New antibiotics against bacterial resistance

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Abstract
The evolution of bacterial resistance is generating a serious public health problem due to the indiscriminate use of antibiotics, the application of non-optimal doses, the irregularity in the taking of medicines sent by the health professional, factors that have affected the increase in the rate of antimicrobial resistance; It is important to generate strategies that contribute to diminishing it, including the rational use of antibiotics and the constant research of new therapeutic alternatives such as teixobactin, which is a product of the Gram negative bacterium called Eleftheria terrae, related to the genus Aquabacterium, is a microorganism that presents extremophile conditions, for which, a multichannel system of semipermeable membranes called Ichip was developed for its isolation. Eravacycline is a new fully synthetic bacteriostatic antibiotic of the tetracycline family, is a potent inhibitor based on the mechanism of the bacterial ribosome and exerts potent activity against a broad spectrum of susceptible and multiresistant bacteria.

Keywords: teixobactin, eravacycline, tetracycline, bacterial resistance, lipid II, Eleftheria Terrae, Ichip.

Introduction
Currently, pharmaceutical industries are lacking therapeutic alternatives against pathogenic bacteria, due to multiresistance mechanisms developed by these ones against the different commercialized antibiotics1. In the last decade, several key organizations, as the Infectious Diseases Society of America (IDSA), Centers for Disease Control and Prevention (CDC), the World Health Organization (WHO), and the World Economic Forum (WEF), have made of the antibiotic resistance the focal point of the reports, by conferences to accelerate the restriction and control activity faced with antimicrobials2. Bacterial resistance evolution is generating a serious problem in public health3, as a result of the indiscriminate use of antibiotics, the application of non-optimal doses, the irregularity in medication taking referred by the professional in health, these are factors that have influenced in the antimicrobial resistance rate rising4, being relevant to generate strategies that contribute to decrease it, including the rational use of antibiotics5. That is why it has been necessary constant researches and the perseverant development of new therapeutic alternatives6. Approximately five years ago, professor Kim Lewis from Northeastern University in Boston, directs the research that discovered a new natural antibiotic extracted from the ground called teixobactin; this research has been developed together with NovoBiotic Pharmaceuticals, which headquarters are located in Cambridge, Massachusetts and are the owners of the patent7. The objective of this review is to describe new antibiotics generated as therapeutic alternatives faced with the bacterial resistance emergence, their obtaining methods and actions mechanisms.

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Materials and methods

It was made a bibliographic search on data bases as Science Direct, Redalyc, Google Scholar, NCBI, Pubmed, Pro-quest, Dialnet, Lilacs and Toxnet; they were used the next key words teixobactin, eravacycline, tetracycline, bacterial resistance, lipid II, Eleftheria Terrae,echip validated in MeSH. It was established for the search of thirteen combinations, finding a total of 70 articles. They were selected original and revision articles published from 2012 to 2018 available in Spanish, English and Portuguese.

Results

Teixobactin

Teixobactin is a depsipeptide that consists of eleven residues of amino acids (aa), including seven L-aa residues and four D-aa ones, among them it is found an unusual L-allo-enduracididine (L-allo-End), which is part of the C-terminal tetrapeptide lactone substructure formed by an ester bond between D-Thr8 and L-Ile11. (Figure 1). This is a product of a Gram negative bacterium called Eleftheria terrae, which belongs to the proteobacteria group (betaproteobacteria), related to Aquabacterium gender, from this bacterium, it has been isolated a compound called teixobactin with a molecular weight of 1.242 Daltons, which biosynthetic route is made up of two genes txo1 and txo2; the technique used to discovered it, entails a higher impact that is able to originate a new encouraging era in the discovery of new natural antibiotics, because of the description of its action mechanism in the organism which possibly avoids the creation and expression of any kind of bacterial resistance.

Technology for microorganisms obtaining: IChip Method

The microorganisms are cultivated in a laboratory where it is analyzed its pathogenicity, its capacity of producing antibiotics and its evolution, through the control of growth conditions, taking into account those necessary for an optimal development and proliferation, however, approximately 99% of the bacteria is not able to be cultivated in the laboratory due to extract growth demands.

