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## Pathogenic and opportunistic microorganisms in caves

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### Abstract:

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With today's leisure tourism, the frequency of visits to many caves makes it necessary to know about possible potentially pathogenic microorganisms in caves, determine their reservoirs, and inform the public about the consequences of such visits. Our data reveal that caves could be a potential danger to visitors because of the presence of opportunistic microorganisms, whose existence and possible development in humans is currently unknown.

**Keywords:** *Caves, bacteria, fungi, virus, opportunistic pathogens*

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### INTRODUCTION

Europe is an exceptional continent for its abundance and variety of subterranean karstic forms comprising the natural geological heritage. Many of them are Protected Sites, and today many countries have programmes for the protection of caves and geodiversity. Some caves can be visited, and are, either ecologically or culturally, a tourist attraction. Today, many caves have been invaded by, and are important centres of, mass tourism. Nevertheless, while certain caves have around 500,000 visits annually (Grotta del Vento, Italy; Postojna caves, Slovenia; Nerja, Spain), others are difficult to get to, and are visited only by speleologists (Calaforra Chordi & Berrocal Pérez, 2008).

With today's leisure tourism, the frequency of visits to many caves makes it necessary to know about possible potentially pathogenic microorganisms in caves, determine their reservoirs, and inform the public about the consequences of such visits for people who are immunosuppressed, undergoing chemotherapy,

or have lowered defences. Unfortunately, information on this topic is not well known, and visitors remain unaware. Determining the extent of the potential danger is of great interest – not only scientific, but also medical and social. Our data reveal that caves could be a potential danger to visitors, because of the presence of opportunistic microorganisms, whose existence and possible development in humans is currently unknown.

### CAVE MICROORGANISMS

Microorganisms occupy all the niches of the biosphere, including subterranean ones. Underground habitats, represented essentially by caves, have no light, little or no organic nutrient load, a relatively constant temperature, and extensive areas of mineral surfaces.

The literature on microbial communities in subterranean environments is scant and chiefly restricted to caves found in Spain, Italy, France, Rumania, and the USA. Most of the existing literature refers to specific aspects, such as the taxonomic or geomicrobiological ones (Hernandez-Marine & Canals, 1994; Northup & Lavoie, 2001; Engel et al., 2003; Barton, & Northup, 2007; Barton et al., 2007; Porter et al. 2009), to the effects on rock-art paintings (Schabereiter-Gurtner et al., 2002 a, b), or colonization by fungi (Dupont et al., 2007; Bastian et al., 2009b).

A review on the biodiversity and distribution of

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bacteria in subterranean environments reveals the scant knowledge of these topics. *Proteobacteria* represent the major portion of bacteria in caves when using molecular tools, but *Actinobacteria* constitute the majority of isolates (Groth et al., 1999; Barton et al. 2007), although it was formerly believed they were confined mainly to soils, their natural habitat (Porter, 1971; Goodfellow & Williams, 1983). In the last decade, actinobacteria have been shown to be abundant in caves (Groth et al., 1999; Schabereiter-Gurtner et al., 2002 a,b; Jurado et al., 2005 a,c), suggesting that this is a habitat particularly favourable for this group of bacteria (Figure 1).

Table 1 shows the new species of actinobacteria isolated from subterranean environments reported since 2000, as gathered in the International Journal of Systematic and Evolutionary Microbiology. The identification of new species of the genera *Agromyces*, *Amycolatopsis*, and *Kribbella* (Figure 2) indicates that these environments constitute an ecological niche with a solid potential for the study of biodiversity. The conditions of high salinity in certain niches, and the stability of the microbial communities over the years, make these habitats of great interest and a target for in-depth study, to obtain an understanding not only of the processes forming natural microbial communities and of their role in the interaction with minerals, but



Fig. 1. *Pseudonocardia* sp. colonies in stalactites (Doña Trinidad Cave, Ardales, Spain).

