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Non-antibiotic treatment for infectious diseases

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ABSTRACT

The abuse and uncontrolled use of antibiotics has resulted in the emergence and spread of resistant bacteria. The utility of conventional antibiotics for the treatment of bacterial infections has become increasingly strained due to increased rates of resistance coupled with reduced rates of development of new agents. As a result, multidrug-resistant, extensively drug-resistant, and pan-drug-resistant bacterial strains are now frequently encountered. This has led to fears of a "post-antibiotic era" in which many bacterial infections could be untreatable. Alternative non-antibiotic treatment strategies need to be explored to ensure that a robust pipeline of effective therapies is available to clinicians. The new therapeutic approaches for bacterial infections (beyond antibiotics) may provide a way to extend the usefulness of current antibiotics in an era of multidrug-resistant (MDR) bacterial infections.

Tratamiento no antibiótico de las enfermedades infecciosas

RESUMEN

La utilidad de los antibióticos convencionales en el tratamiento de las infecciones bacterianas se ha visto comprometida debido a las elevadas tasas de resistencia junto con la reducción en el número de nuevos agentes en desarrollo. Como resultado, ahora es frecuente encontrar cepas bacterianas multirresistentes, extensamente resistentes o panrresistentes. Esto nos transporta a una era post-antibiótica en la cual muchas infecciones bacterianas podrían ser intratables. Se necesitan explorar estrategias de tratamiento alternativas a los antibióticos que aseguren un "pipeline" robusto de terapias efectivas que lleguen a estar disponibles para los clínicos. De esta forma, las nuevas estrategias terapéuticas (más allá de los antibióticos) aportarán una vía para extender la utilidad de los antibióticos actuales en una era de infecciones por bacterias multirresistentes (MDR).

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INTRODUCTION

It is undeniable that antibiotics have had an enormous impact on global human health by drastically reducing infection-associated mortality. Nonetheless, the abuse and uncontrolled use of antibiotics has resulted in the emergence and spread of resistant bacteria. The utility of conventional antibiotics for the treatment of bacterial infections has become increasingly strained due to increased rates of resistance coupled with reduced rates of development of new agents. As a result, multidrug-resistant, extensively drug-resistant, and pan-drug-resistant bacterial strains are now frequently encountered. This has led to fears of a "post-antibiotic era" in which many bacterial infections could be untreatable. Whilst resistance to antibiotics has escalated steadily, the number of new antimicrobial drugs approved, especially those with novel modes of action, continues to decline. Among the vast number of Gram-positive and Gram-negative bacteria, the 'ESKAPE' group of pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* species) represent the most common antibiotic-resistance pathogens.

Given the rise of antibacterial resistance and the challenges of common antibacterial agent discovery and development that have led to a very small pipeline of new therapies, it would be prudent to consider the potential role of non-conventional approaches[1]. Alternative non-antibiotic treatment strategies need to be explored to ensure that a robust pipeline of effective therapies is available to clinicians[2].

Table 1 Agents that inhibit toxins (effector proteins) and secretion systems (chemical inhibitors and antibodies)

Name of product	Type	Target	Bacterium	Development phase
Shigamab	Monoclonal antibodies	Stx-1, Stx-2	*E. coli*	Phase 2 clinical trial
Raxibacumab	Monoclonal antibody	Cellular receptor anthrax toxin	*B. anthracis*	Animal model
Bezlotoxumab	Monoclonal antibody	Toxin B	*C. difficile*	Completed phase 3 clinical trial
MEDI4893	Monoclonal antibody	α- hemolysin	*S. aureus*	Phase 2 clinical trial
Compounds 1-9	Small molecules	Type 2 secretion systems	*P. aeruginosa, B. pseudomallei*	Preclinical (inhibition of bacterial secretion)
Compounds 7086, 7832, 7812	Small molecules	Type 3 secretion systems	*Yersinia pestis*	Preclinical (efficacy in cell culture)
Salicylidene acylhydrazides	Small molecules	Type 3 secretion systems	*Salmonella, Shigella, Chlamydia, Yersinia, Pseudomonas*	Preclinical (efficacy in mice)
CHIR-1	Small molecules	Type 4 secretion systems	*H. pylori*	Preclinical (efficacy in mice)

Adapted from reference 4 (Hauser AR, et al. Clin Infect Dis 2016; 63: 89–95).

