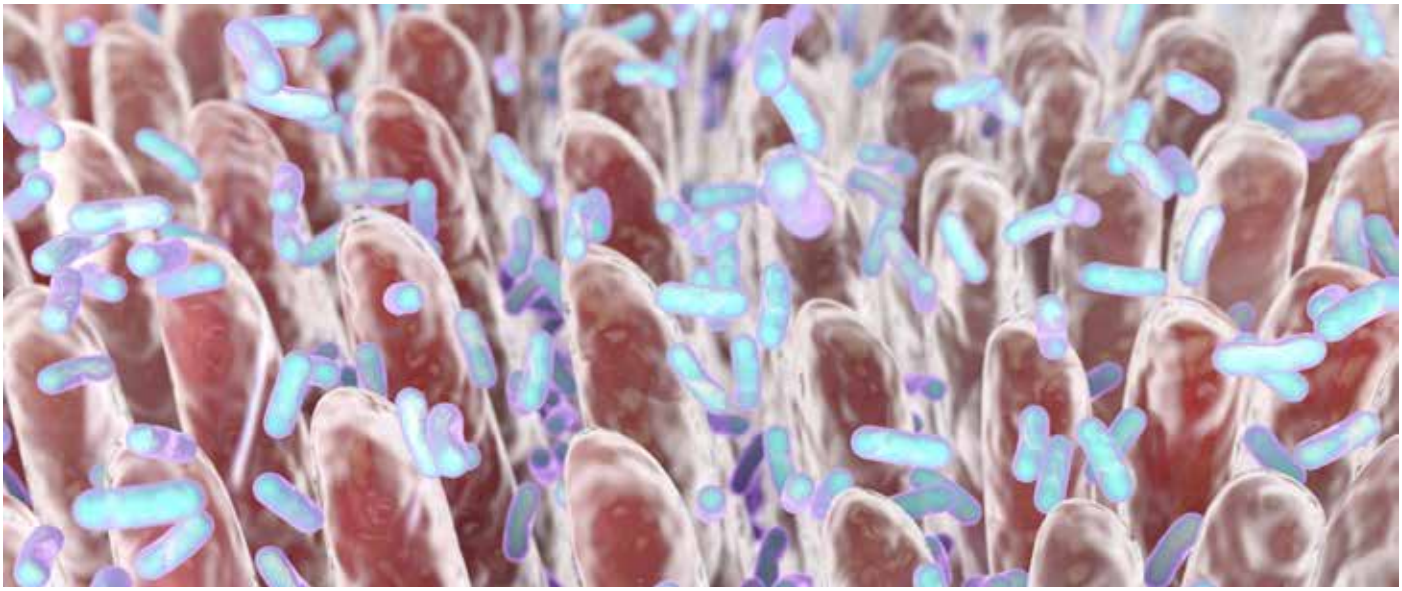
Secrets of the Intestines

In recent years, intestinal bacteria have become more and more resistant to antibiotics. The bacteria that cause diarrhoea are also increasingly resistant. Antibiotic-resistant bacteria are a global threat. Professor Anu Kantele is interested in knowing what happens in the intestines as people travel to the tropics and back home again.



Regular Finns have been sent to Benin as subjects to test a new diarrhoea vaccine. The ETVAX vaccine is administered as an oral solution, similarly to heartburn medicine. Once the vaccine is on the market, it is planned to be sold to developing countries at a low price.

For tourists, diarrhoea is usually just an unpleasant experience, but it is life-threatening for the children in developing countries. Diarrhoeal disease is the second largest cause of death in children under five years old in the world. Every year, more than 1.7 billion children fall ill with diarrhoea. Of them, more than half a million under 5-year-olds die according to the World Health Organization (WHO). Diarrhoea is also the main cause of malnutrition, short stature and impaired learning ability in small children.

Diarrhoea is a symptom brought on by disease-causing bacteria, viruses and parasites that have reached the intestines. They generally spread through water and food contaminated by faeces. Diarrhoea is transmitted especially when there is a lack of adequate hygiene and clean water for drinking and household consumption. According to UNICEF, in 2010, up to one fifth of the world's population had to relieve themselves outdoors. Almost 900 million people suffer from a lack of clean drinking water. On trips to the tropics, the share of those who fall ill with diarrhoea may be up to 60%. Bacteria cause 80% of the cases of traveller's diarrhoea. Enterotoxigenic coliform bacteria (ETEC) are one of the most common causes of severe diarrhoeal disease. That is why there is demand for a vaccine that works against diarrhoea. The pathogens have been studied extensively both in developing countries and with tourists. The information has been used to develop a new vaccine designed to train the human immune system to identify and obliterate pathogens before they can cause symptoms.

The Escherichia coli bacteria, or coliform bacteria, normally live in the intestines of humans and animals. More than 700 types of E. coli have been identified. They are part of the normal microflora of everyone's intestines and are mainly useful. For instance, they protect us against many disease-causing microbes.

"E. coli is the most studied bacterium in the world. It is usually not dangerous, but some are disease-causing. There are several known so-called diarrhoea coliform organisms that cause diarrhoea.

ETEC is one of them. It causes severe watery diarrhoea", says **Anu Kantele**, Professor of Infectious Diseases from the University of Helsinki.

ETEC differs from other types of coliform bacteria in that it produces two toxins that cause significant fluid secretion from the small intestine, that is, watery diarrhoea.

