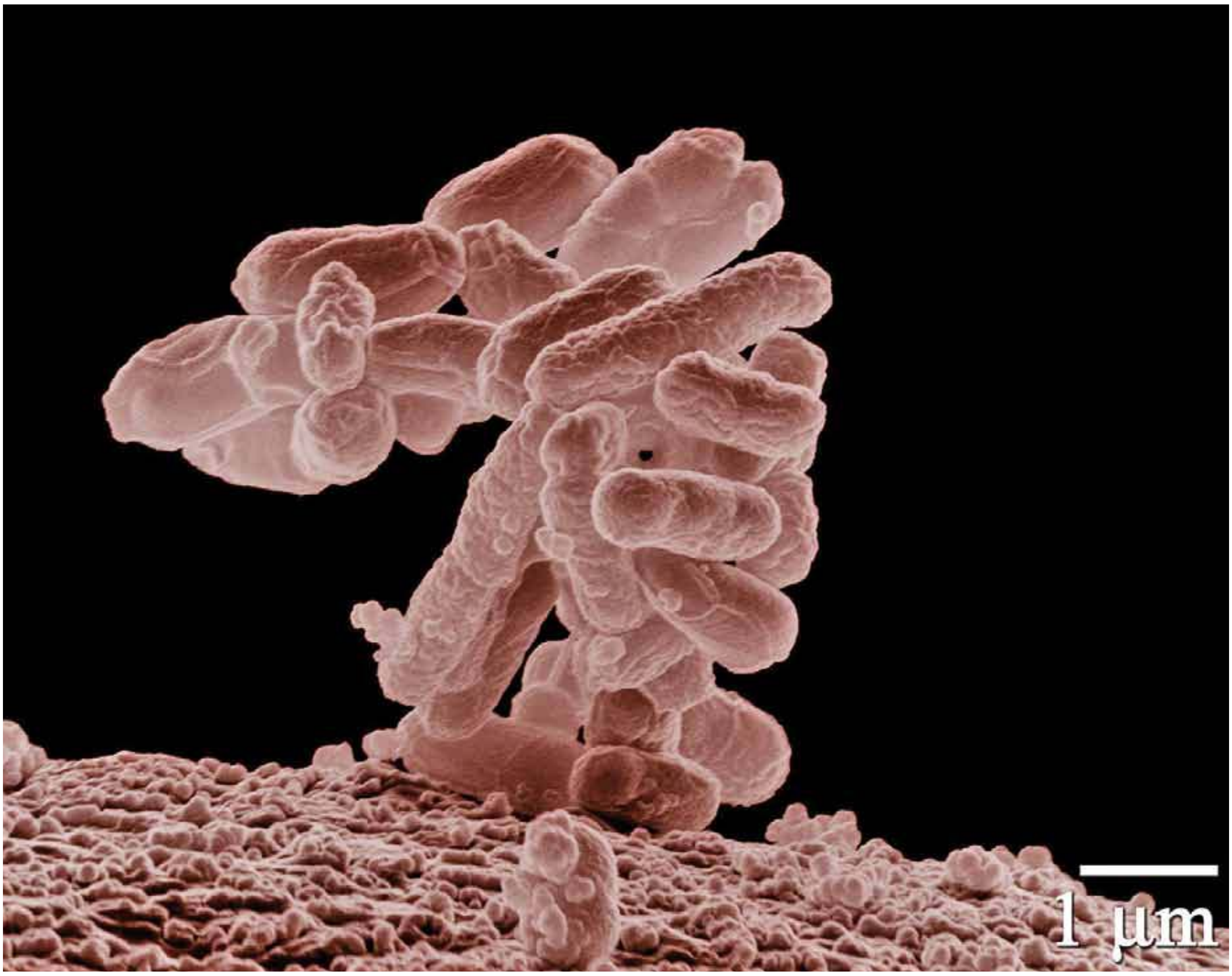
ANTIBIOTICS

When fluorescence tackles resistances

Ever since penicillin was discovered by Alexander Fleming in 1928, then marketed as a drug in the 1940s, the availability of antibiotics has meant that diseases such as tuberculosis and leprosy can now be treated and it is estimated that populations with access to antibiotics have extended their life expectancy by 15 years. Yet, some infectious diseases are still difficult to control. Also, in the fight against bacteria, some of them have developed various forms of resistance. Several research groups, galvanized by these worrying trends, have taken advantage of the facilities on the DISCO beamline to carry out UV fluorescence studies.



Low-temperature electron micrograph of a cluster of *E.coli* bacteria, magnified 10,000 times. Each individual bacterium is oblong shaped.