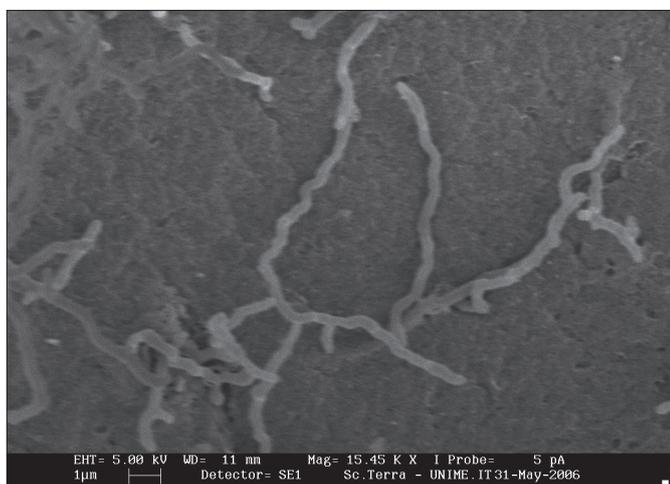


Fig. 2. Morphology of *Kribbella catacumbae* isolated from the catacomb of Saint Callistus, Rome, Italy.

also of the biodiversity of such ecosystems.

At the same time, the ecological study of environments other than terrestrial and marine ones is important because of the potential of actinobacteria as producers of new bioactive substances derived from their secondary metabolism. In fact, the actinobacteria are an important source of naturally occurring (non-synthetic) antibiotics, besides substances with antiviral, antitumoural, immunological, and agrobiological (herbicidal) activity. All of these compounds are of high commercial value (Lemriss et al., 2003). A mathematical model estimates the number of undiscovered antimicrobials from the genus *Streptomyces* to be of the order of a 100,000 – a tiny fraction of which has been unearthed so far (Watve et al., 2001).

According to Williams et al. (1993), one possible way to tackle the isolation of new actinobacteria would be to focus on environments not studied or to investigate habitats in which one or various environmental factors (for example, temperature, pH, aeration, osmotic stress, etc.) are extreme. Accordingly, the exploration of caves and their exploitation as reservoirs of bacteria is of great interest to the pharmaceutical industry. Groth & Saiz-Jimenez (1999, 2002) pointed out the importance of caves as a new habitat in which actinobacteria producing bioactive substances could be found. Later, Herold et al. (2005) reported the production of cervimycin A-D by *Streptomyces tendae*, isolated from the Grotta dei Cervi, Porto Badisco, Italy. These novel polyketide glycosides have significant activity against multi-drug-resistant staphylococci and vancomycin-resistant enterococci.

Another important consideration is the presence of potentially pathogenic bacteria and fungi in isolations from caves, as can be observed in studies made by Groth et al. (1999), who isolated species of the genera *Amycolatopsis*, *Aureobacterium*, *Brevibacterium*, *Nocardia*, *Nocardioides*, *Rhodococcus*, *Streptomyces*, and the family *Micrococcaceae*. Some of the species of these genera have been catalogued as pathogenic – the diseases they cause come from contact with soils, waters, animal excrements, etc., which are their natural habitats. Human pathogenic fungi and yeasts are also common in caves (Sugita et al., 2005). We will focus our review on pathogenic microorganisms in caves.

## PATHOGENIC BACTERIA

In recent years, the actinobacteria – and in particular the genera *Nocardia*, *Mycobacterium*, *Gordonia*, *Rhodococcus* and *Streptomyces* – have been the object of numerous studies at the international level (Nasher et al., 1989; Blanc et al., 2007; Mignard & Flandrois, 2008; Rodríguez-Nava et al., 2008; El Karoui et al., 2009). All the above-mentioned genera, and many others, are widely found in isolations from caves.

The description of new species is in constant flux because of the new molecular methods developed in the last few years, considerably altering the taxonomic classification of these microorganisms.

Table 1. New species of actinobacteria isolated from subterranean environments reported since 2000.

