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The Cave Ecosystem in the Research of New Antibiotic Discovery and Development †

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Abstract: The increasing resistance of micro-organisms to antibiotics is a serious problem of public health. Furthermore, during the COVID-19 pandemic, there was an additional increase in fungal infections from rare fungi, which burdened immunocompromised patients. Climate change, and the resulting high temperatures and humidity, is predicted to worsen this situation, as such conditions accelerate the emergence and spread of these "superbugs" and fungi. The need to produce new antimicrobial agents is therefore essential and imperative. Recent data support the idea that the pristine ecosystems of caves may become an important potential source of novel drugs.

Keywords: cave; actinobacteria; antibiotics; ecosystem; micro-organisms

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1. Introduction

Multi-drug resistant (MDR) micro-organisms are major causes of life-threatening infections. Their capacity for antimicrobial resistance is dependent on overexposure to antibiotics, which leads microbes to develop defense mechanisms against them. The selection and amplification of resistant strains further limits the efficacy of antimicrobial therapies. This problem is dramatically amplified by the overuse of pesticides and triazole insecticides in agriculture and the antibiotic consumption of food-producing animals, from which the multi-drug resistant micro-organisms can spread to the environment and human food. According to the World Health Organization (WHO), infections due to antibiotic resistance may cause, by 2050, 10 million infections every year and could impose a burden of 100 trillion dollars on the world economy [1]. Moreover, the new emerging viruses—Zika, SARS, Ebola, MERS-CoV and SARS-CoV-2 2019—threatened public health and caused thousands of deaths worldwide since there were no appropriate antiviral treatments. Additionally, the use of antibiotics, and the increase of chronic diseases (autoimmune diseases, cancers) and viral infections such as AIDS, have also dramatically increased the rate of fungal infections.

The need for effective and reliable drugs is therefore essential and imperative. Several studies emphasize the importance of the pristine cave ecosystem in the research to discover and develop new antimicrobial agents.

2. Cave Ecosystem

Caves are divided into primary—created by the deposition of environmental rock (volcanic, coralline etc.)—and secondary—created by the solidification of rocks (aeolian, tectonic and karst). A total of 95% of caves are created by carbonate rocks (mainly limestones and secondarily dolomites) that have been dissolved by water containing carbon dioxide (Karst phenomenon). Limestone caves were formed over centuries by the sedimentation of shells of marine organisms containing calcium carbonate. Therefore, caving may be the result of precipitation, wind effects, tectonic effects, the mechanical erosion of rocks by water, or the chemical erosion of rocks by water (Karst phenomenon) [2]. There are land, ice, volcanic and sea caves, and, as mentioned above, most of them are composed of limestone, gypsum and dolomite rocks. Aragonite, selenite, sodium and magnesium sulfates, limonite, etc., are also sometimes found [3].

The sedimentary deposits of caves belong to two categories:

1. Speleothems, which are deposits of calcite or aragonite and form stalactites, stalagmites, moon-milk, helictites and eccentrites;
2. Detrital sediments, which may include lithogenic materials of limestone origin from cave walls, and outdoor materials that have entered with wind or water, but also organic materials such as pollen, plant remains, microfauna, coprolites, or fragments of human or animal bones and teeth [4].

In the cave environment there is darkness, constant temperature, high humidity, low pressure, low oxygen concentration and minimal nutrients. It is divided into three zones: the bright entrance zone, which is approximately five meters long, with similarities to the outside environment; the twilight zone, which is approximately ten to twenty meters long, with less sunlight, no vegetation and a relatively constant temperature; and the dark zone, which is without light and has an absolutely constant temperature [3]. This unique climatic stability makes caves ideal environments for the study of climate change in nature. The temperature in them depends on the temperature of the external environment. Scientists have noticed that climate warming is able to modify subterranean microclimates. This situation is further aggravated by the decrease in rainfall, which reduces the water infiltrated in karst and cave humidity. In the future, these conditions may fatally affect subterranean species due to their inability to withstand temperature and other microclimate changes [5].

