THE USE OF SPECIFIC ASSAYS TO QUANTIFY OXIDATIVE STRESS IN MULTIDRUG-RESISTANT BACTERIA

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ABSTRACT
During oxidative stress the balance between the production of reactive oxygen species and antioxidant protective systems is disturbed. Reactive oxygen species (chemical species with an odd electron) include: hydroxyl radical (OH^-), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), while antioxidant protective systems are classified into: enzymatic antioxidants (superoxidismutase, glutathione peroxidase, glutathione S-transferase) and non-enzymatic antioxidants (reduced glutathione, vitamin E, ascorbic acid, selenium, beta-carotene and vitamin A). In the face of microbial invasion, cells defend themselves by releasing reactive oxygen species, but almost all bacteria have evolved and are able to form and use a range of antioxidants to manage the toxic effects of oxidative stress. To understand the defense mechanisms of bacteria, bacterial models were used that managed to characterize their adaptive responses. The development of methods capable of correlating oxidative stress with the bacterial response could contribute to obtaining a perspective on the development of new antibacterial strategies.
Keywords: oxidative stress, reactive oxygen species, enzymatic antioxidants, non-enzymatic antioxidants

INTRODUCTION

Oxidative stress is defined as the situation in which there exists an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and antioxidant defense [1]. Reactive oxygen species are produced by living organisms as a result of normal cellular metabolism and of environmental factors - such as air pollutants or cigarette smoke. At low to moderate concentrations, they work in physiological cellular processes, however at high concentrations, they are able to damage cellular structures such as carbohydrates, nucleic acids, lipids, proteins, and, in addition, they can alter their functions. Aerobic organisms have developed two systems for controlling reactive oxygen species: the first and most important - the prevention of their formation and the second - their neutralization as soon as they form. Integrated antioxidant systems include enzymatic and non-enzymatic antioxidants that are usually effective in blocking the harmful effects of ROS, but under certain conditions, the balance between oxidants and antioxidants is disturbed in favor of oxidants. This oxidants/antioxidants imbalance is the definition of oxidative stress [2]. Although ROS are cell defense systems against microbial invasion, it has been shown that almost all bacteria have evolved and are able to use an arsenal of adaptive and inducible antioxidants, so that they can manage the toxic effects of ROS [4].

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Bacterial cells have been shown to possess a number of proteins that are able to repair oxidized proteins resulting from ROS attack [3].

REACTIVE OXYGEN SPECIES (ROS) are chemical species that have an odd number of electrons. Being highly reactive molecular fragments, free radicals react with organic molecules, macromolecules and membranes, disrupting intracellular homeostasis and damaging subcellular structures (mitochondria and endoplasmic reticulum) and their decomposition products enter the structure of lipofuscin pigments (also called wear and tear pigments) or of age pigments. They are produced in the cell, either accidentally or during phagocytosis. ROS occur in certain redox reactions in which changes occur, as a result of which the substance often changes its biological function (becomes more hydrosoluble or takes part in another chain of metabolic reactions). ROS intervene as the main amplification factor in the production of "oxidative lesions": peroxides, hydroperoxides, endoperoxides and epoxides, to which a new amplification resulting from the oxidants/antioxidants imbalance is added: through excess of pro-oxidants, doubled by lack of antioxidants. The amplification and accumulation over time of free radicals and oxidative stress (chronic stress), expressed by the imbalance of the ratio of excess pro-oxidants/deficiency of antioxidants, causes the shift of the anabolism/catabolism balance towards anabolic stress (hypo-anabolism)/catabolic stress (hyper-catabolism) with intracellular accumulation of insoluble waste. Oxidative damage can have a devastating effect on the structure and activity of proteins and can even lead to cell death. Amino acids which contain cysteine and methionine sulfur are particularly sensitive to reactive oxygen species and reactive chlorine species, which can damage proteins [3].