	Actinobacteria Class	Origin	Reference
1	<i>Kribbella koreensis</i>	Gold-mine Cave, Republic of Korea	Lee et al., 2000 a; Sohn et al., 2003
2	<i>Catelliglobospora koreensis</i>	Gold-mine Cave, Republic of Korea	Lee et al., 2000 b; Ara et al., 2008
3	<i>Saccharothrix violacea</i>	Gold mine cave, Republic of Korea	Lee et al., 2000 c
4	<i>Saccharothrix albidocapillata</i>	Gold mine cave, Republic of Korea	Lee et al., 2000 c
5	<i>Pseudonocardia kongjuensis</i>	Gold mine cave, Republic of Korea	Lee et al., 2001
6	<i>Knoellia sinensis</i>	Reed Flute Cave, Guilin, China	Groth et al., 2002
7	<i>Knoellia subterranea</i>	Reed Flute Cave, Guilin, China	Groth et al., 2002
8	<i>Pseudonocardia spinosispora</i>	Gold-mine Cave, Republic of Korea	Lee et al., 2002
9	<i>Arthrobacter psychrophenicus</i>	Alpine ice cave, Salzburg, Austria	Margesin et al., 2004
10	<i>Agromyces salentinus</i>	Grotta dei Cervi, Italy	Jurado et al., 2005 a
11	<i>Agromyces neolithicus</i>	Grotta dei Cervi, Italy	Jurado et al., 2005 a
12	<i>Agromyces italicus</i>	Roman catacombs, Italy	Jurado et al., 2005 b
13	<i>Agromyces humatus</i>	Roman catacombs, Italy	Jurado et al., 2005 b
14	<i>Agromyces lapidis</i>	Roman catacombs, Italy	Jurado et al., 2005 b
15	<i>Agromyces subbeticus</i>	Cave of Bats, Córdoba, Spain	Jurado et al., 2005 c
16	<i>Isopterocola hypogeus</i>	Roman catacombs, Italy	Groth et al., 2005
17	<i>Myceligenans crystallogenes</i>	Roman catacombs, Italy	Groth et al., 2006
18	<i>Amycolatopsis jejuensis</i>	Natural cave on Jeju Island, Republic of Korea	Lee, 2006 a
19	<i>Amycolatopsis halotolerans</i>	Natural cave on Jeju Island, Republic of Korea	Lee, 2006 a
20	<i>Nocardia jejuensis</i>	Natural cave on Jeju Island, Republic of Korea	Lee, 2006 b
21	<i>Actinocorallia cavernae</i>	Natural cave on Jeju Island, Republic of Korea	Lee, 2006 c
22	<i>Amycolatopsis saalfeldensis</i>	Medieval alum slate mine, Thuringia, Germany	Carlssohn et al., 2007 a
23	<i>Kribbella aluminosa</i>	Medieval alud slate mine, Thuringia, Germany	Carlssohn et al., 2007 b
24	<i>Nocardia speluncae</i>	Natural cave on Jeju Island, Republic of Korea	Seo et al., 2007
25	<i>Amycolatopsis nigrescens</i>	Roman catacombs, Italy	Groth et al., 2007
26	<i>Kribbella catacumbae</i>	Roman catacombs, Italy	Urzi et al., 2008
27	<i>Kribbella sancticalisti</i>	Roman catacombs, Italy	Urzi et al., 2008
28	<i>Nocardia altamirensis</i>	Altamira Cave, Cantabria, Spain	Jurado et al., 2008
29	<i>Fodinicola feengrottensis</i>	Medieval mine, Thuringia, Germany	Carlssohn et al., 2008
30	<i>Jiangella alkaliphila</i>	Natural cave on Jeju Island, Republic of Korea	Lee, 2008
31	<i>Thermobifida halotolerans</i>	Salt mine, Yunnan, China	Yang et al., 2008
32	<i>Hoyosella altamirensis</i>	Altamira Cave, Cantabria, Spain	Jurado et al., 2009
33	<i>Fodinibacter luteus</i>	Salt mine, Yunnan, China	Wang et al., 2009
34	<i>Ferrimicrobium acidiphilum</i>	Sulfur mine in North Wales, UK.	Johnson et al., 2009

For example, the genus *Mycobacterium* includes more than 130 species (Mignard & Flandrois, 2008), the genus *Nocardia* around 70 species (Rodríguez-Nava et al., 2008), the genus *Rhodococcus* 32 species, the genus *Gordonia* 21 species (Blanc et al., 2007), with other genera described in the literature having fewer species, such as *Brevibacterium* with 19 species, and *Micrococcus* with nine species. Some strains of these bacteria are responsible for different skin, lung, and/or brain infections in man. The most common primary location is the pulmonary system, with most clinical cases being reported in immunosuppressed patients, mainly associated to corticoids therapy, although immunocompetent hosts can also be affected. The secondary location is the central nervous system. In this case, the microorganisms also form abscesses inducing sensory, motor, and behavioural disturbances that differ depending on their location and the aetiological agent responsible, as well as nausea, headaches, and vomiting. Other, less common, locations are the eyes, the auditory

apparatus (Iida et al., 2005), the lymphatic ganglia, the myocardium, the liver, etc. (Ghosheh et al., 2007 ; Rodríguez-Nava et al., 2007; Regnier et al., 2009; El Karoui et al., 2009).

