

Adjunct antimicrobial therapy – prospects for the future

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The bacterium is a relatively simple microorganism compared with mammalian cells, generally ranging in size from just under 1200 to over 6000 genes, but has evolved an amazing repertoire of defenses against environmental insult, toxins and other microbial life. The standard of care has been to kill the bacterium when found in the host as a pathogen, and a large arsenal of drugs has been developed to do just that. However, the adaptability of these pathogens to evade the host immune system defenses and classical antibiotics has presented an evolving scenario of continuous emergence of resistance against virtually all antibiotic drug classes. It may be time to explore other options in this infection battle through the use of adjunct therapy such as immunotherapeutics and antivirulence agents.

The landscape of infectious diseases looks promising as one sees the expanding drug therapy options for viral infections worldwide [1], improved therapy options in development for serious fungal infection [2], and some recent drug approvals and a somewhat robust pipeline of biotech company niche products [3–6]. Where the emphasis by many large pharmaceutical companies has shifted away from antimicrobials (antibacterials and antifungals), the continuing hope for short-term relief of emerging drug resistance in the clinic is from the biotechnology company pipelines [7–9]. However, within the pool of bacterial pathogens reported as responsible for the majority of infections in a particular environment – intensive care unit (ICU), hospital (non-ICU), outpatient or community – are numerous resistance-emergence issues for each therapy setting involving multiple pathogens [10–13]. It is clear to many that something must be done to expand the options for treatment of bacterial infections in light of increased emerging resistance [14,15] and the solution may reside in alternate approaches.

Whereas this evolution of susceptibility patterns in the clinic is nothing new as the standard-of-care therapy has evolved over a 60-year period from the streptomycins and penicillins, to the tetracyclines in the 1950s, through cephalosporins in the 1970–1990s and through the macrolides and quinolones of the late 1980s onward, the one common event in almost all drug classes is the inevitable selection of resistance among the drug-treated pathogen population in the host [14–17]. Whereby the most obvious ‘classical’ solution to future drug therapy is the identification

of a better version of an existing drug class that overcomes resistance, or a novel drug class without pre-existing resistance or cross-resistance, there are additional options available for therapy but not routinely used in clinical practice. Among them, increased discovery and development and use of vaccines against these resistant pathogens [18,19] and the use of biologics as adjunct therapy [20–22]. One high usage adjunct therapy has been the nonantibiotic first generation β -lactamase inhibitors (BLIs) used with several β -lactam combinations to restore β -lactam susceptibility [23–24].

Antibacterials/antibiotics

The success of antimicrobial therapy, specifically antibacterial or antibiotic therapy, has been documented in multiple recent reviews [3–6,8,9]. Whether referring to antibiotics (natural, product-derived agents) or antibacterials (totally synthetic agents), these ‘wonder’ agents have probably saved more lives threatened by acute mortality than any other therapeutic area class. Yet, it is becoming apparent that there is a situation developing worldwide in which two events are contributing to what some researchers are calling ‘a return to the preantibiotic era’ due to both a decrease in industrial interest/support for the discipline of antibiotics/antibacterials [7,9], and the emergence of antibiotic drug resistance [13–17]. For the sake of brevity, the use of ‘antibiotics’ will refer to either an ‘antibacterial’ or an ‘antibiotic’ for the remainder of this review.

Aside from the politics and business aspects of supporting this discipline, the approach to drug therapy for the majority of therapy options has

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been to identify agents that kill the bacterium. Mechanisms of inhibiting bacterial pathogens may be through a bacteriostatic or bactericidal mechanism [25], but ultimately it has entailed the use of bacterial ‘killing agents’ – with one noteworthy exception – that of BLIs [23,24]. Designed to restore the susceptibility of select β -lactams from their loss of susceptibility, the use of BLIs has provided a proof-of-principle on the use of adjunct therapy, an ‘antiresistance’ or ‘antivirulence’ approach, in the antibiotic market place and clinical setting [24].

