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Alternatives to overcoming bacterial resistances: *State-of-the-art*

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ABSTRACT

Worldwide, bacterial resistance to chemical antibiotics has reached such a high level that endangers public health. Presently, the adoption of alternative strategies that promote the elimination of resistant microbial strains from the environment is of utmost importance. This review discusses and analyses several (potential) alternative strategies to current chemical antibiotics. Bacteriophage (or phage) 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. Lysin therapy is also recognized as an innovative antimicrobial therapeutic option, since the topical administration of preparations containing purified recombinant lysins with amounts in the order of nanograms, in infections caused by Gram-positive bacteria, demonstrated a high therapeutic potential by causing immediate lysis of the target bacterial cells. Additionally, this therapy exhibits the potential to act synergistically when combined with certain chemical antibiotics already available on the market. Another potential alternative antimicrobial therapy is based on the use of antimicrobial peptides (AMPs), amphiphilic polypeptides that cause disruption of the bacterial membrane and can be used in the treatment of bacterial, fungal and viral infections, in the prevention of biofilm formation, and as antitumoral agents. Interestingly, bacteriocins are a common strategy of bacterial defense against other bacterial agents, eliminating the potential opponents of the former and increasing the number of available nutrients in the environment for their own growth. They can be applied in the food industry as biopreservatives and as probiotics, and also in fighting multi-resistant bacterial strains. The use of antibacterial antibodies promises to be extremely safe and effective. Additionally, vaccination emerges as one of the most promising preventive strategies. All these will be tackled in detail in this review paper.

Contents

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1. Introduction

Worldwide, bacterial resistance to antimicrobial therapy has increased dramatically over the past few years, reaching a "new pre-antibiotic era", where society is put in danger. Currently, according to the World Alliance against Antibiotic Resistance (WAAR), antibiotics may completely lose their effectiveness over the next five years due to a combination of both self-medication and irrational prescription and use of these therapeutic agents, which has led to the development of multi-resistant bacterial strains, and in fact, some of them are resistant to all available antibiotics. Therefore, the need to develop feasible alternatives to antibiotics becomes more and more of utmost importance, so as to protect and promote global public health (Goossens et al., 2005; Carlet et al., 2011, 2012b; Carlet and Mainardi, 2012; Escobar-Paramo et al., 2012; Oldfield and Feng, 2014; WHO, 2015).

The European Centre for Disease Prevention and Control (ECDC) reported that, each year, 25000 people die from infections caused by multi-resistant bacteria and also added that these microorganisms costs about 1.5 billion euros in extra healthcare services and productivity losses per year to Europe (Carlet and Mainardi, 2012). Additionally, as published in a report by the World Health Organization (Leung et al., 2011), in the USA alone such costs represents ca. 35 billion USD per year. It was found that, certain bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* (which are both pathogenic and commensal bacteria in humans or animals), that in an initial phase were susceptible to conventional antibiotics, started to acquire resistance to the antimicrobial treatment, including to the third-generation cephalosporins (Carlet et al., 2011; Carlet and Mainardi, 2012).

The "globalization of resistance" to antibiotics occurred, for example, with the spread of New Delhi Metallo-beta-lactamase-1 (NDM-1), an enzyme produced by *Klebsiella pneumoniae* or *Escherichia coli*, which can make them resistant to β-lactam antibiotics. This enzyme was first discovered in India, and later it was disseminated to Pakistan, USA, Canada, Japan and the United Kingdom (Yong et al., 2009; Walsh, 2011; Charan et al., 2012; Tsang et al., 2012; Ojala et al., 2013). Another example of "globalization of resistance" to antibiotics is the appearance, back in 2009, of KPC (*Klebsiella pneumoniae* carbapemenase), an enzyme responsible for the degradation of carbapenem antibiotics (Nordmann et al., 2011). The panorama is more alarming when bacterial resistance linked to bacterial virulence factors, leading to an outbreak as what took place with the haemolytic-uremic syndrome associated with the *Escherichia coli* serotype O104:H4, which occurred in Europe in May 2011, affecting more than 3400 people and killing ca. 50 patients. This bacterial strain was found in salads, causing renal failure, thrombocytopenia and haemolytic anemia (Buchholz et al., 2011).

Bacteria can resist to antibiotics via different resistance mechanisms, viz. (i) reduction in bacterial uptake of the antibiotic; (ii) production of hydrolytic enzymes, such as β-lactamases, that inactivate the antimicrobial drug; (iii) modification of the antimicrobial drug receptor; (iv) reduction of the antimicrobial drug concentration in the intracellular environment by the efflux pumps present in bacterial membranes; (v) modification of the enzymatic pathway, leading to a decrease of the bacterial susceptibility to the antibiotic; and (vi) loss of intracellular enzymes used in the activation of the prodrug (Łęski and Tomasz, 2005; Piddock, 2006; Rouveix, 2007; Stavri et al., 2007; Rang et al., 2012; Romanelli et al., 2010; Wardal et al., 2010; Martínez-Júlvez et al., 2012; Ojala et al., 2013).

Over the last decades, it has been observed an increased use of antibiotics, in large part caused by the increasing numbers of people needing healthcare, as a result of an ageing population and the consequent increase of chronic diseases and healthcare-associated infections (HAI), also known as nosocomial infections, which represents a worldwide public health problem (Pina et al., 2010a,b; Fair and Yitzhak, 2014).

Self-medication provides another "weight" factor that contributes to a widening of this problem and leads to an overconsumption of antibiotics. Drugs are incorrectly used by the population, and are frequently used to treat common colds and/or respiratory tract infections, mainly caused by viruses (Campos et al., 2007; Mainous et al., 2009). Furthermore, the overuse of