Multidrug Resistant Gram-Negative Bacteria

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Antibiotic resistance has increased markedly in Gram-negative bacteria, causing severe infections intractable with traditional drugs and amplifying mortality and healthcare costs. Consequently, to find novel antimicrobial compounds, active on multidrug resistant bacteria, is mandatory. In this regard, cationic antimicrobial peptides (CAMPs)—able to kill pathogens on contact—could represent an appealing solution. However, low selectivity, hemolytic toxicity and cost of manufacturing, hamper their massive clinical application. In the recent years—starting from CAMPs as template molecules—less toxic and lower-cost synthetic mimics of CAMPs, including cationic peptides, polymers and dendrimers, have been developed. Although the pending issue of hemolytic toxicity and biodegradability is still left not completely solved, cationic antimicrobial polymers (CAPs), compared to small drug molecules, thanks to their high molecular weight, own appreciable selectivity, reduced toxicity toward eukaryotic cells, more long-term activity, stability and non-volatility. With this background, an updated overview concerning the state of the art of the main manufactured types of CAPs, active on Gram-negative bacteria, is herein reported, including synthetic procedure and action's mechanism. Information about the antibacterial activity, advantages and drawbacks of the most appealing compounds was also provided.

antibiotic resistance | Gram-negative bacteria | hemolytic cytotoxicity | membrane disruption | positively charged polymers

1. Introduction

The increasing replacement of antibiotic-susceptible bacteria (ASB) with antibiotic-resistant bacteria (ARB) is one of the most concern of microbiologists and over the last two decades, antibiotic resistance has increased markedly in Gram-negative bacteria and has determined an improvement of mortality and of healthcare costs.

Gram-negative bacteria pose a major threat to human health, since they are the most critically resistant and rapidly spreading bacteria, frequently responsible for severe and often deadly infections, not only in the general population, but also in the hospital settings or among people with weak or not yet fully developed immune systems, such as newborns, elderly, people undergoing surgery and cancer treatment.

Gram-negative bacteria, such as *Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa, Burkholderia cepacia* and *Escherichia coli*, are responsible of severe infections including pneumonia, bloodstream infections, wound or surgical site infections and meningitis in healthcare settings.

Unfortunately, as very recently outlined by two reports published by the World Health Organization (WHO) on new antibiotic agents, among the 50 innovative molecules in development, very few target Gram-negative species .This findings raise deep concern, especially if considering a previous report published by WHO in 2017 indicating 12 classes of bacteria that are highly critical for human health, due to their extraordinary resistant traits, where, in addition to *Mycobacterium tuberculosis*, Gram-negative pathogens clearly outnumber the Gram-positive ones. The prevalence of Gram-negative bacteria over Gram-positive is evident in all the priority groups identified in the report, such as the “other priority pathogens” group (where *A. baumannii, P. aeruginosa* and *Enterobacteriaceae* are included), the “high priority” group (encompassing *Helicobacter pylori*, Campylobacter specie, Salmonella species and *Neisseria gonorrhoeae*) and the “medium priority” group (that include *Hemophilus influenzae* and Shigella species).

2. Resistance

Lastly, Gram-negative bacteria, unlike Gram-positive bacteria, are characterized by high and similar resistance levels, both in Europe and in the United States. In fact, citing the same report: “when compared to the US data, the European Center for Disease Prevention and Control (ECDC) surveillance network showed overall lower rates of resistance in Gram-positive bacteria (although with large differences between countries) and the same worrying rates among Gram-negative bacteria”

These reports, developed by a WHO-led group of independent experts, encourage the medical research community to develop innovative treatments for these resistant Gram-negative bacteria, which are spreading rapidly and, more than Gram-positive ones, require urgent solutions.