Defining our terms

For clarity, the use of the term ‘adjunct therapy’ is herein defined as a nonantibiotic agent used in combination with an antibiotic in an attempt to improve therapy. The requirement for these agents – adjunct therapy – is simply to be non-antibiotic at clinical use drug levels, and to be used with another specific drug, such as an antibiotic. This definition distinguishes this review from the use of the term ‘nonantibiotic’ used in the context of recent reports in which other therapeutic area drugs have been identified as having antibiotic activity (Table 1) and have been suggested for use in combination therapy [26–33]. It may be of interest to explore the mode by which these ‘nonantibiotic’ drugs actually inhibit bacteria based on the suspicion that it could be a starting point for a novel antibiotic scaffold. This latter usage of ‘adjunct therapy’ in the context

of the agents described in Table 1 will not be discussed further in this review.

Why bother with adjunct therapy?

The success of the first generation BLIs and other nonobvious efforts at using adjunct therapy in treating patients merits expanded consideration – if nothing else, at least from a theoretical standpoint [23,24]. Where adjunct therapy would not be presumed to replace first-line antimicrobials in the clinic, the placement of BLIs in the clinic prompts consideration of similar ‘antivirulence’ approaches [34,35]. Imagine the ability of a nonantibiotic agent to reverse vancomycin resistance [36], or a nonantibiotic agent to inhibit alginate capsule formation to improve antibiotic therapy in cystic fibrosis patients by permitting increased drug access [37,38], or an inhibitor of biofilm formation in staphylococci to also increase drug access to the pathogen [39,40], or an efflux pump inhibitor to reverse quinolone resistance [41–43] (see Table 2 for representative examples of some of these potential adjunct therapy approaches).

There may be other reasons for using adjunct therapy in the treatment of bacterial infections. From a theoretical standpoint, there may be less selective pressure on bacterium from a nonantibiotic rather than an antibiotic. As an alternative to killing the microbe, one or more nonessential gene products/functions may be affected by the adjunct therapy treatment, which may result in a synergistic, improved treatment outcome [34,35].

Table 1. Nonantibiotic drugs being investigated for antibiotic activity.			
Drug name	Class of agent	Antibacterial activity	Refs.
Amlodipine	Cardiovascular agent	Broad spectrum (Gram-positive, Gram-negative) synergy with streptomycin	[26]
Taurolidine	Intravitreal anti-inflammatory	<i>S. epidermidis</i>	[28]
Dodecyl gallate (C12–3,4,5-trihydroxybenzoate)	Antioxidant	MRSA	[29]
Diclofenac & trifluoperazine	Microbicides	Gram-positives	[30]
Methotrexate	Cytostatic	<i>S. aureus</i> (MSSA & MRSA)	[31]
Theobromine Coumarin-152 Theophylline Trifluoperazine, Fluophenazine	Miscellaneous	<i>Burkholderia cepacia</i> (synergistic with gentamicin and ceftazidime)	[32]
Simethicone	Anti-foaming agent	<i>Helicobacter pylori</i>	[38]
Gabexate mesylate	Protease inhibitor	<i>H. pylori</i>	
Ketoconazole	Antifungal	<i>H. pylori</i>	

Table 2. Miscellaneous adjunct therapy options with combination drug pairings.

Target	Mode of action	Drug pairing	Pathogen(s)	Refs.
Modification of D-ala-D-ala to D-ala-D-lactate (two-component system)	Anti-VanR/VanS	Vancomycin	VanA ^R -enterococci	[36,112]
Inhibition of capsule	Antialginate capsule	Antipseudomonal agent(s)	<i>P. aeruginosa</i>	[37,38]
Toxin inhibition	Antilethal factor Antitoxin	Anti- <i>B. anthracis</i>	<i>B. anthracis</i>	[121]
		Antistaphylococcal agent	<i>S. aureus</i>	[122]
Efflux pumps	Inhibition of efflux of antibiotics, specifically: AcrAB-tolC pumps MexAB-OprM pump MexAB-OprM pump AcrB	Macrolides	<i>E. aerogenes</i>	[92]
		Levofloxacin	<i>E. coli</i>	[92]
		Meropenem	<i>P. aeruginosa</i>	[41,42,91]
		Quinolones	<i>P. aeruginosa</i>	[123]
			<i>Salmonella enterica</i>	[43]
Antitarget modification	Inhibition of <i>erm</i> methylase	Macrolides	<i>S. pneumoniae</i> <i>Streptococcus pyogenes</i>	[124]
Antibiofilms (antiattachment)	Inhibition of assembly of biofilms, Specifically, <i>agr</i> -regulon	Antistaphylococcal agents	<i>S. aureus</i>	[40,112,113]
Antiquorum sensing	Inhibition of multiple 'community-associated' virulence functions	Quinolones, aminoglycosides	<i>P. aeruginosa</i>	[106,107]