Types of ROS produced *in vivo*

1. The hydroxyl radical – $OH^{\cdot}$ is one of the most reactive free radicals, it appears by exposing the body to ionizing radiation. It can react with almost any molecule of the living cell; once formed it destroys everything around it, but it cannot migrate long distances into the cell. Almost all the destructive effects caused by excessive exposure to ionizing radiation are initiated by the attack of the hydroxyl radical on proteins, carbohydrates, DNA, lipids [6].

2. The superoxide anion - $O2^{\cdot -}$ is produced by phagocytes (neutrophils, eosinophils, monocytes, macrophages), as well as other cells such as lymphocytes, fibroblasts, vascular endothelial cells in order to inactivate viruses and bacteria. $O2^{\cdot -}$ is thought to be involved in the transmission of the intracellular signal as well as in the regulation of cell growth. The superoxide anion, as well as hydrogen peroxide, can be generated by autooxidation reactions in which compounds such as catecholamines, tetrahydrofolate react with oxygen to form the superoxide [6]. It will oxidize other compounds initiating reaction chains with the formation of other free radicals. The intimate mechanism of formation involves the addition of an electron to molecular oxygen. This process is mediated by nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase or xanthine oxidase or by a mitochondrial electron transport system. The main site for the production of the superoxide anion is the mitochondria, that is that part of the cell specialized in the production of adenosine triphosphate. Normally, electrons are transferred through the mitochondrial electron transport chain to reduce oxygen to water, but about 1 to 3% of all electrons leak out of the system and produce superoxide. NAD(P)H oxidase is found in polymorphonuclear leukocytes, monocytes and macrophages. During phagocytosis, these cells produce an explosion of superoxide leading to bactericidal activity. Superoxide is converted to hydrogen peroxide by the action of superoxide dismutase (SOD). The superoxide anion is capable of causing a range of damaging effects, including destruction of endothelial cells with increased microvascular permeability, formation of chemoattractants such as leukotriene B4, recruitment of neutrophils for inflammation, lipid peroxidation, destruction of DNA, and formation of the peroxynitrite anion ($ONOO^{-}$). which in its turn can cause a number of changes [5].

3. Hydrogen peroxide (H_2O_2) occurs in natural habitats through reactions between sulfur and oxygen at the oxic/anoxic interfaces, by photochemical reduction of oxygen by chromophores or the redox cycle of pigments. In addition, plants and animals can excrete H_2O_2 to destroy microbes [7]. H_2O_2 can be considered an ideal weapon because it is small and not electrically charged. These characteristics facilitate its passive crossing of the cell membrane at speeds similar to those of water [8]. Once inside the cytoplasm, it disrupts the iron metabolism, virtually destroying the cell. An experimental model using the Escherichia Coli bacterium was used to identify the mechanisms by which H_2O_2 affects cell survival. The results showed that exogenous H_2O_2 damages the cell's DNA and is therefore mutagenic. This effect is known as the Fenton reaction, in which H_2O_2 reacts with intracellular iron [9].

$Fe^{2+} + H_2O_2 \rightarrow [FeO^{2+}] \rightarrow Fe^{3+} + OH^- + HO^{\cdot}$

The explanation why H_2O_2 is so harmful to cells when it interferes with the iron metabolism comes with the discovery that it inactivates two families of iron-containing enzymes: non-redox mononuclear enzymes and dehydrases [10, 11]. Basically, H_2O_2 oxidizes iron from these enzymes and suppresses their activity. It has been observed that only 0.5 μM H_2O_2 is sufficient to destroy the cells in this manner. It has also been observed that following the Fenton reaction, a series of lesions appear in the cell that can cause mutagenesis [10].

ANTIOXIDANT PROTECTIVE SYSTEMS

The increase in the O_2 concentration of the Earth's atmosphere 3 billion years ago forced the primitive forms of life to adapt by creating antioxidant protective systems. These unicellular algae and bacteria not only adapted to the increasing concentration of O_2 but begun to use it as a promoter of the evolution of life and initiator of many essential processes [12]. The body defends itself against excess free radicals with the help of non-enzymatic (natural and synthetic) or enzymatic antioxidants. To increase efficiency, enzymatic and non-enzymatic antioxidants located in various environments - membrane, cytoplasm, extracellular fluid, and in several subcellular fractions - act on the same free radical species [6].