In this review, we explore a range of therapeutic strategies that could be employed in conjunction with antibiotics and may help to prolong the life span of these life-saving drugs. In this article, we highlight some of the recent developments in this area, such as the targeting of bacterial virulence factors, utilization of bacteriophages to kill bacteria, vaccines to prevent healthcare-associated infections (HAI) and manipulation of the microbiome to combat infections. Thus, the new therapeutic approaches for bacterial infections (beyond antibiotics) may provide a way to extend the usefulness of current antibiotics in an era of multidrug-resistant (MDR) bacterial infections.

ANTI-VIRULENCE STRATEGIES

Bacterial pathogens produce virulence factors, molecules that allow them to resist clearance by the host, to invade and gain access to deeper tissues, and to damage host cells. Several innovative alternatives under development interact with virulence factors, making it easier for the immune system to fight them[3]:

Inhibition of toxins and secretion systems. Agents developed can be chemical inhibitors or antibodies (table 1). **1**) Most gram-negative bacteria release toxins via their type III secretion system (T3SS), a complex multiprotein, needle-like apparatuses to inject toxins directly into human cells. T3SS' inhibitors may be active against multiple different bacteria (e.g. KB001 (KaloBios), a pegylated, humanized anti-PcrV antibody Fab' fragment, was safe and showed a trend toward decreasing the development of ventilator-associated pneumonia in *P. aeruginosa* colonised patients; **2**) MEDI3902 (AstraZeneca), a chimeric bispecific monoclonal antibody that recognizes both PcrV and the polysaccharide Psl located on the surface of *P. aeruginosa;* **3)** Raxibacumab (GlaxoSmithKline), a fully humanized immunoglobulin G1 (IgG1) monoclonal antibody that prevents anthrax toxin binding to its host cell receptor, is now recommended for the adjunctive (along with conventional antibiotics) treatment of inhalational anthrax[4], **4)** Antibodies H3H, F3A and F4H suppress the catalytic domain of neurotoxin serotype A in *Clostridium botulinum*).

Targeting biofilms and adherence. Novel methods are being developed that are designed to prevent biofilm formation and to disaggregate biofilms once formed; however, to date these newer strategies have not reached the clinical testing stage[5], although previous modifications of inert substances have been described. **1**) Catheters coated with the zwitterionic polymeric sulfobetaine had reduced amounts of both *S. aureus* and *Escherichia coli* adhesion, and animals treated with these catheters experienced fewer infections. **2**) c-di-GMP, a small signaling molecule, has also been a recent target to prevent infections by *biofilm*-forming pathogens because it regulates the switch that allows planktonically grown bacteria to form biofilms. **3**) Inhibitors of the *pili* biosynthesis (pilicides) reduce the adhesion of bacteria to the epithelium and consequently reduce *biofilm* formation; uropathogenic *E. coli* (UPEC), which causes urinary tract infections, uses a lectin-type fimbriae adhesin to attach to epithelial cells. Small molecules have been developed that interfere with the binding of the fimbriae to sugar moieties on epithelial cell surfaces. For example, ZFH-04269 molecule caused a 1000-fold reduction in the number of UPEC bacteria in the bladders of chronically infected mice.

Targeting signaling and regulation. Quorum-sensing (QS) is a cell density–dependent communication system that utilizes low-molecular-weight signaling molecules (autoinducers) to regulate virulence in many bacterial pathogens. In general, gram-negative species use

N-acylhomoserine lactones (AHLs) or related compounds, and gram-positive species use ribosomally produced autoinducing peptides for QS. M64, a phenoxy derivative of a substituted benzamide moiety with endocyclic aromatic amines, follows the former strategy by inhibiting MvfR, a transcriptional regulator of the 4-hydroxy-2-alkylquinoline QS system of *P. aeruginosa*. Interference with QS of bacteria via 5'-methylthio-DADMe-ImmucillinAs, 5'-ethylthio-DADMe-ImmucillinAs and 5'-butylthio-DADMe-ImmucillinAs, which inhibit the 5'-Methylthioadenosine nucleosidase (MTAN), an enzyme involved in QS of *E. coli* and *Vibrium cholerae*, reducing the biosynthesis of autoinducers AI-1 and AI-2 (signaling molecules), the ability to form biofilms, reducing the infection capacity and the resistance to antibiotics[6].