Other potential approaches may be to halt the ability of the bacterium to be a pathogen (e.g., antiattachment, anti-invasion factor, antiprotease, antibiofilm production and antitoxin) [34,35,44,45], to stop the production of a factor that the bacteria needs to survive (e.g. induction of transport systems or porins that enable survival in the host's environment), or to stop the production of that which the bacteria needs to resist antibiotic therapy (efflux pumps, porin down-regulation and expression of plasmid-mediated antibiotic hydrolytic enzymes) [46].

Adjunct therapy

First wave – unanticipated success

The use of BLIs, which began in the mid-to-late 1980s, was never anticipated to produce the blockbuster agents that amoxicillin–clavulanic acid or ampicillin–sulbactam have become [47,48]. However, the initial medical need-based positioning of these agents expanded into increased usage in the community and hospital, respectively – as each member of this adjunct therapy class of agents found its place in reducing the impact of the

emerging resistance against the β -lactamase(s) occurring in the clinical setting. A BLI is a non-antibiotic agent, used in combination with a β -lactam to inhibit the hydrolysis of the β -lactam by a bacterial-produced β -lactamase [23,49]. Three major β -lactam–BLIs commercial successes have been used worldwide:

- Amoxicillin–clavulanic acid
- Ampicillin–sulbactam
- Piperacillin–tazobactam [50]

A fourth BLI, but not as commercially significant, is ticarcillin–clavulanic acid which has also been used in the clinic. The strategy of matching a BLI with a β -lactam is a remarkable achievement, not necessarily in its scientific marvel, but more so in the vision that a nonantibiotic in combination with a β -lactam could be a viable commercial product.

Wave II – recombinant cytokines as adjunct therapy

In a similar vein, the identification of human, recombinant cytokines, frequently used as adjunct therapy in multiple disease states (including anti-

cancer therapy, other immunocompromised disease states, and in infectious disease therapy) was a remarkable achievement [20,21,51,52]. Multiple cytokines followed the initial success of erythropoietin such as granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage (GM)-CSF to increase endogenous granulocyte production and interleukin (IL)-11 to increase host platelets [20,21,51,52]. Additional biologics are being considered in multiple therapeutic areas as either therapy, such as interferons (IFNs) and cytokines [53]; as adjunct therapy, such as immunoglobulin (Ig), monoclonal antibodies (mAbs) [54,55,56,57]; or immunomodulation, such as helping to treat lung infections [58,59]. However, the use of cytokines in the management of infection is not well documented, with most data being preclinical [20,52,60–64]; and has not yet become a routine adjunct therapy regimen in antibiotic therapy [65]. Recent reviews on this topic have provided encouragement for expansion of these treatment regimens into the infectious diseases arena [61,62].

The use of immunoglobulins as direct or as adjunct therapy has been successful with multiple reports and reviews of their initial successful application in the clinic in the 1990s in areas other than antimicrobial therapy [54–57,66,67]. More recent efforts with adjunct treatment of sepsis has led to re-exploration of multiple cytokines and alternate immunotherapy regimens to intervene in this high mortality disease state [22,67–71]. An outstanding review on the attempts to immunomodulate the immune system and inflammatory cascade of cytokine activators in sepsis patients is provided by Narawasy [22]. Both Masihi and Pratt provide excellent updated reviews of the current approaches for immunotherapeutics and immunomodulators in infectious diseases [61,62].

Wave III – new generation niche adjunct therapies?

