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The Future of Antibiotic: From the Magic Bullet to the Smart Bullet

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Editorial

The antibiotics have represented a great revolution for humankind, the development after the World War II of a magic bullet (the antibiotic molecule), as imagined by Paul Erlich, the pioneer of chemotherapy, with the property to kill or inhibit the growth of microorganisms by hitting the microbial structures with low toxicity for host cells and tissues, has determined a new era in the treatment and prophylaxis of infectious disease and in the quality of human life.

However, starting from '50 of last century up to recent decades and currently, a large number of antibiotics, due to the emergence of multidrug-resistant bacterial strains (both Gram-negative and Gram-positive), have become scarcely effective and not-useful. It is estimated that drug-resistant strains of bacteria are responsible for 5,000 deaths a year in the UK and 25,000 deaths a year in Europe. The World Health Organization, in the recent (2014) report on antimicrobial resistance in common bacterial pathogens, states that a post-antibiotic era is a close possibility for the 21st century [http://www.who.int/drugresistance/documents/surveillancereport/en/]. Moreover, there is a lack of investments by pharmaceutical companies in the development of new antibiotics, but new antimicrobials for counteracting the pathogens are needed. This scenario has to stimulate the research of alternative strategies to conventional antibiotics. How could we imagine the antibiotic of future, which additional characteristics should it have? Starting from a good selectivity index (that is the ratio between toxic dose for the host and efficient dose against microbial cells), other important properties to obtain a "smart bullet" will be needed: the ability to hit pathogens without killing beneficial microbiota; a low selectivity pressure to promote the rise in antibiotic-resistance strains; the property to tackle natural form of resistance like multi-stratified microbial population growing on surfaces, the so-called biofilms; the capacity to eliminate "dormant" cells, that is microbial cells metabolically inert and for this naturally resistant to current antibiotics.

The first two objectives could be obtained throw the use of anti-pathogenic agents. Over the last decade, many studies focused on agents that target the virulence of pathogens without killing or inhibiting the growth of microorganisms and therefore with limited selective pressure to promote the antibiotic resistance development [1,2]. A fundamental step of Gram-positive and Gram-negative pathogenesis is the bacterial adhesion to the host tissue involving a direct and a specific interaction between bacterial surface molecules and host ligands. Interfere with adhesion, the first step of pathogenesis, could be an efficient way to prevent or treat infections. The adhesion is a fundamental step for microbial colonization and infection and through it the pathogens also avoid to be mechanically removed from the host [3].

Gram-positive and Gram-negative pathogens adhere to the host tissues through filamentous organelles known as pili [4,5]. The pili function on initial bacterial attachment, invasion and biofilm formation, has been mainly studied for Gram-negative bacteria [5]. Some new agents, known as pilicides, have been synthesized to target the chaperone–subunit interaction [6] and the chaperone interaction with a protein involved in the biogenesis of the pili in Gram-negative known as fimbrial usher protein [7]. Uropathogenic *Escherichia coli* (UPEC) is the major aetiological agent of Urinary Tract Infections (UTIs) and is often studied as model of Gram-negative pathogen for the development of pilicides compounds.

There are many works on the synthesis of pilicides [6,7], in a recent article on this subject, it has been reported the synthesis of N-(4-chlorophenyl)-2-{5-[4-(pyrrolidine-1-sulfonyl)-phenyl]-[1,3,4]oxadiazol-2-yl sulfanyl}-acetamide as inhibitor of the assembly of type 1 pili interfering with the subunit incorporation cycle of the chaperone–usher pathway [8].

Similar structural motifs of pilin components has been found in an important family of Gram-positive surface proteins, the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), able to recognize host's extracellular matrix proteins, such as fibrinogen, fibronectin, and collagen [4,9].

If we consider the important part played by MSCRAMMs in the first step of Gram-positive pathogenesis and of biofilm formation, we believe that new anti-virulence agents could be developed by using as a target the enzyme responsible of linking such proteins to cell wall, that is the Sortase A (SrtA), rather than any single surface protein involved in the mechanism of virulence [10]. The SrtA is a membrane-bound cysteine transpeptidase that is responsible, in Gram-positive bacteria, for the covalent anchoring of surface proteins to bacterial cell wall.

