Current approach and methods

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.

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. 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.

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Rev Esp Quimioter 2017;30 (Suppl. 1): 66-71
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:

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 anthrax4; 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; 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 pil 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

<table>
<thead>
<tr>
<th>Name of product</th>
<th>Type</th>
<th>Target</th>
<th>Bacterium</th>
<th>Development phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigamab</td>
<td>Monoclonal antibodies</td>
<td>Stx-1, Stx-2</td>
<td>E. coli</td>
<td>Phase 2 clinical trial</td>
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<td>Raxibacumab</td>
<td>Monoclonal antibody</td>
<td>Cellular receptor anthrax toxin</td>
<td>B. anthracis</td>
<td>Animal model</td>
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<td>Bezlotoxumab</td>
<td>Monoclonal antibody</td>
<td>Toxin B</td>
<td>C. difficile</td>
<td>Completed phase 3 clinical trial</td>
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<tr>
<td>MEDI4893</td>
<td>Monoclonal antibody</td>
<td>α-hemolysin</td>
<td>S. aureus</td>
<td>Phase 2 clinical trial</td>
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<td>Compounds 1-9</td>
<td>Small molecules</td>
<td>Type 2 secretion systems</td>
<td>P. aeruginosa, B. pseudomallei</td>
<td>Preclinical (inhibition of bacterial secretion)</td>
</tr>
<tr>
<td>Compounds 7086, 7832, 7812</td>
<td>Small molecules</td>
<td>Type 3 secretion systems</td>
<td>Yersinia pestis</td>
<td>Preclinical (efficacy in cell culture)</td>
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<tr>
<td>Salicylidene acylhydrazides</td>
<td>Small molecules</td>
<td>Type 3 secretion systems</td>
<td>Salmonella, Shigella, Chlamydia, Yersinia, Pseudomonas</td>
<td>Preclinical (efficacy in mice)</td>
</tr>
<tr>
<td>CHIR-1</td>
<td>Small molecules</td>
<td>Type 4 secretion systems</td>
<td>H. pylori</td>
<td>Preclinical (efficacy in mice)</td>
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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-alkylquinolone QS system of P. aeruginosa. Interference with QS of bacteria via \(5'-\text{methylthio-DADMe-ImmcillinAs},\ \ \overline{6}^\text{-ethylthio-DADMe-ImmcillinAs}\) and \(5'-\text{butylthio-DADMe-ImmcillinAs},\) which inhibit the \(5'-\text{Methylthioadenosine nucleosidase (MTAN)},\) 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 10^30, 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\(^7\).

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. 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. 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. There is currently a developing program for an antigen 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) has taken center stage in the last years. *C. difficile*, *S. aureus* and *P. aeruginosa* mAbs are currently in phase 2-3 clinical trials, 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, sporulating, toxin former, gram-positive bacilli which represents the leading cause of healthcare and antibiotics-associated diarrhoea and pseudomembranous colitis.

- **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.

- **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. 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*, 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.

**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\textsuperscript{16}. 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\textsuperscript{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 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\textsuperscript{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 subclasses, 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.

REFERENCES

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