Enzymatic antioxidants

1. Superoxidismutase (SOD) - is found in any aerobic cell, in the mitochondria and cytosol. It converts the superoxide anion to hydrogen peroxide [13]:

$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$

There are several forms of SOD: Fe-SOD in lower organisms and in higher organisms Cu-Zn-SOD in the cytoplasm and Mn-SOD in the mitochondria. Although SOD is found quite abundantly in mammalian cells, diseases have been observed in which the activity of the enzyme is low, either due to the presence of inhibitors, or due to reduced biosynthesis. Decreased SOD has been found in certain foci: inflammation, tumors, cataracts and ischemia of large organs (heart, brain). Attempts have been made to introduce purified SOD in the treatment of diseases, with variable results [14]. Outstanding results were obtained with synthetic SOD, but the high toxicity of this product limits its clinical use.

2. Catalase is found in all aerobic cells, in mitochondria and peroxisomes. It acts on hydrogen peroxide molecules, with a detoxifying effect:

$H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O$

The large amount of catalase present in red blood cells and liver seems to justify the theory of its involvement in processes where high amounts of H_2O_2 are produced [15].

3. Glutathione peroxidase contains selenium as its active part. The accumulation of the oxidized form of glutathione, formed both through the action of GSH peroxidase or non-enzymatically, seems to be an indicator of oxidative stress at the cellular level. GSH peroxidase also acts as a regulator in prostaglandin biosynthesis by inhibiting lipoxygenase. The importance of GSH peroxidase in prostaglandin biosynthesis is so great that in selenium deficiency endothelial lesions of some vessels and platelet aggregation disorders occur, due to the impairment of prostacyclines biosynthesis [6].

4. Glutathione S-transferase is found intracellularly. It catalyzes the conjugation of glutathione with different compounds under conditions of chemical pollution [6]. A wide variety of isoenzymes of this antioxidant exist, that are ubiquitously expressed in humans. In addition to their catalytic role in conjugating GSH to a variety of harmful components, several isoenzymes reduce hydroperoxides and detoxify the end products resulting from lipid peroxidation.

Non-enzymatic antioxidants

Unlike enzymatic antioxidant systems, which are limited to the four enzymes, non-enzymatic antioxidants comprise a very large group of substances whose list is constantly growing.

1. Reduced glutathione (L-alpha-glutamyl-L-cysteinyl-glycol) (GSH) is the main intracellular non-enzymatic antioxidant. It is a carrier of free -SH groups. Both non-enzymatically and acting as a coenzyme of GSH peroxidase or transferase, GSH is involved in the neutralization of endogenous or environmental harmful substances, of free oxygen radicals generated by acute and chronic intoxications and in reactions with organic compounds and free radicals [6].

2. Vitamin E (α-tocopherol) is recognized as being the most important liposoluble antioxidant, whose basic role is to maintain the integrity of membranes. Vitamin E is a target for free radicals and is an effective inhibitor of the evolution of the oxidative chain [16].

Vitamin E has been shown to regenerate continuously, and this increases its effectiveness in pathological conditions in which cell lysis occurs [17].

3. Ascorbic acid. Discussions on the therapeutic utility of vitamin C have had a great echo with the general public. The main involvements of vitamin C are the anti-infective role based on the modulating effects of ascorbic acid in cellular immunity, the detoxifying role based on accelerating the metabolism of xenobiotics, the antioxidant role – its most controversial since a number of papers list vitamin C among free radicals, while other papers argue in favor of its pro-oxidant role, especially with in vitro experiments [18].

4. Selenium acts not only by GSH peroxidase, but also independently or in association with vitamines [19].

5. Beta-carotenes and vitamin A, while having strong antioxidant effects, are known especially well among anticancer compounds. Numerous works have demonstrated their antioxidant character, peroxidation propagation blocker, and singlet oxygen scavenger [6].

