

Nutrition and Multiresistance Alert

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Abstract

In the last 70 years, the advent of antibiotics radically changed human life, avoiding epidemic infections caused by microbial agents on mankind and loss of enormous quantity of food. Nowadays, most of antibiotics are used in intensive livestock. Furthermore, the contamination of food and spoilage by microorganisms is a major concern to consumers, government authorities and food industries. Therefore, antibiotics actually play a role of crucial importance for future production of food. Bacteria efficiently counteracted with multiresistance and the Antibiotic Era risks a rapid decline. It is time to explore new solutions, like the use of natural substances, novel mechanisms of action and multi-components drugs, against microorganisms attacks. Urgently, new antimicrobial agents are needed for animal and livestock wellbeing and nutrition, before the ban of actual products, due to the microbial multidrug resistance and habitat damages. In addition, animal products are the main pathogenic and technological microorganisms target. This review reports about the potential utilization of selected natural products against resistant strains of microorganisms.

Keywords: *Multiresistance; Antibiotics; Nutraceuticals; E. coli; Nanoparticles*

Introduction

Most of antibiotics are not used for human health, but for the production of food, and to diseases in intensive breeding farms. Therefore, antibiotics actually are of crucial importance for future production of food. Microorganisms are going out of control. The eternal fight against our most dangerous enemies could be rapidly loosen. Several studies are prospecting the end of the Antibiotic Era, when most of the actual antibacterial drugs will be without any effect against microorganism attacks as consequence of increasing multi-resistance. This situation, already clear to the scientists, becomes emergency when media and important institutions alert the populations about the incoming scenarios. As soon as an emergency is considered over, like Ebola, other ones appear in secret niches of the world, like Xylella [1, 2]. Considering the difficulty of producing new active molecules by the pharmaceutical industry if they continue to insist in the established model, it is time to explore new solutions, like the use of natural substances, novel mechanisms of action and multi-components drugs. Technology could be decisive to enhance the natural products activity, so far considered too inferior respect the synthetic products.

When we focus on human health, the tendency is to simplify by the use of the adequate medicine for the connected disease. However, this is not the real challenge. Health depends mainly by the quality of food [3,4]. Less evident, silent and underscored, the unique and basic competition with microorganisms is always in act: the fight for food. The endless army, made by the composite troops of Monera, Protista and Fungi, was in any time our main competitors for acquisition and utilization of the organic matter, in conclusion for survival.

Incredibly, the problem of food is still reduced to the Malthusian prophecy, meaning production of food in amount sufficient for the human needs. We know nowadays that the goal can be reached only with enormous stretches and deviances from the natural limits. Nowadays, most of the production of meat is obtained in unnatural conditions and agriculture menaces natural habitats. Consequences of monoculture are the increasing of desertification and the remaining useful part of the planet is subjected to over exploitation. To maintain the level of production, increasing quantities of chemical treatments are continuously used and discharged everywhere, poisoning the environment. Even if we will be able to multiply again and again the cultivated hectares and amplify the production, behind any limit of

imagination, still there is the problem of the conservation of crops. In each of these steps, the role of antibiotics is crucial, exactly at the centre of our life. On the livestock side, increasing quantity of chemicals, in particular antibiotics are used to maintain levels of production.

The State of Art of Antibiotics

The Pre-Antibiotic Era

In 1929, penicillin was reported as antibacterial agent by Fleming, produced by the mould *Penicillium*. In 1938, Howard Florey, Professor of Pathology at Oxford University, began research on the use of penicillin, as medical drug. As a matter of fact, doctor Florey started his treatment in consideration of the need to face the consequence of the war with Germany and the possible invasion of Britain. Therefore, he focused on cultivating mould and purifying penicillin.