3. Use of Cave Ecosystem in Human Health

3.1. Speleotherapy

Since the 19th century, salt caves have been used in the treatment of respiratory diseases. Speleotherapy has its roots in the salt mines and caves of Eastern Europe, where it was first observed that due to the inhalation of small particles of salt during mining, workers had acquired some kind of protection against respiratory diseases, as well as a youthful appearance to their skin. In 1839 in Poland, Dr. Feliks Boczkowski opened the first health resort at the Wieliczka Salt Mine, where he studied the beneficial effects of underground natural brine in the treatment of asthma [6]. In 1949, German physician Dr. K.H. Spannahel, after observing that people who hid in salt mines and caves during World War II bombings acquired benefits to their respiratory system, proceeded to create the Klyutert Cave, in order to study the effects of the underground environment on inpatients. Dr. K.H. Spannahel and Hungarian geologist Dr. H. Kessler are considered the founders of modern speleotherapy. In 1958, Professor Mieczyslaw Skulimowski started a therapy program called the "Skulimowski Method" for patients at the Wieliczka Salt Mine [6]. In 1964, the underground "Kinga" Allergy Treatment Spa was created in the Wieliczka Salt Mine, which was later called the "Kinga" Health Resort Hospital. In 1968, the first cave-hospital was opened in the Solotvyno Salt Mine in Ukraine [6]. In 1985 in Odessa, Russia, the first halotherapy device was created. After the fall of the Soviet Union in 1991, the treatment protocols became accessible to the rest of the world [6]. Since then, many studies have demonstrated the benefits of speleotherapy for human health [7]. Cave ecosystems are particularly beneficial for the elderly, who lose the ability to maintain proper posture and body balance, the control of shoulder blade movements, the ability to bend the chest wall, and the elasticity of the lungs. These conditions are particularly aggravated in elderly sufferers of chronic obstructive pulmonary disease (COPD) and asthma due to reduced muscle strength and the inability to exercise. In these patients, but also in others with upper respiratory diseases such as chronic rhinitis and sinusitis, speleotherapy, the underground air and the stable microclimate has been demonstrated to be effective [8]. In these caves, the temperature is relatively low (12.9–14.5 °C), the humidity is moderate to high (60–75%), there is a high concentration of minerals and ionization, and a minimal level of dust and pollution. The speleotherapy protocol includes breathing exercises in the clean environment of caves or mine chambers, with their constant air temperature, moderate to high humidity, high levels of sodium, potassium, magnesium and calcium, low levels of bacteria and fungi, and the absence of atmospheric pollutants and pollen. Under these conditions, speleotherapy, with pulmonary rehabilitation, and endurance and strength training, has been shown to increase the dynamic balance and thoracic mobility in older adults with chronic respiratory diseases [9]. Entering these environments, the air changes, with a decrease in oxygen levels, an increase in carbon dioxide levels and the appearance of other elements such as radon, methane, or hydrogen sulfide, and sodium, calcium or magnesium chloride aerosols. The aerosols are created by the union of small solid or liquid particles (charged due to the high ionization) with cave air. In salt mines, sodium chloride aerosols result from salt rocks, brackish stagnant water, brine springs and water streams. The caves and salt mines of Wieliczka and Bochnia in Poland, Turda in Romania, Zlote Hory in the Czech Republic, and Berchtesgaden in Germany, are still used today for speleotherapy [10].

3.2. Cave Ecosystem in Drug Discovery and Development

Although caves are extreme, harsh environments, they present a multitude of microbial populations, which develop different strategies in order to survive. Some micro-organisms interact, forming biofilms on cave walls, stalagmites and stalactites. These biofilms consist of algae, cyanobacteria, bacteria and fungi, and facilitate the flow of minimal nutrients. Other micro-organisms compete with each other, adapting secondary metabolisms and producing metabolites, which inhibit the growth of other bacteria and fungi in the same area. These micro-organisms could be used in research to discover and develop new drugs [3].