OXYGEN REACTIVE SPECIES IN BACTERIA

Oxidative stress in microbial cells shares many similarities with other cell types, but has its specific characteristics that may differ between prokaryotic and eukaryotic cells [22]. A series of studies aimed at elucidating the mechanism by which bacteria react to ROS attack, used bacterial models (Escherichia Coli and Pseudomonas aeruginosa), thus succeeding in characterizing their adaptive responses, resulting in three conclusions [4, 20]:

- Firstly, each species of ROS triggers its own type of response;
- Secondly, the ROS response is under regulatory control in terms of inducing proteins and enzymes with a role in antioxidant defense;
- Thirdly, the response mechanisms are relatively preserved in most bacterial species. Therefore, it appears that the results obtained using the Escherichia Coli and Pseudomonas aeruginosa models can be extrapolated to visualize the general bacterial responses to ROS. Both bacterial species seem to react in the same way: they achieve antioxidant defense by acting at all possible levels [4]:
  - bacteria are constantly trying to prevent ROS attacks;
  - once the ROS offensive is launched, attempts are made to intercept and block the radicals;
  - if damage has been caused, repairs are made.

Thus, bacterial cells prepare their potential defense by maintaining a basal level of enzymes capable of eliminating ROS such as $O2^-$ and H_2O_2, and thus keeping their concentration within safety limits. The defense against ROS is also performed by antioxidant enzymes, metals, radical scavengers, protective proteins and repair enzymes [21]. Given the recent increase in antibiotic resistance, certain antimicrobial strategies based on the generation of ROS may be considered. To elaborate on the way this could be achieved, we will present a brief review of how antibiotics act on bacteria [23, 24]. It is not yet clear whether this is achieved through a single mechanism or multiple ones, which once triggered will eventually lead to the death of the bacterium. What is certain is the fact is that **the main antibacterial drugs have three targets in bacteria:**

- Bacterial cell wall
  Penicillin, for example, promotes transpeptidation, that is inhibiting the formation of peptidoglycan-type cross-links in the bacterial cell wall. The result is a very fragile cell wall that disintegrates, killing the bacteria.
- Bacterial synthesis of proteins
  Antibiotics can inhibit the synthesis of certain proteins. Tetracycline, for example, can cross the bacterial wall membrane and accumulate in high concentrations in its cytoplasm. It then attaches to the 30S ribosomal subunit and blocks RNA and thus stops the elongation of the protein chain [23, 25].
- Replication and repair of bacterial DNA
  Bacterial chromosomal topology is maintained through the activity of three enzymes: topoisomerase I, topoisomerase IV and DNA gyrase (topoisomerase II). The action of these enzymes is disorganized by synthetic quinolone, an antibiotic that targets DNA-topoisomerase complexes. This action leads to complete inhibition of cell division, with bacteriostatic effect and eventually the death of the bacterium [25].

A study conducted by Kohanski and colleagues in 2007 showed that three of the main classes of bactericidal drugs use a common inactivation mechanism that stimulates the production of lethal doses of hydroxyl radicals by the Fenton reaction [26]. Triggered oxidative stress contributes to antibiotic-mediated cell death. Also in this study it was shown that oxidative stress is involved in acquiring antibiotic

resistance of pathogenic bacteria by altering the antioxidant defense mechanisms of cells. In conclusion, oxidative stress can be considered as a possible mechanism for antibiotics' action, and, on the other hand, as a factor involved in the development of bacterial resistance to antibiotics [27].