In 1941, a police constable Albert Alexander was the first person to be clinically treated with penicillin, as antibacterial drug. Constable Alexander was a human volunteer, being in a terminal condition, because of an infection accidentally achieved from a rose scratch two months earlier. 24 hours after intravenous infusion of 160 mg of penicillin, the infection had began to heal. After four days treatment, Alexander was well on the way to recovery, but the stock of penicillin ran out. He died one month later. Therefore, the treatment was switched to sick children, who required smaller quantity of the drug, demonstrating the efficacy of the “miracle drug”. In 1944, penicillin was followed by streptomycin, chloramphenicol (1947), cephalosporin (1948), etc. The Age of Antibiotics was borne.

The Antibiotic Age

Later on, antibiotics has been successfully used to cure a series of infections, such as septicaemia, meningitis, pneumonia, infection of sinuses, joints and bones. The main achievement was that the life time of ordinary people raised, in a short time of decades, from about 45 to 60. The use of antibiotics was extended to any kind of animal of interest and even in agriculture. The value of antibiotics was without any shadow and the abuse everywhere. However, microorganisms did not like the role of insensible targets. They are trained to react to environmental changes. Exactly, from the moment antibiotics were first used, some bacteria resulted resistant to antibiotics. In other words, when a population of sensitive bacteria are exposed to antibiotics, they will massively die except the few resistant bacteria, that can continue to grow and continuing the use of the antibiotic they will be favoured until they will be the dominant ones.

In 1946, a year after the widespread use of penicillin, some *Staphylococcus aureus* strains had already began resistant to it. During the next decades, the cases of resistance raised exponentially, including strains of the most common bacteria, starting the phenomenon of the multidrug resistance. Resistance means that in a population of organisms some of them develop capacity to render harmful the substance or drug currently used. Multidrug resistance is the result of appearance of Superbugs, consisting in bacterial strains that could survive exposure to several different classes of antibiotics.

In 1970, at least 440,000 cases of multi-drug resistance tuberculosis were detected in 69 countries, resulting in around 150,000 deaths. In 2011, around 25,000 deaths a year in the EU were caused by multidrug resistant infections, with the paradox that two-thirds of which were caught while hospital patients. Resistant bacteria from hospitals are also causing more “community-acquired” infections. Difficulty in treating infections with effective antibiotics are increased because some resistant bacteria have also acquired toxins that make them more virulent, like leukocitin, which causes necrotic lesions that can kill patients in 72 hours [5].

Post-Antibiotic Era

What happens when there are not more antibiotics left to treat infections? This possibility is not so far. In 2011, Margaret Chan, General Director of World Health Organization (WHO), choosing the theme “Combat Drug Resistance”, reported: “We are now in the brink of losing its precious arsenal of medicines. The use and misuse of antimicrobials in human medicine and animal husbandry over the past 70 years have increased the number and types of microorganisms resistant to these medicines, causing deaths, greater suffering and disability. If this phenomenon continues unchecked, many infection diseases risk becoming uncontrollable. In the absence of urgent corrective and protective action, the world is heading towards a post-antibiotic era, in which many common infections will no longer have a cure”. This sentence evidences the need of a change in the antibiotic story, when the institutions enter in the problem, with the important entrance

of the institutions. If we continue focusing on human health, associating antibiotics with human disease, we risk to lose the real entity of the problem.

The Antibiotic Emergency

More than 150 antibiotics belonging to at least 17 different classes are now available. They are used in medical treatments, mainly for farm animals and pets. The quantity of antibiotics used in farms increased progressively, because of the aforementioned resistance. Each antibiotic operates on a specific target or site within the bacterial cell. On the other side, the microorganism has a defence to contrast the effect of the drug [6-9].

Very common antibiotics attack the cell wall, inhibiting the wall synthesis. This class includes the beta-lactams, including glycopeptides, cephalosporins, carbapenems, monobactams, and glycopeptides and cyclic lipopeptides, including daptomycin. The target of the bacterium is the enzymatic cleavage of the beta-lactam ring. Other antibiotics act on cell membranes (like polymyxins) or at the metabolic level inhibiting synthesis of proteins (e.g., aminoglycosides, chloramphenicol and tetracycline), nucleic acids (e.g., fluoroquinolones, rifamycins), or target particular biochemical pathways (e.g., methotrexate, sulphonamides, etc.) or by cross-link to cysteines on enzymes (e.g., metronidazole). The mechanism of antibiotic action includes alteration of target site, by passing an inhibited reaction by alteration of the metabolic pathway, reduced drug accumulation by decreasing the drug permeability. The last defence mechanism is very efficient and the basis of the multidrug resistance: using special channels in the wall, the bacteria are able to expel the drug out of the cell, decreasing the drug concentration to a not effective.