In France, the “Observatoire Français des Nocardioses” (OFN) is a research centre whose main role is the identification at species level of bacteria of the genus *Nocardia* and the study of their patterns of sensitivity and resistance to antibiotics. The diagnosis of *Nocardia* is performed mostly in cultures and clinical samples from French hospitals. The result of such studies enables the OFN to conduct an epidemiological monitoring of nocardiosis in France (Rodríguez-Nava et al., 2008). The OFN also offers specific assistance to laboratories both in France and in the rest of the world.

At the same time, the research group “Bactéries Pathogènes Opportunistes et Environnement” of the “CNRS 5557 d’Ecologie Microbienne” carries out studies on the distribution and abundance of pathogenic species of actinobacteria, in particular of the genus

*Nocardia*, in French soils, aimed at evaluating the risk of community infections associated with certain human activities (agriculture, speleology, mining, gardening, etc.), especially in immunosuppressed persons. In addition, there are other projects to characterise and define new actinobacteria species of clinical and environmental origin, in cooperation with various laboratories (Betrán et al., 2009).

The OFN annually records from 150 to 250 clinical cases due to *Nocardia*. The main pathogens identified are *N. farcinica* (26%), *N. nova* (20%), *N. abscessus* (18%), and *N. cyriacigeorgica* (12%) (Rodríguez-Nava et al., 2008). The isolation of *Nocardia* species in caves is relatively frequent (Groth et al., 1999; Lee, 2006 b; Seo et al., 2007; Jurado et al., 2008), although, at present, there is no information on the pathogenicity of these new species of *Nocardia*.

Species of the genus *Gordonia*, such as *G. bronchialis*, *G. otitidis*, *G. aichiensis*, and *G. terrae* are described in the literature as opportunistic pathogens responsible for bacteraemias and bronchopulmonary diseases (Iida et al. 2005; Blanc et al. 2007). Species of *Gordonia* have been isolated from Grotta dei Cervi, Italy, and some of them could represent new species, waiting to be described. This genus is of great interest in biotechnology because of its biodegrading and bioremediation capacities, as is the case of the species *Gordonia nitida*, able to desulphurate fuels (Lee et al., 2005).

In the genus *Rhodococcus*, the main pathogenic species is *R. equi* (Paasche, 2009). Nevertheless, *R. erythropolis* is also a pathogenic species that has been reported in clinical cases (Vernazza et al., 1991). The latter is frequently isolated from caves in northern Spain (Groth et al., 1999). The genera *Brevibacterium* and *Micrococcus* have been little studied, and few clinical cases are reported in the literature. Species of the genus *Brevibacterium*, such as *B. casei* and *B. epidermis*, are found in the human skin flora and are responsible for opportunistic infections (Reinert et al., 1995). Of the genus *Micrococcus*, the species *M. luteus* is known to be related with skin diseases, and to affect mainly immunocompromised patients (Salar et al., 1997). *Streptomyces somaliensis* is the most incidental aetiological agent of actinomycetoma in countries such as Sudan and India (Nasher et al. 1989). Actinomycetoma is also caused by *Nocardioopsis dassonvillei* (Ajello et al., 1987). This actinobacterium was found on the stalactites of Grotta dei Cervi, Italy (Figure 3), and it should be regarded as a potential nosocomial pathogen (Beau et al. 1999).