PROTECTIVE MECHANISMS OF BACTERIA AGAINST OXIDATIVE STRESS

Bacteria live in a toxic world in which their competitors excrete hydrogen peroxide or redox compounds that generate superoxide. To cope with the ROS attack, bacteria developed a series of defense strategies. The most frequently used study model is with E. Coli and the enzymes involved are SOD in the study of superoxide anion degradation and peroxidases and catalases for the degradation of hydrogen peroxide. Mutants that lack these enzymes also suffer from alterations of enzymes that have iron as a cofactor (catalases, peroxidases and ribonucleotide reductases). Also, following the reaction between intracellular bivalent iron and hydrogen peroxide, hydroxyl radicals result, which in turn will react with all biomolecules. At the level of DNA, these radicals produce lesions that are incompatible with the activity of DNA polymerase and with replication, and if the lesions are major, the end result is cell death.The results of the study published by Imlay JA in 2015 suggest that bacteria protect themselves by activating so-called regulons controlled by a number of transcription factors [11]. A key objective of the current activity is to identify the natural circumstances in which regulons are activated. They perceive peroxide when they oxidize key portions of thiolate or iron, they then induce the formation of sets of proteins that will trigger the defense of vulnerable enzymes. In some non-enteric bacteria, some of the transcription factors control the synthesis and export of redox cycle compounds, while in enteric bacteria the cell defends against the same agents. In addition, some bacteria can induce enzymes that defend the cell against the superoxide produced by these compounds. The results of studies on non-enteric bacteria are promising and seem to provide some clarity in the future on how microbes protect themselves against the attack of ROS.

QUANTIFICATION OF OXIDATIVE STRESS IN PROKARYOTIC SYSTEMS (BACTERIA)

The development of methods capable of correlating oxidative stress with the bacterial response could contribute to obtaining a perspective on the development of new antibacterial strategies. Unfortunately, the ROS's tendency to acquire electrons makes them very reactive, with a short lifespan and therefore very difficult to detect. However, a number of methods useful in this regard do exist.

1. Methods based on chemiluminescence

Several studies have used chemiluminescence to test for the presence of ROS in bacteria. The principle of the method is to bind the dye/enzyme complex (luminophores) to the target reactive oxygen species (ROS) and to emit light, the intensity of which is directly proportional to the ROS. The measurement is made with luminometers, and the values are expressed in relative units of light (RLU) [28] or by flow cytometry [29].

Albesa and colleagues used chemiluminescence to assess the involvement of oxidative stress, especially the role of superoxide in the action of antibiotics against various bacteria, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis [30]. Using chemiluminescence, this group of researchers found that various antibiotics can increase the release of superoxide in different strains, but only those antibiotic-sensitive strains respond to oxidative stress. Other studies have shown the role of hydroxyl radicals (OH•) as an essential factor in the response to oxidative stress. A study by Kohanski et al. tracked E. coli treated with norfloxacin, ampicillin and kanamycin and Staphylococcus aureus treated with norfloxacin and chloramphenicol and demonstrated the release of hydroxyl radicals by the Fenton reaction [26]. A specific dye - hydroxyphenyl fluorescein (HPF) was used to evaluate the formation of hydroxyl radicals. Similar results were observed with norfloxacin-treated E. coli after HPF addition. A group of researchers coordinated by Yeom J demonstrated in 2010 that antibiotics could accelerate cell death by promoting the Fenton reaction that leads to an oxidative stress response in ampicillin-treated Pseudomonas aeruginosa [29]. The results of the study show that the antibiotic action is affected by modulating the reduction of nicotinamide-adenine dinucleotide (NADH) levels and iron chelation. In addition to direct or indirect measurements of ROS, the involvement of ROS in cell death and antibiotic resistance can also be assessed through the use of ROS scavengers.