Inside the caves, there are mainly *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, *Firmicutes*, yeasts and saprophytic fungi such as *Penicillium* and *Fusarium*. Studies into several caves around the world have shown that *actinobacteria* (mainly *Streptomyces*) prevail in these extreme ecosystems. *Actinobacteria* are Gram-positive bacteria that are found everywhere in nature, both in aquatic and terrestrial environments, in plants, insects, marine organisms and mammals, and also in human tissues, such as the skin, lungs and intestines [11]. More than 70% of antibiotics are produced using *actinobacteria*, mainly using the *Streptomyces* species. In the extreme environments of caves, *actinobacteria* colonize the ceilings and floors, the waters, the moon-milk (deposits of carbonate minerals on the cave wall, which have been used since prehistoric times in the treatment of diseases), the speleothem (stalagmites and stalactites) and even bat guano [12]. These micro-organisms are not negatively affected, but, on the contrary, are favored by the constant temperature and humidity conditions, the low light and the limited availability of nutrients. Cave micro-organisms can use energy from gases and from the oxidation of the cave rock elements. In addition, they have the ability, through the enzymes or substances that they produce, to cause mineral precipitation and lithification. *Cyanobacteria* are prokaryotic, photosynthetic, oxygen-producing Gram-negative bacteria. They have the unique ability to bind atmospheric nitrogen, convert it into ammonia and form nitrogenous substances such as amino acids and proteins, surviving even in the most adverse conditions. These micro-organisms produce a large number of secondary metabolites (peptides, fatty acids, hydrocarbons and other organic substances) with properties against HIV, cancer, fungi and bacteria [13]. The *cyanobacteria* of Greek caves, such as *Scytonema juliaoum* and *Chroococcidiopsis* sp., have isolated lipoids with properties similar to the platelet activating factor or with inhibitory action against it. *Toxopsis calypsus* and *Phormidium melanochroun* have lipoids with action against Gram-positive bacteria, mainly *enterococci* [13]. The mechanism is unknown, but it seems that, through these lipoids, they selectively penetrate the cell walls of Gram-positive bacteria.

As mentioned above, cave microbes, due to the extreme conditions, particularly the lack of nutrients, become more competitive and produce metabolites in order to survive. This has been found in the bioactivity of several metabolites such as undecylprodigiosin, xiachemycin, hunglongmycin, haxalactin B, diazepinomicin, produced by *Streptomyces* sp. and *Bacillus* sp., polyene and non-polyene metabolites by *Streptomyces* sp. and *Penicillium* sp., lanthipeptides, polymyxin B, paenicidin B, fusaricidin, tridecaptin and colistin A by *Paenibacillus*, lipids, etc. [14].

More specifically, the bioactive metabolites of *Streptomyces* sp. that have been isolated from caves are Cervimycins A,B,C,D (with antibacterial effect) from the Grotta dei Cervi cave in Italy, Undecylprodigiosin (antibacterial and antioxidant effects) from a Serbian cave, Xiachemycin A (antibacterial and anticancer effects) from the Chongquing cave of China, Haxalactin B (antibacterial effect) from the Bolshaya Oreshnaya Cave, Siberia (Russia), Hypogeamycins A (anticancer effect) from the Hardin Cave System in Tennessee, Huangkongmycin (HLM) A (anticancer effect) from the Xiangxi Cave in China, and Gyrophoric acid (with inhibitory action against lipid metabolism) from the Bolshaya Oreshnaya Cave, Siberia (Russia) [15]. In the Altamira cave in Spain, the rare actinobacterium *Crossiella* sp., with action against bacteria and fungi, was isolated [16]. The actinobacterium *Bifidobacterium* also has a protective effect on the gastrointestinal system and produces anticancer metabolites [11].

Cave *actinobacteria* inhibit the growth of a multitude of pathogenic microbes, helminths, fungi, Gram-positive and -negative bacteria, such as *Salmonella*, *Escherichia coli*, *Micrococcus luteus*, *Proteus*, *Listeria monocytogenes*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and even vancomycin-resistant *enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), STEC (Shiga-toxin-producing *Escherichia coli*), and many others [3].