With the same open-mindedness that led to the clinical use of BLIs and human growth factors (cytokines, IFNs and Igs), one can pose the question, what might comprise the next phase of adjunct therapy – wave III? With several unmet medical needs upon us, let me suggest that it may be time to expand antimicrobial therapy, specifically antibiotics, beyond the ‘kill the microbe’ approach, to consider novel adjunct therapy beyond the first generation BLIs [23,24].

Undoubtedly, the outcome of the next phase of adjunct therapy combination drugs will have an uphill battle to achieve the established success of the primarily class A BLIs and β -lactam combinations that saw two of these four drug combinations (amoxicillin–clavulanic acid and ampicillin–sulbactam) achieve the so-called ‘blockbuster’ status for an antibiotic by securing peak sales of over US\$1 billion dollars. However, the intention of this manuscript is not to specifically argue for the commercial viability of these agents, but more to conceptualize options than may fulfill an unmet medical need and provide an alternative to the cycle of one-after-the-other class of antibiotic agents, all of which are at risk for the inevitable development of resistance to the incumbent class [3,6,9,14]. This resistance emergence, as part of the collateral damage to the use of antibiotics, can result from the selective pressure on a microbial population that permits the outgrowth of mutant bacteria that are selected for in the presence of antibiotics [72,73]. There are different frequencies of this resistance emerging, but many agents have had resistance emergence in just 4 years postlaunch [9] and even during the clinical trial period as has been demonstrated with linezolid [74].

Multiple medical situations provide unmet medical needs

To enable the containment of several key approaches, we can divide the therapeutic regimens of adjunct therapy into at least three opportunities:

- Adjunct therapy in the presence of an antibiotic agent that reduces or eliminates a bacterium’s ability to defeat the drug therapy
- Adjunct therapy in the presence of an antibiotic agent that reduces or eliminates a bacterium’s ability to be pathogenic
- Adjunct therapy of a nonantibiotic agent in the presence of an antibiotic that enhances the host’s defense system against the bacterium

Where do opportunities exist for the first option?

Foremost in do-ability of an emerging unmet medical need is a new generation of BLIs [75–81]. The emergence of highly divergent Class A TEM-1 lineage β -lactamases into extended-spectrum β -lactamases (ESBLs) is eroding the widespread use of many β -lactams, including the penicillins and cephalosporins [49,81,82]. There are also reports of first-generation BLI-resistant β -lactamases, or inhibitor-resistant

TEM-1 β -lactamases (IRTs) being identified in *Klebsiella*, *Escherichia coli* and *Enterobacteriaceae* [78–80]. The Class C β -lactamases are also now widespread in Gram-negative pathogens, especially in *Pseudomonas aeruginosa*, *K. pneumoniae* and *Enterobacter cloacae* [81–83]. The Class B β -lactamases, frequently referred to as carbapenemases due to their substrate specificity, are becoming recognized as an up-and-coming problem for the next decade [84,85]. Class D β -lactamases such as the cytochrome oxidase biogenesis (OXA)-family and variants thereof are reported to be key resistance factors in the multiply drug-resistant *Acinetobacter baumannii* identified in ICUs [86]. Finally, there are multiple, unusual β -lactamases being identified throughout the world, such as the KPC-2 [78], SME-1 [87], SME-2 [88], as well as the expanding multitude of ESBLs that continue to demonstrate the selective pressure of antibiotic therapy on an evolving bacterial ecology drug susceptibility [81–83]. Simply put, first-generation BLIs provided a model for the placement and clinical usage of new classes of BLIs which would restore the activity of multiple generations of penicillins, cephalosporins and carbapenems to historical susceptibility levels.