2. Methods which use flow cytometry

This type of method is relatively new in quantifying oxidative stress in antibiotic-treated bacteria. In 2018, a group of researchers from the University of Geneva led by Daniel Manoil set out to evaluate the oxidative stress in bacteria using flow cytometry [31]. They used the DeepRO CellROX® kit which combines an ROS-sensitive fluorophore (CellROX® Deep Red) and a dye for assessing cell viability (SYTOX® Blue) to allow the detection of non-oxidized, oxidized and damaged cells. The bacteria Enterococcus faecalis and Fusobacterium nucleatum were studied and subjected to oxidative stress. An optimal concentration of CellROX® was determined on Enterococcus faecalis and Fusobacterium nucleatum exposed to oxidative stress (H_2O_2). Bacteria were exposed to different concentrations of H_2O_2 and labeled with CellROX® to check if fluorescence increased with oxidative stress. Bacteria exposed to H_2O_2 were also double stained with CellROX® and SYTOX® Blue and analyzed by flow cytometry. Both strains were labeled with CellROX® Deep Red. The fluorescence of CellROX® Deep Red-labeled bacteria increased accordingly with oxidative stress. Flow cytometry analysis of double-stained samples showed subpopulations of bacteria with increased CellROX® signal when stressed and a higher absorption of SYTOX® Blue under higher oxidative stress. The results of this study indicate that CellROX® Deep Red can be applied to measure oxidative stress in E. faecalis and F. Nucleatum and the combination of CellROX® and SYTOX® Blue allowed the discrimination of non-oxidized, oxidized and damaged bacteria.

3. Methods based on the use of free radical scavengers/antioxidants

The principle of this method is based on the addition of antioxidants (thiourea; 2,2-bipyridyl; glutathione; ascorbic acid) in bacterial cultures exposed to antibiotics that cause oxidative stress that protects cells from the harmful effects of ROS and thus increases the survival rate of bacteria. Goswami and colleagues investigated the involvement of ROS in the action of Ciprofloxacin on Escherichia coli [32]. Ciprofloxacin is an important and frequently used member of the fluoroquinolones family of drugs. Its mechanism of action is based on the inhibition of the activities of DNA topoisomerase II and DNA topoisomerase IV respectively, eventually leading to the death of bacterial cells. In addition, an increase in ROS in bacterial cells in response to the action of ciprofloxacin has been demonstrated. These researchers investigated the role of ROS in the antibacterial action of ciprofloxacin by studying the effects of various antioxidant compounds on the susceptibility of Escherichia coli to ciprofloxacin. Among the antioxidants tested, glutathione and ascorbic acid provided substantial protection against ciprofloxacin. The involvement of the superoxide anion (O_2) and hydrogen peroxide (H_2O_2) in the antibacterial action of ciprofloxacin was analyzed using superoxide dismutase, catalase and alkyl hydroperoxide reductase. The results of this study suggest that O_2 and H_2O_2 may be involved in the antibacterial action of ciprofloxacin, that ROS species may have a similar role in the antibacterial action of all fluoroquinolones, and that glutathione-mediated protection is not a general phenomenon but one that is specific to fluoroquinolones.

4. Methods based on the use of mutant strains

Investigating the processes related to oxidative stress and the role of ROS in bacteria exposed to antibiotics provides valuable information on the mechanism of cell death mediated by bactericidal antibiotics and the involvement of ROS in this process. Another strategy for evaluating the release of ROS in antibiotic-treated bacteria is the use of mutant strains that have a modified antioxidant defense mechanism. The antioxidant defensive system of bacteria includes specific antioxidant enzymes, including superoxide dismutase (SOD), catalase and peroxidase. By manipulating E. coli strains, a knockout strain can be generated to create a state of artificial imbalance that allows the selective study of oxidant/antioxidant mechanisms.

The principle of this determination method is based on:

1. use of antibiotic-sensitive strains compared to resistant or knockout strains (bacteria whose oxidative stress defense genes, in particular those responsible for SOD, catalase and peroxidase, have been inactivated through genetic manipulation)
2. highlighting certain genes that are expressed in response to oxidative stress

5. Proteomic investigations

Are based on the use of the following methods:

- Mass spectrometry - ESI (electrospray ionization) and MALDI (ionization by laser desorption assisted by a solid matrix) – identification of proteins/isoforms affected by oxidative stress and their grouping according to the function they perform
- Gel electrophoresis - identification of proteins/isoforms affected by oxidative stress, based on molecular weight.

- PCR - identification and subsequent expression of DNA sequences responsible for the occurrence of OS.