Most isolated bacterial strains still remain susceptible to two antibiotics, colistin and tigecycline. However, both of them have serious collateral effects. Colistin, a polymyxin that dates back to the 1940's, has toxic effects on kidneys and does not penetrate well in the tissue [10]. Tigecycline, released in 2005, is part of a new class of antibiotics called glycylcyclines, but it does not diffuse well through blood and the bladder. Furthermore, the tigecyclines were practically banned by FDA because of "an unexplained increased risk" of death [11].

However, this is not enough to understand the scenario. Actually, the most important bacteria weapon is an economic one. They have targeted the profitability of antibiotic development by drug companies. Bacteria are able to rapidly inactivate any new drug. The prospect is that the efficient and rapid capacity of the bacteria to inactivate any new drug [13-18] is now sufficient to discourage pharmaceutical companies from joining the battle [18].

Towards the End of the Antibiotic Age

Fight Multiresistance

US President Obama administration is stepping up its efforts to combat the rising problem of antibiotic resistance. The President started in 2014 a series of acts to face multiresistance phenomenon. First, he signed an executive order establishing a new inter-agency task force charged with developing a national strategy to combat antibiotic-resistant bacteria. Dr. John Holdren, director of the White House Office of Science and Technology Policy and assistant to the President, said the problem is a serious challenge to public health and national security. "We are clearly in a fight against bacteria where no permanent treatment is possible." The order also established a Presidential Advisory Council made up of nongovernmental experts who will provide advice and recommendations to strengthen surveillance of infections, research new treatments and develop alternatives to antibiotics for use in agriculture. The administration released "National Strategy on Combating Antibiotic-Resistant Bacteria", a five-year plan to prevent and contain outbreaks and develop the next generation of tests, antibiotics and vaccines. The President's Council of Advisers on Science and Technology known as PCAST also released a report on combating antibiotic resistance.

There are three main components to the report: improve surveillance of antibiotic-resistant bacteria and stop outbreaks; increase the life of current antibiotics and develop new ones, as well as promote research accelerating clinical trials and increase economic incentives to develop new antibiotics. In fact, a \$20 million prize will be given to spur development of tests health care professionals can use to identify highly resistant bacterial infections.

The task force will be co-chaired by the secretaries of Health and Human Services, the Department of Defence and the Department of Agriculture. The task force submitted its national action plan to the President by February 15, 2015. As a consequence in March 2015 the President declared the fight against multiresistance and he is going to ban definitely every use of antibiotics for farm animals.

Obama's resolution is the results of an incredible situation. Most antibiotics used in the US and UK are given to animals, and not for therapeutic purposes. In 2001, the Union of Concerned Scientists estimated that greater than 70% of the antibiotic used in the US was given to food animals (chickens, pigs and cattle) also in absence of diseases. The idea is that antibiotics at low doses promote the growth in farm animals, although there is not any scientific evidence of these effects. The situation in EU is not very different despite the regulations [19].

The Obama's resolution is a crucial sign. It ends a long strike with farm industries, allied with food and pharmaceutical industries. It is the time to face the possible end of Antibiotics Era? Considering the consequences of the absence of these products and the necessity of their presence, we have only one possibility. Declare the end of the first period of Antibiotics and start immediately to open the second period. We must hurry, we have only 30 years to solve the problem going in other directions and help the mankind to progress, once more, against any dystopia.

Of course, there are already signs of reactions. The WHO called for "push" incentives to encourage certain classes of antibiotics [20]. The US Senate has recently introduced the Generating Antibiotic Now Act, to "spur development of new antibiotic to combat the spread of antibiotic resistant bacteria" [21].

