3945-59.

25. Tran N, Mir A, Mallik D, Sinha A, Nayar S, Webster TJ: **Bactericidal effect of iron oxide nanoparticles on Staphylococcus aureus.** *Int J Nanomedicine* 2010, **5:**277-83.

F1000Prime RECOMMENDED

26. Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A: **Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study.** *Int J Nanomedicine* 2012, **7:**6003-9.

F1000Prime RECOMMENDED

27. Raghupathi KR, Koodali RT, Manna AC: **Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles.** *Langmuir* 2011, **27:**4020-8.

F1000Prime RECOMMENDED

28. Xie Y, He Y, Irwin PL, Jin T, Shi X: **Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni.** *Appl Environ Microbiol* 2011, **77:**2325-31.

29. Gajjar P, Pettee B, Britt DW, Huang W, Johnson WP, Anderson AJ: **Antimicrobial activities of commercial nanoparticles against an environmental soil microbe, Pseudomonas putida KT2440.** *J Biol Eng* 2009, **3:**9.

30. Wang Z, Lee Y, Wu B, Horst A, Kang Y, Tang YJ, Chen D: **Antimicrobial activities of aerosolized transition metal oxide nanoparticles.** *Chemosphere* 2010, **80:**525-9.

F1000Prime RECOMMENDED

31. Baek Y, An Y: **Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb2O3) to Escherichia coli, Bacillus subtilis, and Streptococcus aureus.** *Sci Total Environ* 2011, **409:**1603-8.

F1000Prime RECOMMENDED

32. Lin C, Fang Y, Al-Suwayeh SA, Yang S, Fang J: **Percutaneous absorption and antibacterial activities of lipid nanocarriers loaded with dual drugs for acne treatment.** *Biol Pharm Bull* 2013, **36:**276-86.

F1000Prime RECOMMENDED

33. Hazan R, He J, Xiao G, Dekimpe V, Apidianakis Y, Lesic B, Astrakas C, Déziel E, Lépine F, Rahme LG: **Homeostatic interplay between bacterial cell-cell signaling and iron in virulence.** *PLoS Pathog* 2010, **6:**e1000810.

F1000Prime RECOMMENDED

34. Ng W, Bassler BL: **Bacterial quorum-sensing network architectures.** *Annu Rev Genet* 2009, **43:**197-222.

35. Que Y, Hazan R, Ryan CM, Milot S, Lépine F, Lydon M, Rahme LG: **Production of Pseudomonas aeruginosa Intercellular Small Signaling Molecules in Human Burn Wounds.** *J Pathog* 2011, **2011:**549302.

F1000Prime RECOMMENDED

36. Lesic B, Lépine F, Déziel E, Zhang J, Zhang Q, Padfield K, Castonguay M, Milot S, Stachel S, Tzika AA, Tompkins RG, Rahme LG: **Inhibitors of pathogen intercellular signals as selective anti-infective compounds.** *PLoS Pathog* 2007, **3:**1229-39.

F1000Prime RECOMMENDED

37. Shlaes DM: **New β-lactam-β-lactamase inhibitor combinations in clinical development.** *Ann NY Acad Sci* 2013, **1277:** 105-14.

38. Levasseur P, Girard A, Claudon M, Goossens H, Black MT, Coleman K, Miossec C: **In vitro antibacterial activity of the ceftazidime-avibactam (NXL104) combination against Pseudomonas aeruginosa clinical isolates.** *Antimicrob Agents Chemother* 2012, **56:**1606-8.

39. Sabet M, Tarazi Z, Lomovskaya O, *et al.*: **In Vivo Efficacy of the Beta-lactamase Inhibitor RPX 7009 combined with Biapenem (RPX 2003) Against KPC-producing K pneumoniae.**

[abstract]. Presented at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy, 2012; San Francisco, CA

40. **Eravacycline:Eravacycline (TP-434).** [http://tphase.com/pipeline/overview/eravacycline/default.cfm.]

41. Lomovskaya O, Zgurskaya HI, Totrov M, Watkins WJ: **Waltzing transporters and 'the dance macabre' between humans and bacteria.** *Nat Rev Drug Discov* 2007, **6:**56-65.

42. Machado D, Couto I, Perdigão J, Rodrigues L, Portugal I, Baptista P, Veigas B, Amaral L, Viveiros M: **Contribution of efflux to the emergence of isoniazid and multidrug resistance in Mycobacterium tuberculosis.** *PLoS ONE* 2012, **7:**e34538.

43. Zhang Q, Buckling A: **Phages limit the evolution of bacterial antibiotic resistance in experimental microcosms.** *Evol Appl* 2012, **5:**575-82.

44. Patel G, Bonomo RA: **"Stormy waters ahead": global emergence of carbapenemases.** *Front Microbiol* 2013, **4:**48.