SrtA inhibitors can be classified into three groups: natural compounds, peptides and synthetic small molecules. Natural compounds with SrtA inhibitory activity are obtained principally from plants as *Fritillaria verticillata* [11], *Rhus verniciflua* [12], *Curcuma longa* L [13-15], *Coptis chinensis* [16], *Sophora flavescens* [17] and from marine invertebrates like the sponges Spongosorites sp [18], *Aaptos aaptos* [19], Sceptrella sp [20], Coscinoderma sp [21] and like the ascidian Synoicum sp [22,23].

The first peptides described as SrtA inhibitors were the peptidyl-diazomethane and peptidyl-chloromethane analogues, Cbz (benzyloxycarbonyl)-Leu-Pro-Ala-Thr-CHN_2 and Cbz-Leu-Pro-Ala-Thr-CH_2Cl which found to act as time-dependent irreversible inhibitors of recombinant sortase (SrtAΔN). The inhibitor sequences mimic the substrate recognition motif of the SrtA (-Leu-Pro-Xaa-Thr-Gly-) with the difference that the scissile amide bond between threonine and glycine residues was replaced with a diazoketone or chloromethyl ketone group, groups able to alkylate the Cys 184 of the

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enzyme active site [24]. Peptide acting as irreversible inhibitor of SrtA was obtained by Connolly et al. replacing the scissile Thr-Gly in the substrate recognition motif of SrtA with a vinyl sulfone group (C=C-SO_2Ph) [25].

Regarding synthetic small molecules the most representative were obtained via High-Throughput Screening by Suree et al. that described new derivatives belonging to the three chemical classes of rhodanines, pyridazinones and pyrazolethiones with SrtA IC50 values, for the most active molecules, of 3.7 μM, 0.20 μM and 0.30 μM, respectively. These molecules probably act on the enzyme through a thiol-disulfide exchange reaction with Cys 184 [26].

Other cell surface molecules in Gram-positive bacteria, involved in the adhesion process, without cell wall anchorage, are non-proteinaceous adhesins like Wall Teichoic (WTA) and lipoteichoic acids. Since WTAs are required for host infection and play important role in biofilm formation, it has been suggested that they are important virulence factors required for the estabilishment and spread of infection in a host. Therefore, the enzymes involved in WTAs biosynthesis can be considered as good targets for novel antimicrobials that interfere with Gram-positive pathogenic process. One possible target is the WTA biosynthetic pathway because strains of *S. aureus* and *B. subtilis* mutants in WTAs are not able to colonize the host tissue and show a greatly diminished ability to establish infection in animal models [27,28].

Current antibiotics can be efficacious against planktonic (free living) pathogens but are poorly effective against bacteria growing as biofilms. Biofilms structured bacteria develop multifactorial mechanisms of antibiotic-resistance and one of the most important factor of tolerance is the slow growth and low metabolic activity of bacterial cells (dormant cells) in the internal layer of community, so they are intrinsically resistant to current antibiotics, which target dividing and metabolically active cells, and represent a reservoir for recurrent infections. The Antimicrobial Peptides (AMPs) [29] generally defined as cationic, amphipathic peptides, with no more than 50 amino acids, are very promising agents in the struggle against pathogenic biofilms, in fact they permeabilize and form pores within the cytoplasmatic membranes, so they can act on slow-growing or even non growing bacteria that exhibited a reduced susceptibility to many antibiotics. The AMPs also have a high potential for interfering with biofilm formation, in fact they could minimize initial adhesion of microbial cells to abiotic surfaces (medical devices etc.) by altering the adhesive features, or by inhibiting quorum-sensing, that is the communication system used by many pathogens to control collective behaviours, such as virulence factors production and biofilm formation.

There are many gaps of knowledge in the research of anti-virulence drugs, for example, the lack of assessment of the efficacy of most SrtA inhibitors by using *in vitro* or *in vivo* models of infections. Attenuation of virulence as antimicrobial strategy needs a good host immune defence for bacterial clearance, so immune-compromised patients could not be treated. That is a point of weakness of anti-virulence approach and further studies to improve the pharmaceutical potential of anti-virulence agents are needed. The possibility of using novel anti-virulence agents in combination with AMPs that have also immune modulatory functions [30], could contribute to overcoming the above mentioned point of weakness, and make this strategy effective to combat the developing risk of pathogens that current antibiotics cannot defeat.

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