In the struggle, they are able to sacrifice their precious past. These so simple and microscopic organisms ruled the Earth from the beginning and now they claim the absolute dominion

The beginning or the end?

Remember that, until the human genome will slowly change acquiring resistance to bacteria, we absolutely need antibiotics. Use of antibiotics in the last 70 years has transformed human health. With them, we are able to survive bacterial infections that routinely killed our ancestors, but without new antibiotics we may soon be exposed once more to terrible epidemics. In any case, we need a solution, urgently.

The recent studies on our microbiota are changing radically our point of view about bacteria. Usually, people is concerning about microorganisms only in case of infection. Attention is focused on effects of the presence of pathogens and we only are interested in killing the bad bacteria as soon as possible to reach again the status of health. The problem is that the consequence of antibiotic treatment are not only beneficial [24-28]. We have in our body more bacterial cells (accounting for 1-2.5 Kg of our body weight) than eukaryotic cells. The bacterial biodiversity is far more abundant, considering that in our gut more than 500 different species were found, albeit 10 are predominant, and in the mouth we have other hundreds of species different from those of the gut and the human skin carries other several hundreds [29,30]. Most of them acts in symbiosis, being responsible of our survival. Extermination of friend bacteria in favour of resistant ones generates a dangerous disequilibrium in the microbiome [31,32]. Introducing huge numbers of fast-growing and virulent bacteria, any antibiotic resistant genes that appear in response to selection pressure could become established and continue to spread. However, the most important alarm do not concern their misuse as medical drugs, but the environmental input [28].

The Natural Products Opportunity

Several factors must be considered in case of a product based on natural substances. In theory, the plant could be available for everyone and therefore it cannot be patented, discouraging industry investment. The chemical production of a plant is strictly subjected to the environmental conditions that can highly influence this production [33-35]. Therefore, the exact species must be used and determined in composition [36-38]. Once insured the raw material, the process of transformation can deeply change the composition of the product. The technological transformation is essential to the quality and the efficacy of the product. Therefore, this is a second essential step that must be considered for the success of the product. The third step consists into the target of the product and the consequent marketing. To this third step is dedicated this part, because of its essential importance.

Natural products are the key passage to envisage the drugs, food and feed of the future. Technology is the password. Therefore, if so far the attention in feed was focused on proteins and carbohydrates, now it is evident that feed must be integrated and enriched with other substances, as in the original raw materials and tailored in accordance with the target.

The New Products for Food and Feed

Intensive Vs Extensive Breeding

If we aspire to a better food, we must consider the production steps. Most of the meats we eat derive from intensive breeding, where the animals are allowed to live only in the growing period and in abnormal conditions and habitat. Only the quantitative result in increasing weight is considered. Health of animals in industrial breeding, like chickens or fish or cattle and others need to be continuously medically supported. Actually, most of the meat, milk, eggs and other foods in the market are the result of abnormal living conditions. The poultry meat is obtained from young poultry living for four months at maximum. This is because in that period there is the maximum of increasing of weight, due to the ageing of the animal. In chickens breeding, the conversion between feed and growing can reach the 60% that means that for 100g of feed, 60 g remain inside the animal. After fourth weeks, the poultries are ready to be converted in meat and consumed in different forms. The main problem is that chickens are crowded in a little space with the lights always open to avoid continuous mortal attacks. In such incredible conditions, these organic machines of conversion of vegetable matter into meat need continuous help because the diseases and plagues are the normality.

The problem is that the antibiotics pass through the alimentary chain, transferred in the marketed products, until they reach to us, where they accumulate. Therefore, the meat we eat is full of estrogens, hormones and overall antibiotics, usually highly over the dictated limits. This precarious connection between feed and food is already evident on many sectors. Fish consumption is nowadays deeply depending from aquaculture. Again, the production is supported by a heavy utilization of antibiotics, but this will not anymore be possible in the future. Many fish producers, supported by legislation, are already partially replacing antibiotics with selected natural products introduced in food, in order to increase natural immuno defenses and some important results are obtained. Other breeding sectors are following the example. This could be the compulsory future of the breeding and meat production not considering the already present and increasing market of ecologically produced products.