Antifungal drug development is a particular challenge, as fungi are eukaryotic microorganisms and must be treated selectively, without causing damage to the eukaryotic human organism [14]. Metabolites from cave bacteria could provide the solution to this problem. *Streptomyces*, *Micromonospora*, *Dactylosporangium* and *Streptosporangium* species produce metabolites against the fungi *Colletotrichum gloeosporioides*, *Alternaria*, *Fusarium oxysporum*, *Magnaporthe grisea*, *Rhizoctonia solani*, *Phytophthora*, *Curvularia oryzae*, *Candida albicans*, etc. In addition, the animals that live in caves (bats, arthropods, insects, etc.) are colonized with bacteria, which also produce metabolites that protect them from pathogenic fungi, and therefore, they could be used in research to discover new antifungal drugs [15].

The recent COVID-19 pandemic, as well as other viral epidemics (HIV, MERS, Ebola, etc.), highlighted the challenges of treatment with drugs and prevention with vaccines, due to the viral ability of mutations. Bats are hosts for 15,000 coronaviruses, the rabies virus and possibly Ebola. *Flaviviridae* (West Nile virus, Zika virus, Dengue virus, etc.), *Adenoviridae*, *Papillomaviridae* (such as *human papilloma virus* HPV), *Reoviridae*, *Parvoviridae*, *Paramyxoviridae*, *Polyomaviridae*, and *Picornaviridae* have been detected in bat feces and urine. Coronaviruses, astroviruses and filoviruses have also been detected in cave bat serum. Nevertheless, the bats do not suffer from viral infections themselves. Due to their immune system, the viruses remain dormant, simply colonizing their body [1]. The rest of the organisms in caves, such as arthropods, rodents, reptiles, etc., are also reservoirs of viruses, while in the rest of the underground ecosystem they are almost absent. Cave bacteria such as *Paenibacillus* sp. and *Bacillus* sp. produce lipopeptides such as octapeptins, polymyxins, iturins, fengycins, polypeptins, fusaricidins, tridecaptins, kurstakins, and surfactins, with antiviral actions. Cave *actinobacteria* could also contribute to the development of antiviral drugs and vaccines [1].

The bioactive products of actinobacteria also show anticancer activity against colon, lung, oral cavity, breast, liver, cervical and prostate cancers [15].

The following drugs/products of actinobacteria with anticancer effects have already been approved by the FDA:

Mitomycin C, isolated from *Streptomyces caespitosus*, is currently used against neoplasms of the breast, bladder, gastrointestinal tract, colon, in glaucoma surgery, etc. It acts mainly by inhibiting DNA synthesis in cancer cells;

Doxorubicin or adriamycin is produced from *Streptomyces peucetius* and exhibits DNA topoisomerase II inhibitory properties. It is used in the treatment of thyroid, breast, lung and ovarian cancers;

Paclitaxel is isolated from the actinomycete *Kitasatospora* sp. and is used against breast, ovarian, prostate, lung and many other cancers;

Sirolimus is produced by *Streptomyces hygroscopicus*. It acts by inhibiting the mTOR pathway and is used to treat diabetes, multiple sclerosis and other diseases;

Everolimus is a derivative of sirolimus and is used to treat kidney and pancreatic cancers. In addition, there have been encouraging results in the use of its derivatives in the treatment of metastatic colon cancer [11].

4. Conclusions

Antimicrobial resistance is a major challenge for the treatment of infectious diseases and currently represents one of the most significant threats to global health. New pathogens, such as *Candida auris*, have also emerged as serious problems for public health, causing global epidemics due to multi-drug resistance, high transmissibility and high rates of mortality. On the other hand, the lack of antibiotic discovery and development is a concern of the scientific community. Recent studies support the idea that the pristine cave ecosystems may provide the solution to the threat of dwindling treatment options to combat these life-threatening infections. In particular, the metabolites produced by cave microbes have demonstrated antibiotic, anticancer, anthelmintic, antifungal, antiviral and immunosuppressive effects. Therefore, it is an imperative for scientists to react and to explore these new antimicrobial compounds by adapting modern techniques and methodologies.

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