A study published by Huang CH and colleagues in 2011, aimed at proteomic analysis of up-regulated proteins of Helicobacter pylori under oxidative stress led to very interesting results [33]. The development of gastric cancer is known to be associated with chronic inflammation that occurs as a consequence of Helicobacter pylori infection. In such inflammations, the oxidative stress induced by reactive oxygen species in vivo can exert bidirectional effects on both hosts and Helicobacter pylori. In this study, ROS-induced oxidative stress was mimicked in coculture of gastric epithelial cells with Helicobacter pylori treated with hydrogen peroxide (H_2O_2). To investigate the effect of H_2O_2 on the Helicobacter pylori proteome, two-dimensional polyacrylamide gel electrophoresis was performed, followed by liquid chromatography with mass spectrometry and bioinformatics analysis of the database. The presence of overexpressed proteins was found: protein A associated with cytotoxin (CagA), vacuolating cytotoxin (VacA), adhesion protein (AlpA), two antioxidant enzymes - alkylhydroperoxide reductase (AhpC) and catalase (KatA), plus a serine protease (HtrA), aconite hydrate and fumarate reductase. In addition, up-regulation of virulence factors and antioxidant proteins in several strains of Helicobacter pylori isolated from patients have been observed. Furthermore, the rate of Helicobacter pylori infection was found to decrease and proliferation increase after exposure to H_2O_2. It has also been observed that gastric epithelial cells can be protected from oxidative H_2O_2 damage in the presence of Helicobacter pylori. In conclusion, this study supports the assumption that reactive oxygen species containing H_2O_2 may induce up-regulation of Helicobacter pylori virulence factors and antioxidant enzymes and may explain the inflammation that contributes to the development of gastric cancer due to Helicobacter pylori infection.

6. Electrochemical sensors

They allow the real-time quantification of oxidative stress in complex biological systems. Disadvantages: difficulties in calibration and sometimes operation, in complex biological systems, due to the interference between the free radicals formed and their instability.

CONCLUSION

The development of methods capable of in-depth analysis of oxidative stress, and of correlating oxidative stress with the bacterial response could contribute to obtaining a perspective on the development of new antibacterial strategies.

Acknowledgement: This work was supported by the grant "Bioeconomic approach of the antimicrobial agents - use and resistance – BIO-AMR", PN-III-P1-1.2-PCCDI-2017-0361.

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UTILIZAREA DE ASSAY-URI SPECIFICE PENTRU CUANTIFICAREA STRESULUI OXIDATIV LA BACTERIILE MULTIREZISTENTE

REZUMAT

În cursul stresului oxidativ echilibrul dintre producția de specii reactive ale oxigenului și sistemele protectoare antioxidante este tulburat. Dintre speciile reactive ale oxigenului (specii chimice care posedă un electron impar) fac parte: radicalul hidroxil ($OH^{\cdot}$), anionul superoxid ($O_2^{\cdot -}$), peroxidul de hidrogen (H_2O_2), în timp ce sistemele protectoare antioxidante se clasifică în: antioxidanţi enzimatici (superoxiddismutaza, glutation peroxidaza, glutation S-transferaza) și antioxidanţii neenzimatici (glutationul redus, vitamina E, acidul ascorbic, seleniul, beta-carotenii şi vitamina A). În fața invaziei microbiene, celulele se apără eliberând specii reactive ale oxigenului, dar aproape toate bacteriile au evoluat și sunt capabile să formeze și să utilizeze o serie de antioxidanți, pentru a gestiona efectele toxice ale stresului oxidativ. Pentru a înțelege mecanismele de apărare ale bacteriilor s-au utilizat modele bacteriene care au reușit să caracterizeze răspunsurile adaptative ale acestora. Dezvoltarea metodelor capabile să coreleze stresul oxidativ cu răspunsul bacterian ar putea contribui la obținerea unei perspective asupra dezvoltării de noi strategii antibacteriene.

Cuvinte cheie: stres oxidativ, specii reactive ale oxigenului, antioxidanți enzimatici, antioxidanți neenzimatici