When we considering the animal not a living organism with its evolution and style-live, but simply a machine conversion of feed in other food, we must considered the epigenetic factor. As a matter of fact, when we change radically the environmental conditions, we are producing animals very different from the starting ones, because of the accumulation in DNA of little but important changes. The changes are translated in the composition of the derived products. Therefore, the present nutritional input is very different from previous one and this must be considered as the cause of several consequences in the alimentary chain and at the end in our physiological equilibrium, and the source of many modern diseases.

The Nutraceutical Opportunities

The studies of our microbiota have completely changed the paradigm of the invisible enemies. Currently, we know that without this microscopic symbiotic help, we could not be able to live and that even our feelings are probably influenced by our microbiota. Therefore, we know that there are good and bad bacteria. Even more, a good bacterium can change and become aggressive and dangerous. The microbe world is every moment subjected to change according to its environment.

Furthermore, it is clear that our microbiota is different and changes in parts or organs of our body. Therefore, probiotic and prebiotic products must be tailored on this consideration. Products must be also tailored for ambient situations. For instance, the keyboard of the computer is usually a preferred field for some aggressive bacteria and several bacteria may be transferred by the use of the same computer by different persons. Therefore, in particular in epidemic situation some places must be monitored and cleaned. The cleaner must consider the type of bacteria usually present in this case. The alternative solution is the use of glove or/and other protections, but the worker usually do not like this solution, especially for long periods of time. In Europe, we are surprise looking the tourists from Orient

wearing protection masks, as usual in their countries. This kind of aspects are not usually considered, because they are not evident in the people experience, but their importance must increase and be crucial when the epidemic alerts will be more common, especially in town's environment. In other words, the need of familiar products to maintain the hygiene at home and prevent the diffusion of the epidemic microbiome of microorganisms will be a key aspect in pest control and an important market in the future.

The Food Pressure

It is now evident that antimicrobial resistance is an environmental problem, interesting many aspects of human life. Zoonotic food and water borne pathogens began resistant to antibiotics, by the abuse of medicinal drugs and consequent spread. Detectable high levels of antibiotic residues are present in waste water and soil from water treatment animals or plants. The cause of contamination may be *inter-alia* the consequence of farming practices. Use of antibiotics, as growth promoters or for prophylaxis in farm animals, selects resistant strains of enterobacteria in gastro-intestinal tract.

Meat contamination by pathogen bacteria may have great health consequence and high impact on consumers. The most known cases are related to HUS, Haemolytic Uremic Syndrome, that was first recognized in 1982 in USA and Canada, with outbreaks associated with fast food restaurants. People experienced gastroenteritis with bloody diarrhoea, caused by the lining of their microbiota. In 1993 a multistate outbreak generated international interest in this disease, popularized by the name "Hamburger Disease". Hamburger disease is based on its association with the consumption of ground beef patties containing a pathogen *Escherichia coli*. This should not be confused with the related benign *E. coli* that is in the gut of every mammal.

Many strains of *E. coli* are part of the nonpathogenic facultative flora of intestinal tract of humans and other mammals. However, some of them induce diseases of the gastrointestinal and urinary tracts or may affect the central nervous system, causing serious economic losses in far animal herds and are widespread in newborns in both developed and developing countries. There is a wide range of transmission possibilities of these pathogens, including direct contact, food, drinks, migrations, environment and others. Epidemiology and clinical symptoms of the disease are similar in various animal species but the majority of strains are species-specific. They differ particularly in the type of the expressed surface "adherence" antigen (adhesin or pilus).

These microorganisms produce two main types of virulence factors i.e., adhesins and enterotoxins. Since consumers look for meat products of upgraded sensory quality and increased functional and nutritional properties, as well as guaranteed safety but yet less processing, and fewer additives or "technological" interventions, the plant derived extracts or phytocomplex offer an alternative to synthetic food additives as effective antimicrobial agents.