Eleftheria terrae is a microorganism that presents extreme conditions, faced with this, it was developed a semipermeable plastic membrane system called IChip (isolation chip) (Figure 2), which creates an analogous natural environment of bacteria and fungi, allowing the growth in its “natural environment” by simulating their environment in the laboratory. IChip is able to cultivate organisms from soil, sea water, saliva, marshes, and sewage water bioreactors.

This multichannel device with 192 compartments is used for an initial microorganisms isolation followed by stimulating the proliferation of bacteria which present a difficult growth in the laboratory; from a soil sample collected in the state of Maine (U.S.A.) it was found an ecosystem composed by all kind of microorganisms (bacteria, fungi, protozoans), this consists of a central dish, which is home to growing microorganisms in their semipermeable membranes in each side of the dish which are separated from the environment and two lateral support panels; the central dish and the lateral panels possesses multiple holes, when the dish is immersed in cells suspension in molten agar, the holes capture small volumes which get solidified forming small agar plugs, then the membranes join together and the I chip is placed on the soil where the strain came from.

By means of this technique, they were isolated about 10,000 bacterial strains from the soil, for this, they were prepared agar extracts and nutrients in order to identify which presented antibiotic activity, discovering in this way, a gram negative bacterium called Eleftheria terrae, which produced a substance that
inhibited *Staphylococcus aureus* growth\(^{19}\); after several tests, they observed that this technique also worked successfully for *Mycobacterium tuberculosis* and *Clostridium difficile*\(^{20}\).

**Teixobactin action mechanism**

Teixobactin exerts its bactericide effects joining to two bacterial polymers precursors from the cell wall: lipid II (peptidoglycan) and lipid III (teichoic acid)\(^{21}\). (Figure 3)

Peptidoglycan is essential to conform the bacterial cell wall structure, teichoic acid has net negative charge, affixing cations that bring rigidity to the cell wall structure\(^{22}\); this attraction to those lipids explains the efficacy of the compound faced with Gram positive bacteria\(^{23}\). Teixobactin Works differently to other antibiotics when attacking not only lipids, but also cell walls\(^{24}\), lysing them quickly, stopping in this way the development of resistance mechanisms to this compound\(^{25}\); literature mentions that when inoculating mice with teixobactin, which were infected with *Staphylococcus aureus* or *Streptococcus pneumoniae*, their infection gets reduced without any toxicity\(^{26}\).

Teixobactin is ineffective against Gram negative bacteria, since the cell wall composition in its external membrane contains phospholipids and polysaccharides that block the Access of lipid II\(^{27}\), besides these bacteria lack techoic acids, main components in order to make the antibiotic exerts its bactericide action\(^{28}\).

Lewis determined the activity of teixobactin by means of methicilin resistant *Staphylococcus aureus* (MRSA)\(^{29}\), deducing that it had an excellent bactericide activity faced with this microorganism, which is superior to vancomycin in the destruction of populations in exponential phase and kept the bactericide activity against intermediate resistance, as it is shown in the figure 4.

**Eravacycline**

Eravacycline is a new bacteriostatic antibiotic entirely synthetic belonging to tetracycline family\(^{30}\), the compound has exposed a powerful activity against a wide spectrum of sensitive and multiresistant bacteria\(^{31}\), in its activity spectrum they
are found Gram positive bacteria as Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus, Streptococcus agalactiae; and Gram negative bacteria as Citrobacter freundii, Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae and anaerobic bacteria Clostridium perfringens, Bacteroides caccae, Bacteroides fragilis, Bacteroides ovatus, Bacteroides thetaiotaomicron, Bacteroides uniformis, Bacteroides vulgatus, Parabacteroides distasonis32.

