Fighting antibiotic resistant bacteria, rationally!

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Today, we do not consider a small scratch to be potentially life threatening. This is because we have the ability to treat bacterial infections by use of antibiotics. Antibiotics have arguably revolutionised modern medicine. Antibiotics made it possible for us to treat previously life threatening infections and allowed us to prevent bacterial infection after invasive and advanced medical procedures such as surgery. The first antibiotic, penicillin, was accidentally discovered by Alexander Fleming who had let one of his petri dishes get contaminated with a fungus. Upon closer inspection, he discovered that the fungus had prevented growth of the bacteria. This was in 1929 and by the D-Day landings of Normandy in 1944 during World War Two, penicillin was in mass production. In this capacity, penicillin saved the lives of many wounded soldiers by treating infections. In the next coming decades many more novel antibiotics were discovered, allowing us to further treat bacterial infections. During his Nobel Prize acceptance speech, Alexander Fleming warned us about how easily the bacteria developed resistance against antibiotics. This is a warning that we perhaps did not heed.

Today, hospital acquired antibiotic resistant bacteria are arguably one of the greatest healthcare challenges to be faced today. Many of the bacterial infections that were previously treatable with antibiotics are resistant and the antibiotics are now obsolete. According to the CDC (Centers of Disease Control and Prevention), in the US it is estimated that 2 million people per year acquire serious infections caused by resistant bacteria and close to 23,000 people die per year due to these infections. In addition to the human suffering caused by these infections, there is an estimated additional healthcare cost of up to $20 billion per year associated with antibiotic resistant bacteria. If nothing is done, there is a danger that we will be returning back to the pre-antibiotic era and lose the possibility to safely carry out life saving medical procedures.

A large majority of hospital acquired antibiotic resistant bacteria are caused by the ESKAPE pathogens. ESKAPE is an acronym standing for: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Psuedomonas aeruginosa,* and *Enterobacter species.* These ESKAPE pathogens cause a great deal of morbidity and mortality and are highly resistant to antibiotics. In sight of the clear and urgent need for novel antibiotics, a metabolic pathway called the non-mevalonate pathway has been identified as a promising target in these bacteria. The non-mevalonate pathway is responsible for isoprenoid biosynthesis in these pathogens and it has been shown that blocking this pathway clears the infection. In this study, we managed to produce a protein involved in this pathway, called IspE, with the goal to study the structure in order to rationally design antibiotics to bind to IspE and block this pathway. IspE from three bacteria from the ESKAPE group were studied. These are *Acinetobacter baumanni, Klebsiella pneumoniae, and Pseudomonas aeruginosa.* In order to study the structure, it is necessary to produce pure protein and generate protein crystals. These protein crystals will then be analysed with a high powered X-ray beam at a synchrotron. The protein crystal will defract the x-rays and from the data collected, it is possible to model a 3D structure of the protein. Once the 3D structure has been modelled, it is possible to rationally design drugs to bind effectively to the active site of the protein. This could give rise to the next much needed antibiotic.

As it is extremely difficult to produce protein crystals, no protein crystals were generated during this project but the procedures for producing these proteins were established, allowing for further experimentation with these proteins. In the future, this will facilitate the development of novel antibiotics against these highly resistant bacteria.

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