The Food Packaging

The contamination of food and spoilage by microorganisms is a major concern to consumers, government authorities and food industries. Spoilage bacteria that negatively influence meat products, causing sour off-flavours, discolouration, gas production, slime production and decrease in pH, belong to Gram positive, Gram negative, anaerobic and facultative genera. These effects have been attributed, among others, to the action of extracellular compounds, such as lipases and proteases, produced by dominant spoilage microorganisms.

The technologies used to increase the storage time and to ensure the safe consumption of highly perishable products, such as meat, have undergone a continuous evolution over the time in response to the needs of consumers and industry. The new sustainable solutions in food packaging have to ensure their safety and quality and to reduce food losses and environmental impact. Food packaging plays a crucial role in preserving the quality and safety of food during distribution and storage from farm to fork, but it also contributes to the generation of waste. The need to use materials more sustainable and more compatible with food represents a new market and leads to an intense activity in the study of natural substances for the production of biodegradable wrapping and edible coatings. Besides, the diffusion of active packaging, systems capable of interacting dynamically with the food and/or with the atmosphere in order to save the healthiness of the product and to extend its shelf life is increasing. The effectiveness of these systems has been improved with the use of film activated by antibacterial substances and chemical or natural preservatives slow release. An antimicrobial packaging, active against

spoilage microorganisms and/or pathogens, can prolong the shelf life and improve the safety for all types of foods, especially those processed [39-42]. The importance of utilization of natural products is inherent with a large spectra of activity and the possibility of avoiding the multiresistance. To obtain such results, it is now clear that the multicomposition of a phytoextract is the only way. Most of the natural products are the result of a long selection derived by the interaction with the environment, including the defense against infection and degradation by microorganism.

In addition, interest in the use of active as well as intelligent packaging systems for meat and meat products has increased in recent years, as well the incorporation of natural antimicrobial substances into edible films has attracted great interest, as alternative to control or reducing the growth of food borne and spoilage microorganisms. Even through recognition of the benefits of both packaging technologies by the food industry, the development of economically viable packaging systems and an increased consumer acceptance are necessary for commercial realization of these packaging technologies.

Food packaging helps to an easier distribution and protects food from environmental conditions, such as light, oxygen, moisture, microorganisms, mechanical injuries and dust. Active packaging acts by preserving the condition of the packed food and leading to an increase in shelf life and improvement in safety and sensory properties [39-42].

In addition, the application of such packaging methods on the product surface before packaging can create an environment that may delay or even prevent the growth of undesirable organisms.

Zoonotic food and water born pathogen resistant strains have been isolated from ordinary food and could be entering the human gastrointestinal tract on an almost daily basis. The increasing incidence of food-borne diseases, coupled with the resultant social and economic implications, causes a constant striving to produce safer feed and food.

The exploration of plants and their agro-industrial waste and by-products constituents can be sources of biologically-active substances as preservatives is an innovative way to find new alternative substances for meat preservation [43]. On the side of market, the use of plant extracts as antimicrobial is important, since they represent a lower perceived risk to the consumer, as well as a replay to consumer's demand for minimally processed, preservative free products increases. To be suitable this antimicrobial should be: low cost, eco-friendly, target tailored, besides being effective [44].

The International Life Sciences Institute, Europe has developed a comprehensive document on the use of plant materials in food products, which stresses that the ingredient for use in food products must be well identified and characterized. The starting material must be accurately identified in order to ensure that the plant materials for food use are consistent with respect to quality and quantity of active ingredient and the method of preparation must meet good manufacturing practices.

Limitations of the use of antimicrobials for meat preservation include inactivation of compounds on contact with the meat surface or dispersion of compounds from the surface into the meat mass. Incorporation of bactericidal compounds into meat products may result in their partial alteration by muscle components knew to significantly affect the efficacy of the antimicrobial substances and their release. So, physicochemical characteristics of muscle could alter the activity of antimicrobials. In addition, the antimicrobial activity and chemical stability of incorporated active substances could also be influenced by water activity of the meat.