The other best chance of short term success may be the use of an efflux pump inhibitor (EPI) to restore the nonsusceptibility (or resistance) of an antibiotic to ‘susceptible’ minimum inhibitory concentrations (MICs) [89,90]. Some of the best examples have been demonstrated in the preclinical use of EPIs to potentiate the activity of fluoroquinolones against Gram-negative bacteria [41–43]. The proof-of-principle of do-ability appears to have come from the efforts of researchers at Microcide Pharm. Co. (CA, USA) whose efflux pump assets are now owned by Mpex Pharmaceuticals (CA, USA), Trine Pharmaceuticals (MA, USA) and Daiichi (Japan). The first generation EPIs were identified as adjunct therapy to be used with the quinolones, that is, levofloxacin, in the treatment of *P. aeruginosa* [43,91]. MC-207,110 is one such example of these peptide EPIs in which the inhibition of efflux lowered the MICs of three quinolones (levofloxacin, ciprofloxacin and ofloxacin) [43,91]. Similar reports have emerged on EPIs restoring susceptibility to macrolides and ketolides against *E. aerogenes* [92] and *E. coli* [92,93] through the inhibition of the AcrAB-TolC pump. The key to optimization of these primarily diamine-containing EPIs as useful clinical agents may lie in achieving a balance between

in vitro, *in vivo*, pharmacokinetics, stability and acute toxicity as thoroughly illustrated by Watkins and colleagues [94].

Where do opportunities exist for the second option?

Adjunct therapy of a nonantibiotic agent in the presence of an antibiotic that decreases the ability of a bacterium to exert its pathogenicity is the second option [95–99]. The best proof-of-principal for this potential approach is the targeting of surface antigens on bacterial pathogens as vaccine candidates and the successful vaccination against *S. pneumoniae*, *Neisseria meningitides*, and *Haemophilus influenzae* [95,96] and anticipated success against staphylococci [18,19,62]. However, there has been a tremendous amount of unrelated work over the past 20 years that suggests this approach is doable [34,35,46,62,97,99–102]. Table 2 lists a number of options for adjunct therapy approaches, including antivirulence efforts, but there are more thorough reviews on the topic available [34,35,46,97,100–104].

Bacterial virulence is one approach that has yet to be accepted by the medical community and exploited successfully by the industrial community [98–100,103,104]. An increasingly clear understanding of the requirements for pathogenicity in bacteria [98,99] and the common mechanisms by which some bacteria achieve and/or maintain the pathogenic state may lead to common themes enabling more than just ‘niche’ applications (which may be less commercially viable) [34,35,97,100]. Among the virulence factors that a pathogen has to exert, its pathogenicity are gene products that facilitate antibiotic resistance. Based on the success of the BLIs, these will most likely be first to be exploited as a nonantibiotic that restores susceptibility (and therefore usefulness) to an existing, successful antibiotic, represents a more definitive end point and possibly a clearer development plan.

Among the more provocative possibilities for adjunct therapy under this approach are:

- Quorum sensing inhibitors: inhibitors of quorum sensing in which a virulence response requires a certain density of the bacterial population [105] and a secondary metabolite such as the homoserine lactone. Initially identified in *Vibrio* sp. [106], are inhibited as nonantibiotic, adjunct therapy. Such a therapeutic intervention may be best illustrated in the role of quorum sensing in *P. aeruginosa* infection in which biofilm formation occurs through the successful interplay of multiple virulence factors, including quorum sensing signals [105–108].

- Anti-plasmid replication/transcription/translation inhibitors: it is well known that there exists numerous plasmid-encoded 'pathogenicity islands' encoding for an assortment of virulence factors in Gram-negative bacteria, as well as multidrug resistance. Thus inhibition of plasmids in bacteria may act as nonantibiotic inhibition of bacterial virulence. Such an approach may actually reduce the resistance burden in the microbial ecology by slowing the spread of mobile resistance elements that frequently deliver multidrug resistance to pathogens.
- Global virulence regulon inhibitors: an approach is described by Kupferwasser and colleagues in which the use of salicylic acid, shown to reduce virulence in *S. aureus in vitro* [111], actually exerts both an antivirulence and antiplatelet mode of action [109,110]. As salicylic acid reduces multiple virulence factors in *S. aureus* – such as *sigB*-controlled global regulator *sarA*, the global regulator *agr*, α -hemolysin secretion and fibronectin binding – as has elegantly been described and discussed elsewhere [111–115], an approach to develop salicylic-acid type, nonantibiotics as an antivirulence platform may expand the existing repertoire of anti-staphylococcal agents.
- Exogenous lytic enzyme: the use of bacterial lytic enzymes such as lysostaphin or bacteriophage lytic enzymes, combined with an antibiotic, may provide additional cell membrane/wall access to an antibiotic by disruption of the pathogen's cell wall [116,117].