In Altamira Cave, Spain, a new genus and species of actinobacterium, *Hoyosella altamirensis*, has recently been isolated and described (Jurado et al., 2009). Phylogenetic data group *Hoyosella* with the *Mycobacterium* genus. The problem lies in knowing whether environmental bacteria are potentially pathogenic or not. It would be of interest to be able to confirm beyond doubt the existence of pathogenic species of these genera of actinobacteria in little-explored natural ecological niches, such as subterranean ones, to evaluate their potential, besides

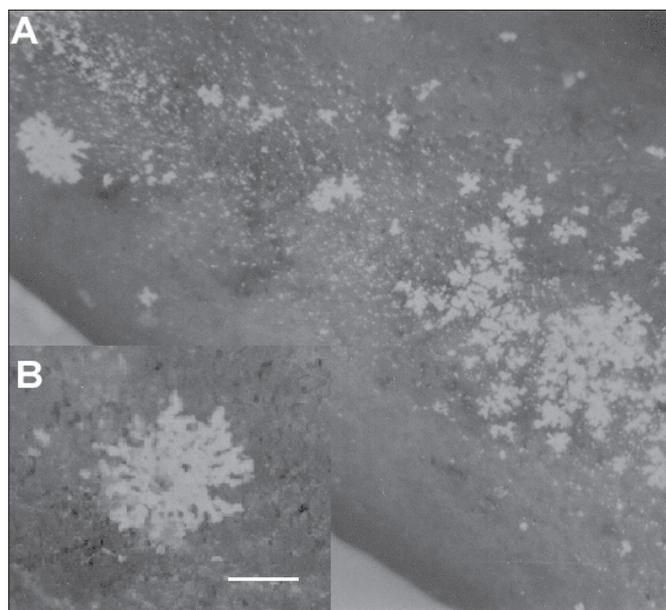


Fig. 3. *Nocardioopsis dassonvillei* on a stalactite (A). Detail (B). Bar is 1 mm. (Grotta dei Cervi, Porto Badisco, Italy).

their diversity and role in such environments.

There is also evidence of the existence of a large reservoir of pathogenic bacteria belonging to the phylum Proteobacteria in visitable caves. For example, recently in Spanish caves have been possible to isolate species of alphaproteobacteria, such as those of the genus *Inquilinus limosus*, a novel emerging pathogen in cases of cystic fibrosis (Schmoltdt et al. 2006) and the genus *Afipia* (associated to protozoa) and likely responsible for nosocomial diseases (La Scola et al., 2002). In addition, reservoirs of *Legionella* (gammaproteobacteria) have been detected in Lascaux Cave, France, by the use of molecular techniques (Bastian et al., 2009a). Lavoie & Northup (2006) found a few bacteria in caves, which were considered indicators of human impacts, such as *Staphylococcus aureus* (Firmicutes).

With regard to the genus *Aurantimonas* (alphaproteobacteria), interesting clinical cases have been reported due to *Aurantimonas altamirensis* – a bacterium isolated and described in 2006 from Altamira Cave (Jurado et al., 2006). Two years later, the second isolation of this species in the world was performed in a Canadian hospital, obtaining it from three different patients suffering from cystic fibrosis, keratitis, or corneal ulcer (Luong et al., 2008). The latest isolation of this species took place in a hospital in Indianapolis, where it was described as an agent responsible for bacteraemia (Mendes et al., 2009). We foresee that in the future, the number of clinical cases due to *A. altamirensis* will increase, as the clinicians now have the possibility to compare and identify patients' strains with the sequences deposited in the GenBank or the type strain deposited in culture collections.

Spirochaetes are frequent in caves. Bats and rodents are reservoirs of leptospirosis and they are numerous in tropical caves, which maximize leptospirosis exposure. High heat and humidity lead to minimal protective clothing, and frequent contact with rock

results in multiple abrasions, a known portal of entry for the organism (Mortimer, 2005).

Relapsing fever borreliosis is another disease related to caves. A tick is the vector of *Borrelia* spp. and is widely distributed throughout India and Kashmir, the southern countries of the former USSR, Iran, Iraq, Syria, Jordan, Turkey, Israel, Cyprus, and Egypt. In Israel, 30–60% of caves were found to be infested by *Ornithodoros tholozani*, the most important tick vector of *Borrelia persica*, causing tick-borne relapsing fever. *O. tholozani* mainly feeds on rodents and small mammals, which constitutes the natural reservoir. As far as the civilian population is concerned, 86% of the infestation foci were caves and 5% were ruins, and individual cases were recorded in areas of rocks and beside animal burrows. There have also been cases where persons who sat or lay on the ground at a distance of several metres from an infestation focus have been infected with *Borrelia* spp. (Assous & Wilamowski, 2009).