Nanotechnology Utilization

After all these considerations, we can now go straight to the key argument. The dominance of synthetic products is based on their higher activity. Natural products potentiality is out of discussion, but an increase of their efficacy is necessary. We must consider the key role of technology. In accordance with the Prometheus prophecy, any important advancement in human exploitation of natural resources has been related to a technological step. Nowadays, nanotechnology is one the most promising field, including its possible application in resistance phenomenon [45]. Nanotechnology has gained popularity in the recent years due to its application in various fields. The biosynthesis of nanoparticles offers numerous benefits of eco-friendliness, cost effective and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols [46]. Nanotechnology research includes a wide array of applications in the fields of biomedical, sensors, antimicrobials, catalysts, electronics, optical fibres, agricultural and bio labelling [47].

In medicine, nanoparticles play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, tissue engineering. Important applications concern parasitology against insect-borne diseases involving microorganism pests [48].

Nanotechnology has the potential to revolutionize the agricultural and food industry with novel tools for the molecular management of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients, among others. On the other hand, nano bio technology can improve our understanding of the biology of various crops and thus can potentially enhance yields or nutritional values, as well as developing improved systems for monitoring environmental conditions and enhancing the ability of plants to absorb nutrients or pesticides. An eco friendly green mediated synthesis of inorganic nanoparticles is a fast growing research in the limb of nanotechnology. Inorganic raw materials have been used to obtain nanocrystals of gold, silver, and their alloys have been synthesized with the assistance of various bacteria [49-53]. Nanoparticles have been obtained also using organic materials like chitosan, fungus or algae [54,55]. Furthermore, microbes are used in nanotechnology for producing nanoparticles and present green synthesis has shown that the environmentally caring and renewable source of fungi used as an effective reducing agent for the synthesis of silver nanoparticles. There is a need to develop safe, reliable, nontoxic, cost effective, clean, and ecofriendly methods for the preparation of nanoparticles [56]. Relevant applications of nanoparticles include size-dependent interaction with virus, bacteria, microorganisms, etc., responsible of important diseases, as well as their mosquito vectors [57].

The key steps are the synthesis, the stability and active content of the nanoparticles. Redox synthesis methods use hazardous chemicals as reducing agents or require significant energy input. Therefore, there is a growing interest in the use of environmentally safe 'green' reducing agents. The chemical reduction of aqueous solution of silver nitrate is one of the most widely used methods for the synthesis of silver nanoparticles. Several reliable examples on synthesis of silver nanoparticles have been reported: biosynthesis using *Aspergillus foetidus* was reported that the sunlight-induced by using animal and fungus biomass [58,59].

In conclusion, natural products require time to act and this is improving their natural degradation, but they need more treatments and a consequent high cost. On the contrary, for the reason, natural substances are eco-friendly and must be preferred to synthetic products. Nanotechnology can be used to improve activity and stability, by insertion of natural products inside various materials.

The Neem Opportunity

Are plants a valuable source of new antimicrobial natural products? The potentiality of plants must be checked and validated by researchers. Among the possible solutions, an interesting place can be considered for neem (*Azadirachta indica* A. Juss). Neem, originated from Indian subcontinent, is now largely present in tropical and subtropical regions of the world, mainly cultivated in large populations for the resistance in arid and desert conditions [60]. The plant is worldwide well known for the use of the kernel oil as natural insecticide [61-63], but also its antimicrobial properties are relevant, including the control of resistant bacterial strains [64-68]. Eco-friendly character and selection in the action was tested by the EPA US agency [69].

Conclusions

Planet population is going to reach the seven billion and in 2050 the next step should be the relevant limit of ten billion. The necessary amount of food can be achieved only by massive introduction of technological solutions. In agriculture the increasing utilization of genetic methodologies to improve resilience of crops and herds should be able to reduce drastically the loss in field. The second challenge concerns the conservation of health of crops as well as the health and well being of herds in order to maintain the nutritional standards and limit further loss. In both cases, the control of microorganisms is decisive and the resource of antimicrobial natural products must be carefully explored in order to optimize the production without dramatic damages in the habitat and increasing of resistance phenomenon. In this paper, the preliminary data in this direction are reported, as real application of a natural substance by nanotechnology.

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