These antivirulence strategies have as main advantage the fact of being specific to virulence factors that only exists in pathogenic bacteria, so they do not affect the commensal flora in the host. In addition, these antibacterial approaches can be administered either topically or systemically. Finally, combining antivirulence compounds with conventional antibiotics may provide synergistic enhancement of efficacy.

BACTERIOPHAGES AND PHAGE THERAPY

Bacteriophages, or phages, are viruses that only infect bacterial cells. They are biological entities known for over a century. Phage particles represent the most abundant biological entities on the planet, and total phage abundance in the biosphere has been estimated at 1030, or more. However, only now a special interest on phages has been rediscovered, as a potential alternative or complement to current antimicrobial chemotherapy due to their highly specific and unique properties to fight bacterial strains resistant to conventional antimicrobial drugs. Phages are biological entities completely devoid of any metabolic machinery, and thus are obligate intracellular parasites that require a bacterium to replicate themselves, through their genetic material, by taking over the biochemical machinery of the bacterial cells. Bacteriophage therapy, although not new, makes use of strictly lytic phage particles as an alternative, or a complement, in the antimicrobial treatment of bacterial infections. It is being rediscovered as a safe method, because these biological entities devoid of any metabolic machinery do not possess any affinity whatsoever to eukaryotic cells[6].

Most phages discovered until the present day are specialized in interacting with bacteria that express specific receptors and, if the bacterium does not show at the surface a specific receptor for a particular bacteriophage, then the phage becomes naturally (and highly) specific for a given bacterial host. It is estimated that for every bacterial cell, there are ten different bacteriophages, some of which are highly specific for their host – meaning that they recognize only one type of receptor (monophage), while others have a broader host range and recognize more than one type of receptor (polyphage). Phage therapy has been applied over the past few decades to the treatment of bacterial infections, in countries where research and development centres were built specifically for bacteriophages aiming at developing phage therapy. Bacteriophages were used for antibacterial therapy in Russia and Eastern Europe before the advent of antibiotics, and recent dramatic increases in infections with MDR bacterial strains are driving new interest in this approach. The studies conducted in these research centres produced remarkable clinical results. However, and despite the immense potential of bacteriophages for eradicating infections caused by bacterial-resistant strains, up to now only a few clinical trials have been performed in human beings and are accepted by public health authorities.

Phages offer several important advantages over traditional antibiotics. They are specific for bacteria and even particular strains and species of bacteria, they do not infect human cells, and they have little or no effect on normal microbial flora[4]. Limitations include the development of bacterial resistance and immune responses, difficulties in purification from bacterial endo- and exotoxins, and formulation and stability issues in systemic delivery. For these reasons, most studies have been done with topical, gastrointestinal, or pulmonary deliveries. As mentioned, phages have the advantage of being exquisitely specific, but this is also a disadvantage, as cocktails of multiple phages are required to target multiple species and even most strains within a species. Nevertheless, several phage cocktails have exhibited efficacy in animal infection models. Although not a complete litany of their disadvantages, of primary concern is the integration of phage DNA into the bacterial genome, the failure of bacteriophage therapy due to restrictive specificity, and the development of bacterial resistance based on alteration of the bacterial cell surface receptor. A cocktail containing several different phages, especially when used in conjunction with a traditional antibiotic may circumvent these disadvantages. Alternatively, using bacteriophage components (e.g., virolysin, antimicrobial peptides, etc.) may avoid many, if not most, of these pitfalls, serving as a significant source of potent new antimicrobials. Furthermore, using a modified phage coat to display an antigenic peptide, in conjunction with its natural bacterial targeting specificity, may potentially provoke the host immune response at the site of infection[2].

Recent technological advances in this field open the door to the possibility of customizing bacteriophages and improve their characteristics, particularly: (i) expand the ability of bacteriophages to penetrate bacterial biofilms; (ii) enlarge their potency and effectiveness; (iii) adapt the spectrum of activities of bacteriophages to infections caused by numerous bacterial species and strains; and (iv) make them more stable and specific. Strategies to improve phage therapy have involved engineering phages to increase their infectivity and host range and purifying individual phage components to target bacteria[7].