### PATHOGENIC FUNGI

With regard to fungi and yeasts, there are references to histoplasmosis caused by the fungus *Histoplasma capsulatum*, found in soils and caves inhabited by bats. This fungus produces pulmonary histoplasmosis, relatively frequent in cave explorers (Nieves-Rivera et al., 2009).

Skoulidis et al. (2004) reported that a patient regularly exposed to soil in bat caves was infected by the fungus *Penicillium marneffeii*. Airborne infection was probably the main route of acquisition.

In the last few years, white-nose syndrome has caused a devastating epizootic among bats of the north-eastern US, and the disease continues to spread rapidly. The causal agent of the syndrome is a new fungus, *Geomyces destructans*. It appears that bats have lowered immune responses during hibernation torpor, which may predispose them to infection by *G. destructans* (Gargas et al., 2009). Another *Geomyces*, *G. pannorum*, is a ubiquitous saprophytic fungus frequently isolated from cave sediments, air samples and bat guano. This species is widely distributed in some European caves (Bastian et al. 2009 b; Nováková, 2009). It is an occasional aetiological agent of superficial infection of skin and nails in humans (Christen-Zaech et al., 2008).

The dermatophytes *Microsporum gypseum* and *Trichophyton mentagrophytes* have been isolated from soils and caves inhabited by bats (Kajihiro, 1965). An abundance of species of the genus *Trichosporon* (yeasts) has recently been found in Ardales Cave, Málaga, Spain (Stomeo, 2008). Bats are present in Ardales Cave and in Murciélagos Cave, in Córdoba, Spain. This genus is commonly isolated from guano, and bat guano is a rich source of yeasts and still-undiscovered species (Sugita et al., 2005).

To date, 21 species of *Trichosporon* have been described, some of them psychrophiles, others associated to animals, and five of them have a marked clinical nature (de Hoog et al., 2000). *T. laibachii* is the most common species, with a distribution in soils, sands, mud, and plant residues (Domsch et al., 2007). It is not associated

with humans, and can be clearly distinguished from the pathogenic species *T. asahii*, *T. asteroides*, *T. cutaneum*, *T. inkin*, and *T. mucoides* by molecular methods. Sugita et al. (2005) found new species in bat guano in Japanese caves, and included them in four different serotypes. *T. laibachii* and *T. multisporum* are included in the group I-III serotype of Sugita et al. (2005). Each serotype is associated to the production of pneumonitis. None of the five pathogenic species have been found in preliminary studies carried out in Ardales Cave, but the number of samplings was small (only two), requiring subsequent studies and a more exhaustive sampling.

Vaughan-Martini et al. (2000), found that high counts of blastomycetes (yeasts and yeast-like fungi) in caves were in direct relation to the frequency of human or animal visits. Apparently, excessive human visits were also responsible for the fact that six of the ten yeast strains isolated have a direct relationship with human pathology.

### VIRUS IN CAVES

Several outbreaks of Marburg hemorrhagic fever have been reported in Africa. Between 1998 and 1999 were identified 154 cases of Marburg hemorrhagic fever (case fatality rate, 83 percent) in a gold-mining village in D.R. of the Congo (Bausch et al., 2005). Fifty two percent of cases were in young male miners, working in the mine. Animals found in the mine included bats, rodents, and others, and the environment was heavily soiled with human and bat excrements. Cessation of the outbreak coincided with flooding of the mine. Bausch et al. (2005) concluded that the reservoir hosts of Marburg virus inhabit caves, mines, or similar habitats.

Towner et al. (2007) identified Marburg virus in common African bats, which were also to be likely reservoirs for Ebola virus. Osborne et al. (2003) identified Kaeng Khoi virus in dead bats in Cambodia.

In 2007, miners working in Kitaka Cave, Uganda, were diagnosed with Marburg hemorrhagic fever. The likely source of infection in the cave was Egyptian fruit bats (*Rousettus aegyptiacus*) based on detection of Marburg virus RNA. The bat colony was estimated to be over 100,000 animals using mark and re-capture methods, predicting the presence of over 5,000 virus-infected bats. The genetically diverse virus genome sequences from bats and miners closely matched. These data indicate common Egyptian fruit bats can represent a major natural reservoir and source of Marburg virus with potential for spillover into humans (Towner et al., 2009). Recently, a Dutch woman who was brushed by a fruit bat in a cave in the Maramagambo Forest, Uganda, died of Marburg hemorrhagic fever. The World Health Organization has warned people not to go into Ugandan caves with bats, after this Dutch tourist contracted the deadly Marburg virus (Timen et al. 2009).