Incessantly, Gram-negative bacteria build-in abilities, to find new ways to be resilient to drugs and are also able to pass along genetic materials that allow other bacteria to become drug-resistant as well. Genotyping and sequencing the whole genome of large groups of isolated clinical bacterial has allowed the scientists to understand how antibiotic resistance develops and transmits both among bacteria and patients. The most clinically important resistance phenotypes include carbapenem resistant *Enterobacteriaceae*, extensively drug resistant (XDR) *P. aeruginosa* and XRD *A. baumannii.*

New Delhi metallo-beta-lactamase 1 (NDM-1) makes bacteria resistant to a broad range of antibiotics, including those from the carbapenem family, which today are the last line of defense against antibiotic-resistant bacterial infections.

Antibiotic degradation, antibiotic target modification, modulation of permeability through the bacterial membrane and structural modifications of bacterial lipopolysaccharide are some of the established mechanisms of resistance and their knowledge have influenced the development of novel antibiotics for replacing ineffective beta lactams and have disposed innovative treatment practices in highly resistant infections.

It was established that the traditional antibiotics in the form of single target small molecules or small hydrophobic drugs, often fail in fighting multidrug resistant bacteria and therefore the search for identifying structurally different and more effective forms of antimicrobial agents, active especially against Gram-negative strains is increasingly necessary and urgent.

In this regard, naturally occurring cationic antimicrobial peptides (CAMPs) are a wide well-performant class of not beta lactams antimicrobial agents, with a broad spectrum of action, active on a wide variety of Gram-positive and Gram-negative bacteria, fungi, protozoa and yeast.

In particular, among CAMPs, polymyxins as colistin and polymyxin B, that differs by colistin only for a single amino acid in the peptide ring, are cyclic polypeptides produced by some strains of *Bacillus polymyxa*, specific to counteract Gram-negative bacteria that nowadays are highly critical for human health. In fact, polymyxins, although totally ineffective on Gram-positive bacteria, are highly active against most members of Gram-negative strains, including the Enterobacteriaceae family, counting *E. coli, Enterobacter* spp.*, Klebsiella* spp.*, Citrobacter* spp.*, Salmonella* spp. and *Shigella* spp. and common non fermentative Gram-negative bacteria, such as *A. baumannii, P. aeruginosa* and *Stenotrophomonas maltophilia*.

These molecules, differently from conventional not cationic antibiotics, thanks to their positive charge, without needing to enter the bacteria cell and interfere with specific metabolic processes, act with a rapid and non-specific disruptive action on bacteria membranes and kill pathogens simply on contact, before they manage to organize adaptive processes for becoming resistant. Unfortunately, despite their considerable activity, the massive clinical application of native CAMPs, as well as of polymyxins, is hampered by their poor stability, high costs of production and strong toxicity for human cells.

Assuming that the cation character can represent a fundamental characteristic for manufacturing antimicrobial devices active where old molecules fail, in the recent years, starting from natural CAMPs, taken as template molecules, the scientists have endeavored to develop less toxic and more low-cost mimics of CAMPs.

Synthetic cationic peptides, natural and synthetic cationic polymers and positively charged dendrimers were proposed, to be used as novel and unconventional antimicrobial devices with potential to counteract infections by multidrug resistant Gram-negative strains.

Among the developed mimic of CAMPs, cationic antimicrobials in the form of macromolecules have gained increasing attention by the scientific community because an antimicrobial polymer if compared to small drug molecules could be endowed with several advantages, such as more long-term activity, limited residual toxicity, chemical stability, non-volatility and incapacity to permeate through the skin thanks to its macromolecular structure and high molecular weight (MW).

In the last decades, antimicrobial polymers have aroused increasing interest among scientific community until becoming a “hot” topic as confirmed and highlighted also by the publications trend in the years 1990–2020.

Over 30 years, the scientific production and therefore the research in the field of antimicrobial polymers went from being very limited until 2000, to growing steadily until it assumed an exponential increase in the last decade, probably hand in hand to how the concern for the dangers represented by multidrug-resistant Gram-negative bacteria has grown.

On this background, in the complete work published recently on Polymers and included in encyclopedia with the title "Antimicrobial Polymers", the most important achievements in the field of cationic antimicrobial polymers (CAPs) were reviewed. An updated information concerning the different types of the industrialized CAPs active on Gram-negative bacteria that are highly critical for human health, their structures, the supposed mechanism of action and their uses or field of applications, were reported.