VACCINES

Over the past few years, the prophylaxis of multidrug-resistant pathogen infections through the use of vaccines and passive immunization has become of great interest

due the high economic and ecological cost of antibiotic treatment, as well as the lack of therapeutic targets for the development of new antimicrobials[8]. Its application in high-risk patients will both prevent infections and reduce the use of antimicrobials and the consequent development of resistance. The lack of appropriate predictive models and the selection of high-risk target population are the main challenges in its development.

Among the pathogens considered as urgent threats to public health, *Clostridium difficile* is the one that has achieved greater progress. Different vaccines based on purified and inactivated toxins A and B are in advanced stages of development, showing to be safe and immunogenic[9]. Different surface antigens are also being studied for its incorporation in future vaccines in order to prevent host colonization and cross-transmission.

S. aureus is known for its wide range of virulence factors and host immune evasion mechanisms. Several vaccines (StaphVAX or V710) have failed in their development for several reasons including the complexity of pathogenic mechanisms, extensive antigenic variability, biofilm formation capacity or immune evasion mechanisms[10]. There is currently a developing program for an antigenic vaccine (SA4Ag), which has shown rapid immunogenicity in early studies, being under investigation in a phase 2b study.

Among the Gram-negative bacteria, the vaccines against *P. aeruginosa* have presented a greater development, having already been concluded a phase 3 study (IC43). The variability of *K. pneumoniae* and *E. coli* capsular polysaccharides limit their potential as vaccine targets, and the extracellular vesicles are currently under investigation as potential immunogenic agents. Studies with *Acinetobacter spp.* still in early stages, focusing on the selection of specific antigens.

There are currently two vaccines in phase 2 to prevent vulvovaginal candidiasis, but no specific vaccines have yet been developed to treat invasive fungal infections.

On the other hand, the use of monoclonal antibodies (mAbs)[11] has taken center stage in the last years. *C. difficile*, *S. aureus* and *P. aeruginosa* mAbs are currently in phase 2-3 clinical trials[12], whereas mAbs against *E. coli*, *Klebsiella spp.* and *Acinetobacter spp.* are still in early stages. MAbs do not require adaptive immune response and could be investigated in immunocompromised patients, being of potential useful as therapeutic or prophylactic treatment. Most of them are targeted to toxins and therefore could be considered as antivirulence strategies that can mitigate the disease without promoting antibiotic resistance, but may also require the use of antibiotics to reduce bacterial load.

MICROBIOME MODULATION

Human microbiota is the amount of microorganisms that are found in human body and microbiome is the collection of all their genomes. In a healthy adult colon, (the most investigated body habitat), there are more or less 160 bacterial species (mainly *Bacteroidetes* and *Firmicutes*) which contribute to regulate physiological functions. Disruption of this ecosystem has been associated with many illnesses like diabetes mellitus, cardiovascular diseases, asthma, autism, inflammatory bowel disease (IBD), antibiotics-associated diarrhoea and cancer.

Microbiome usefulness in medicine. At this moment, the most encouraging application of microbiome in medicine is in the sphere of treating recurrent infections caused by *C. difficile*, an anaerobic, esporulating, toxin former, gram-positive bacilli which represents the leading cause of healthcare and antibiotics-associated diarrhoea and pseudomembranous colitis[13].

A. Prebiotics. They are non absorbible polysaccharides (like inulin and fructo-oligosaccharides) that have positive influence in host health, stimulating biodiversity of human gut microbiome. There are studies in which prebiotics have been taken by patients with antibiotics-associated diarrhoea but results about its effectiveness are contradictory[14].

B. Probiotics. They are live microorganisms that, when used at proper concentrations, give benefit to host health, helping to preserve normal microbiome and preventing the growth of pathogenic bacteria[15]. For example, *Saccharomyces boulardii* and *Lactobacillus* species could reduce the incidence of *C. difficile* infection (CDI); *Lactobacillus salivarius* may inhibit the growth of *Listeria monocytogenes*; *Streptococcus mutans* could confer protection against development of dental caries and vaginal applications of *Lactobacillus jensenii* may defend women from infection produced by *Gardnerella vaginalis*, *Candida albicans* and *E. coli*.