Although, through the use of antibiotics, many infectious diseases have disappeared or are no longer fatal when diagnosed correctly, the choice of antibiotic to use still remains a crucial element. This is the case for endocarditis, an infection of the heart valves, with a primary lesion known as "vegetation" formed from a mass of bacteria, platelets and plasma proteins developing on one of the heart valves.

Endocarditis: Where do the antibiotics go?

The standard treatment for endocarditis is an intravenous injection of two different antibiotics for several weeks, sometimes accompanied by surgery to remove the lesion and associated abscesses. The treatment is often not very effective due in large part to the fact that the vegetation has few blood vessels feeding it and the antibiotics are not well channeled to the site and its bacterial colonies. It is essential to find out how antibiotics spread within lesions in order to control and adapt the treatment for endocarditis. Yet, few studies exist on their diffusion through "vegetation" and none within bacterial colonies. To obtain some answers on these topics, the group headed by Eric Batard, at the University of Nantes, chose to work on two beamlines at SOLEIL: DISCO and SMIS, in order to combine microspectroscopy with both UV and IR.

Eric Batard working on DISCO's microscope.

Combining infrared and ultraviolet light

IR microspectroscopy studies carried out on the SMIS beamline (see also article p24) showed that it is possible to distinguish the spectral signatures associated with, on the one hand, the biochemical composition of bacterial colonies, and on the other, that of the vegetation, as well as the fact that this biochemical composition is modified in the presence of antibiotics. Researchers therefore possess a first tool for following the effect of treatment on the injured area.
Moreover, some antibiotic families have fluorescence properties when excited by UV light, which makes them particularly interesting in relation to microspectroscopy. However, living tissues can also be naturally composed of fluorescent molecules. This means that if a tissue treated with antibiotics is analyzed, both sets of signals risk interfering with each other. So before following the spatial distribution of antibiotics administered to tissues affected by endocarditis, Eric Batard's group had to analyze the spectral responses of non-treated vegetation. Through the judicious choice of antibiotic, ofloxacin, which emits at different wavelengths from that of diseased tissues, the signals obtained were different for treated and non-treated tissues. The use of UV fluorescence microspectroscopy proved to be a pertinent choice.
The next step was to study the spatial distribution of the antibiotic within the vegetation and whether the localization of the bacterial colonies might influence the diffusion of the antibiotic in the lesion. Early results already show that ofloxacin, effectively localized in the vegetation, is not present in a homogeneous fashion. The spatial resolution of the images (about a hundred nanometers), carried out on DISCO, will make it possible to refine these data down to the scale of the bacterial colony.

A major public health problem

Bacterial resistance to antibiotics has become a major public health problem in Europe and the rest of the world in recent years. Resistant hospital-acquired infections result from bacterial adaptation to the large-scale use of antibiotic treatments. These microorganisms have developed numerous strategies on different levels: the antibiotic must first penetrate the bacterial membrane, where its crossing may be limited by modifications to the membrane, or counterbalanced by efflux of the antibiotic present in the bacterium, through the presence of efflux pumps in the membrane. The effectiveness of the treatment can also be hampered by bacterial enzymes that degrade the antibiotic or by mechanisms that reduce its action (e.g. mutation of the target molecule to which the antibiotic must attach itself).
Although it is known that bacteria put up these "barriers", a great deal of information is still missing on the biophysical and biological processes involved. The group headed by Jean-Marie Pagès, from the Université de la Méditerranée (Marseille), focused its attention on the stage when the antibiotic molecules are transported into bacteria. Using UV fluorescence microspectroscopy it was possible to follow the presence of antibiotics in different bacteria, chosen for their particular membrane properties: the transport proteins involved in "antibiotic entry" were found to have been modified, thus rendering the bacteria resistant. In addition, chemical compounds capable of inhibiting bacterial resistance to antibiotics were also used.

Resistant bacteria divulge their secrets

The approach of Jean-Marie Pagès's group consisted of testing increasing concentrations of antibiotic on resistant bacteria with or without the presence of the chemical compounds cited earlier. Due to the performance level of the DISCO beamline, it is possible to observe precisely the spot where the antibiotic molecules are localized within bacteria: membrane, cytoplasm, etc. By testing several bacterial strains, the scientists aim to obtain a complete molecular definition of the mechanism of antibiotic resistance in place, as well as processes that could counteract this mechanism. Researchers are expecting a lot from these biophysical investigation methods, which should soon provide new weapons in this perpetual war against bacterial infections.

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