Where do opportunities exist for the third option?

The third option is adjunct therapy of a non-antibiotic agent in the presence of an antibiotic that enhances the host's defense system against the bacterium [118]. In this scenario, modulation of the immune system is used to provide a variety of supportive therapies for use in combination with antibiotics. One approach elegantly laid-out by Weidenmaier and colleagues describes the host immune system's innate ability to produce molecules to defend against bacterial pathogens and the resulting response of the pathogen to evade these immune response systems [118]. Research has shown that many of the host's immune weapons are cationic, such as lysozymes, defensins and phosphatases; and some bacteria have evolved the ability to shift the charge composition of their anionic cell wall components, such as peptidoglycan, teichoic acid

and lipopolysaccharides, by introducing positively-charged groups, thus fending off these cationic host agents [118]. Targeting these processes with nonantibiotic agents may restore the normal host immune system to kill the invading pathogen [118]. Alternatively, based on preclinical models, the use of cytokines and granulocyte colony-stimulating factor may enhance the efficacy of single agent antibiotics [63,64].

The successful use of the corticosteroid dexamethasone in HIV-infected patients with disseminated *M. avium* complex infection and accompanied with progressive weight loss and persistent fever despite multidrug antimycobacterial therapy, has been reported by Wormer and colleagues, suggesting an immunomodulation of the host immune system with adjunct therapy was occurring [119].

Rock and colleagues have provided a snapshot of the potential for exogenous control of regulatory T-cells in allergic responses, inflammatory bowel disease and autoimmunity where saprophytic mycobacteria, helminths and lactobacilli are hypothesized to be recognized by the innate immune system as harmless adjuvants for regulatory T-cell induction [120]. If these 'harmless' adjuvants can be delivered to the host in such a way in asthma and Crohn's patients so as to boost and maintain the normal regulatory levels of IL-10-secreting macrophages and antigen-presenting cells, there may be a beneficial outcome for patients whose immune system is typically depleted of these cell types [120].

Expert opinion

Without question, the mainstay for control of bacterial infections will remain the use of antibiotics. However, with growing resistance problems worldwide affecting virtually all classes of antibiotics, we have the foundation in basic research, the technology and know-how, and in some cases – the proof-of-principle – that adjunct therapy works and can be commercially viable. The most likely scenario for expanded adjunct therapy will be in the hospital setting where more seriously ill patients may benefit from even the smallest improvement in pathogen control, and where there can be a clear indication of improvement with the use of adjunct therapy. Adjunct therapy, with multiple approaches as described above, represents an untapped opportunity for complementing the arsenal of bacteriocidal and bacteriostatic agents.

Outlook

The approach to discovery and development will continue to be driven by empirical therapy practices in medicine, thus requiring the ‘broad spectrum’ agents for community use. Until diagnostic systems become more definitive, less costly and portable, this is unlikely to change. Thus, short of the discovery of multiple, new, novel classes of safe antibiotics, we will see continuous attempts to build improved versions of existing drug classes which will continue to drive resistance emergence in the same classes of drugs. The emerging biotechnology pipeline will provide some novelty in niche markets, but these may spur larger pharmaceutical companies to reinvest in antibiotic research and development as the

outcome of increased antibiotic resistance – predicted by this author to be increased morbidity and mortality – is recognized as a major medical concern and the commercial paradigm changes to entice increased industrial support [8]. Among the niche market options, particularly in reversing antibiotic resistance with a nonantibiotic agent, lies the opportunity for adjunct therapy. As the first generation BLIs have proven to be commercially viable, the next generation could find equal acceptance if they successfully restore the rapidly-eroding β -lactam class of antibiotics. If antivirulence agents can be designed/developed to provide the same clinical impact as BLIs, then antivirulence agents as adjunct therapy could be a viable option for the future.

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