C. Faecal microbiota transplantation (FMT): In FMT, faeces from a healthy person are employed in order to restore gut microbiome which is disrupted in a sick patient, suppressing *C. difficile* and other microorganisms overgrowth. This procedure has shown a rate of cure of recurrent CDI around 90%. Results in IBD are less consistent and there are a few case series reports of patients with neurological disorders like multiple sclerosis or Parkinson disease who achieved sustained improvement and patients with autism which symptoms ameliorated by this approach. It may be useful to treat infections caused by drug-resistant bacteria like vancomycin-resistant enterococci or multidrug-resistant *K. pneumoniae*[16], as it has been shown in two patients who had undergone hematopoietic cell transplant, but nowadays, further investigations are needed to make a high quality evidence based recommendation[17].

CONCLUSIONS AND FUTURE VISION

The global burden of antimicrobial resistance is rising and is associated with increased morbidity and mortality in

clinical and community setting. Spread of antibiotic resistance to different environmental niches and development of superbugs have further complicated the effective control strategies. International, national and local approaches have been advised for control and prevention of antimicrobial resistance. Rational use of antimicrobials, regulation on over-the-counter availability of antibiotics, improving hand hygiene and optimizing infection prevention and control are the major recommended approaches. Thorough understanding of resistance mechanism and innovation in new drugs, antibiotic delivery systems, other potential non-antibiotic alternatives and vaccines are needed[18]. A multidisciplinary, collaborative, regulatory approach is demanded for combating antimicrobial resistance. Solutions to antibiotic resistance are not trivial to implement, with consequences affecting everyone. While solutions have been proposed, with some even being launched to address the problem, action taken to date is merely token. Antibiotics remain indispensable in all health systems, and the consequences of a lack of a medically, socially, and economically coordinated effort will be dire. Simple antibiotic stewardship is no longer an option in the face of rising drug resistance. Clinical treatments and practices for infection control must evolve in response to epidemiological trends in MDR bacteria and the development of new treatment strategies. Hopefully, repeated emphasis will promote adoption of new research strategies for infection treatments, including adherence to three criteria: (1) invention of effective new drugs, (2) prevention of resistance, and (3) protection of the natural host microbiome. The best strategy to meet these criteria includes the development of new combination approaches coupled with local and smart delivery technologies.

The extraordinary success of conventional antibiotics led to a focus on development of these agents to the exclusion of other antibacterial strategies[19]. A silver lining in the current dark cloud of antibiotic resistance is that these alternative strategies are again being pursued, although significant challenges remain before they can be widely adopted into clinical practice. Many of these compounds are still in the preclinical phase of development, and a substantial investment in time and resources will be necessary before they significantly impact the ability of physicians to treat patients infected with MDR bacteria. The next step is to establish out which alternatives to antibiotics are most likely to deliver new therapies of clinical use. Experts have found that academic researchers and the pharmaceutical industry have successfully generated a diverse portfolio of potential alternatives-to-antibiotics projects. Results from studies of these approaches are still emerging and these approaches hold promise provided that adequate funding is available for researchers to build capacity and create a preclinical evidence base to enable prioritization and progress of optimized drugs to following crucial phase validation. So, this first wave of new approaches will probably best serve as adjunctive or preventive therapies. Therefore, traditional antibiotics will still be needed. In an assessment of the future impact of alternative technologies on antibiotics markets, ten alternative technologies were identified and analysed for their potential impact on the antibiotics market. Of these, rapid point-of-care diagnostics, vaccines, FMT, and probiotics were considered to have a "high" or "medium" potential impact over a 10-20 year horizon[20]. Therapeutic antibodies, antibiotic biomaterials, bacteriophages, antimicrobial nanoparticles, antimicrobial peptides, and anti-virulence materials were rated as having "low" potential impact. Despite the apparent potential of the most promising alternative technologies to reduce demand of antibiotics, that reduction will likely only happen in limited segments of the antibiotics market or, in case of preventing community acquired infections by vaccination, in a low-price generics market segment. Thus, alternative technologies are not expected to represent any disincentive to antibiotics developers. Therefore, it is unlikely that alternative technologies will displace the need for new classes, and sub-classes, of antibiotics in short and medium-term.

Longer term substantial and sustainable funding will be needed to advance and make use of the wider alternatives-to-antibiotics portfolio. Policy and funding should now be linked. Without sufficient funding we can assume that new treatments to replace or supplement antibiotics will not be available, and the consequences of such a prolonged delay for global health-care systems need to be considered now. If these difficulties can be surmounted, alternatives to antibiotics may become important therapeutic options for bacterial infections.

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