### ENVIRONMENTAL VS PATHOGENIC MICROORGANISMS

The pathogens up to day found and/or isolated from caves (*Aurantimonas*, *Legionella*, *Staphylococcus*, *Inquilius*, *Afipia*, etc.) represent a danger for humans

specially to risk groups. However, the virulence level of these microorganisms is not always constant within strains of the same species. This may rely on the characteristics of the ecological niche the strain was isolated from that may lead to acquire or to develop different pathogenicity factors.

Also, the fact that not many clinical reports of these microorganisms exist may give the false sensation that just a few strains of these species are pathogens. Even if this may be true, we must take in mind that this could be because i) the detection methods are relatively new because it refers to a newly described species, ii) it is not very present in the environment, iii) it is an opportunistic pathogen that requires special conditions of the immunity system of the host that fortunately are not so common.

It is important, apart from detecting the presence of pathogens in caves, to be able of quantifying this presence by using culture methods and by quantitative PCR. On the other side, it would also be interesting to obtain information about the infectious capacity of the pathogen strains isolated in caves by the means of animal experimentation, studying their virulence capacities with direct PCR methods using virulence genes as markers and by other hosts interaction tests (amoebae, mices, insects, etc.)

There exists a high risk, due to the presence of man in caves, of a bacteria passage from man to the cave that may lead to the development of new bacterial lines with many different pathogenic levels. This phenomenon can be evaluated by the measure of human indicator bacteria as proposed by Lavoie & Northup (2006). On the other side, the adaptive factors after a strain exchange (from man to cave and vice-versa) can be known by transcriptoma and comparative genomic studies.

### CONCLUDING COMMENTS

Densely visited or animal-populated caves are reservoirs of pathogenic microorganisms. In recent years, a number of bacteria, fungi and viruses inhabiting caves have been reported to be pathogens for humans and animals. This is particularly dangerous in tropical areas. Although it is difficult to associate a specific infection to a single visit to a subterranean environment, several medical reports indicate that caves visits and mining activities in Africa result in diseases and fatal casualties.

Recommendations are often included in several papers. Jülg et al. (2008) reported that “Advice regarding histoplasmosis prevention should be given not only to bat researchers and to tourists planning to visit bat-infested caves but also to those who merely watch large groups of bats. In the case of caves with very large bat colonies, infection is a possibility even in the cave entrance, with no bats present. Histoplasmosis should be considered in the differential diagnosis of febrile illness in returning travelers with a history of epidemiologic or geographic exposure”.

Assous & Wilamowski (2009) stated that “public education concerning the risks involved in entering caves, combined with prevention measures in countries

in which ticks lives primarily in caves and similar sites, is effective in reducing the number of cases. Persons entering caves must wear appropriate shoes and clothes covering all the body. It is recommended to spray shoes and the trouser bottoms with a repellent. Avoidance of long stays in caves reduces the opportunities for ticks to identify the host. Sleeping in caves or at nearby sites must be avoided. If it is necessary to stay in caves or at archaeological or similar sites for an extended period, it is necessary to monitor whether the place is infested with ticks, and to spray the site with suitable residual insecticides. It is recommended to spray the ground until it is wet and the walls of the cave up to a height of 1 m. If prolonged work is necessary, the work should be stopped for several days for respraying”.

The most-frequent diseases produced by cave microorganisms are located in the respiratory system and, therefore, it is recommended that visitors should, at the least, wear protective masks. Visits to any subterranean environment should possibly no longer be looked upon as a simple, riskless tourist activity, rather as one with potential risks for human health, particularly for the ill and elderly.

The study about diversity and abundance of pathogens in caves will allow us to set preventive actions for the exposed populations and for the risk populations overall. To decrease the number of cave-associated diseases it is necessary to increase the awareness and education of the public about the danger of entering caves and the need to take preventive control measures in caves and other subterranean environments.

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