**Technology for the obtention of Eravacycline**

As other members of tetracycline family, it has been demonstrated that eravacycline is a powerful inhibitor based on the bacterial ribosome mechanism33; it has modifications in the positions C-7 (fluoride) and C-9 2- (pyrrolidine-1-yl) ethanamide in the tetracycline nucleus which were possible by the use of an entirely synthetic route34. (Figure 5)

Two additional experiments were made to the third generation tetracycline molecule, with C9 substitutions in ring D and as a result it is obtained the eravacycline molecule, it was shown a wide spectrum activity against sensitive bacterial strains resistant to tetracycline35; the studies with radiolabelled tetracycline indicate that Eravacycline has a merger ten times higher for the ribosome than for tetracycline, and inhibits in vitro translation to concentrations four times lower than tetracycline36.

**Eravacycline action mechanism**

As other tetracyclines, they inhibit the elongation phase of the proteins synthesis joining together to the 30S ribosomal subunit of bacteria specifically 16S rRNA and blocking the merger of aminoacil ARNt to the acceptor site40, it means, mRNA joins together with the bacterial 30S ribosomal subunit38. P-site (peptidyl) of the RNA 50S subunit contains the rising polypeptide chain; in normal conditions, eaminoacil tARN is charged with the next aminoacid (aa) which is added, moves to A-site (acceptor), with complementary bases pairing between the ARNt anticodon sequence and the mRNA codon40, eravacycline joins together with 30S subunit, blocking the merger of ARNt with the A-site, therefore it inhibits proteins synthesis41; this is a bacteriostatic antibiotic, since the interaction between tetracyclines and ribosomes is reversible42. (Figure 6)

Tetracyclines generally enter to bacterial Gram negative cells through the porins of the external membrane by means of passive spreading or active transportation, this last one requires TFA and magnesium for the active uptake43; however, it maintains the activity when there is the presence of common resistance specific tetracycline mechanisms acquired, it means, four known mechanisms to confer specific resistance to tetracycline as flow pumps, ribosomal protection proteins (RPP), drugs deterioration, and rRNA mutations; of this mechanisms, the efflux pumps and the RPP are the most common44, some Gram negative bacteria species demonstrate an inherent resistance to tetracycline due to specific lipopolysaccharide components in their external membranes. Specific tetracycline efflux bombs can be found in the cell membranes of Gram positive and Gram negative bacteria45.

This synthetic process is constantly improving and discovering new tetracyclines with enhanced antimicrobial profiles related to previous generations46; Nowadays several candidates are in different phases of development using Eravacycline as main compound, a wide spectrum antibiotic for serious diseases47.

Currently they are being proposed some antibiotics finding platforms in order to be studied, which follow directly the old practices and can be improved by means of the application of modern tools of validation48. it will be needed the development of new data tools based on biology, which is totally feasible taking into account what is currently known49. The establishment of rules implied in the penetration of molecules in the bacterial wrap will allow that high technology platforms be applied effectively50.

![Figure 4. Destruction of pathogenes depending on the time for teixobactin](Image 66x492 to 287x730)


![Figure 5. Eravacycline chemical structure](Image 318x121 to 546x201)

Conflict of interests

The authors declare there is not any conflict of interests.

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Conclusions

Thanks to the researches that have been made looking for an effective therapy against bacteria, Scientist have had to look for new alternatives for amplifying the therapeutic arsenal and obtaining new molecules that replace the ones that do not work anymore.

The search of new treatments against pathogenic bacteria proposes new strategies, as the development of methods to cultivate and stimulate the growth of this organisms by means of in situ culture, using specific growth factors in chambers that allow the spreading by using electronic chips and the isolation of the chemical compounds with an antibiotic reach, taking into account that currently this new technique is a source not so explored.

Researchers are making modifications to the action mechanisms, to their chemical and molecular compounds, of the antibiotics previously used, transforming them in synthetic molecules for therapeutic use.

The synthesis of new antibiotics has been tested by the FDA (Food and Drug Administration), a fact that has favored to decrease the adverse effects. There are currently new antimicrobials as omadaclycline for bacterial pneumonia and acute skin diseases, arikayce indicated for the treatment of pulmonary diseases caused by Mycobacterium avium complex, plazomicin with activity against Gram negative pathogens, including enterobacteria resistant to carbapenems and and tezolidoz which indication is the treatment of complicated sin infections and its soft structures.

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