"The tolerability of the vaccine and the immune defence it elicits are now being studied, while also investigating its efficacy against traveller's diarrhoea. Developing an ETEC vaccine is also one the goals of the WHO. This so-called ETVAX vaccine generates a good immune response and is the most promising of the current ETEC vaccine candidates."

ono is a border river between Benin and Togo. Photo: Ari Turunen

Two-year protection

ETEC strains of bacteria were among the first pathogenic organisms for which molecular diagnostics were developed. Vaccine researchers are currently interested in several diarrhoea-causing microbes, such as ETEC bacteria, Shigella bacteria and norovirus. Vaccines already exist against other major causes of diarrhoea, such as cholera and typhoid bacteria and rotavirus.

The study is being conducted in collaboration between the universities of Helsinki, Gothenburg and Johns Hopkins and the vaccine manufacturer, Scandinavian Biopharma AB. United Medix Laboratories Ltd is also involved. The safety of the vaccine has previously been tested on 140 Swedish adults and 450 Bangladeshi children. In both studies, the vaccine and placebo groups had an equal amount of side effects, so the vaccine is considered to be very safe.

The results of the Swedish research group have demonstrated that the ETVAX vaccine elicits a strong immune response not seen in those who receive the placebo.

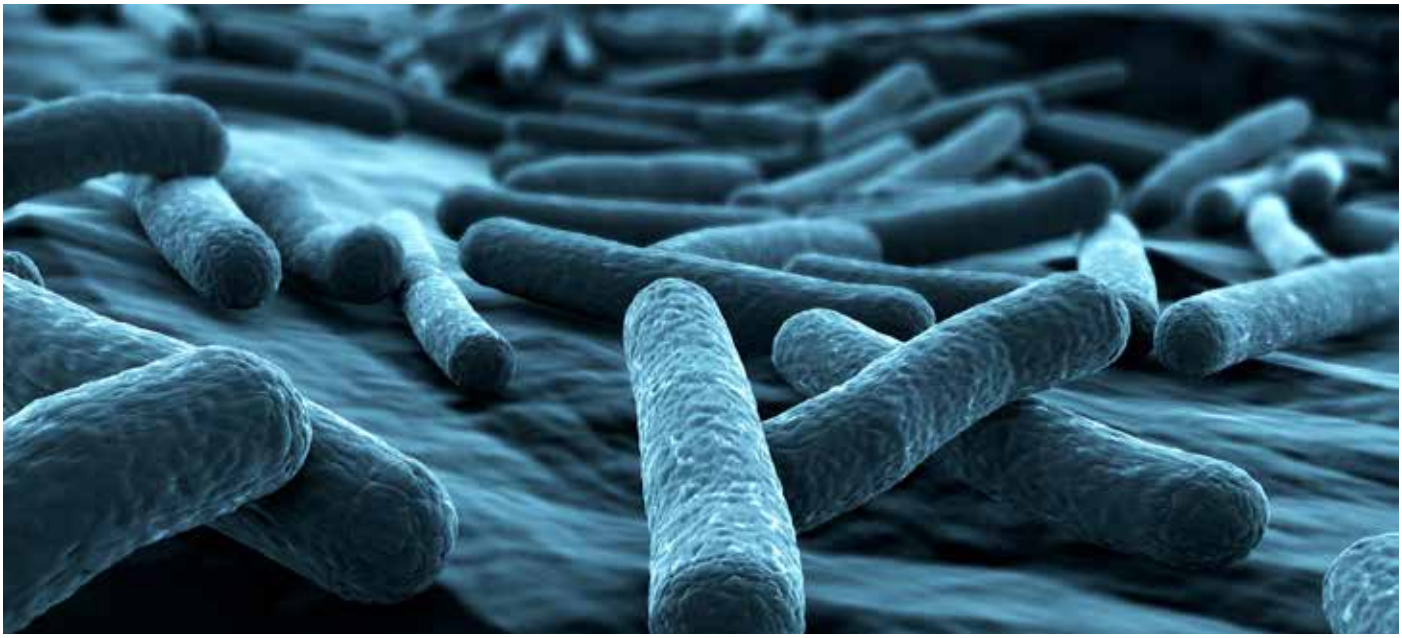
"Among the Bangladeshi children, the vaccine was well-tolerated and the response was good. Its safety was studied previously, and now we are studying the effectiveness of this oral vaccine for the first time", says Anu Kantele.

900 million people suffer from a lack of clean drinking water. During the NEWAW WASH-project taps were installed in Puware Shikhar, Nepal. Photo: Jim Holmes/AusAID

The vaccine has been estimated to provide protection against moderate to severe ETEC diarrhoea in 60–80% of those vaccinated. Studies on the cholera vaccine have been a great help in the development work for the ETEC vaccine. The pathogenic mechanisms of the ETEC bacteria and cholera bacteria (Vibrio cholerae) are very similar. They cause illness by attaching to the surface of the small intestine and producing enterotoxins, or intestinal poison, that are responsible for the symptoms of the disease. The toxin kills cells by preventing their protein synthesis. The toxin makes the mucous membrane of the intestine permeable, whereupon a lot of water passes from the tissues to the intestine. This causes very severe watery diarrhoea. The cholera toxin and ETEC bacteria toxins are structurally, functionally and immunologically similar.

ETEC bacteria produce both heat-labile (LT) and heat-stable (ST) toxin. Both have a protein structure and are toxic to humans. ETEC carries a plasmid, a circular DNA molecule that guides the production of the toxin.

"The ETVAX vaccine contains a number of components: killed ETEC bacteria, so-called colonisation factors that allow the bacteria to reproduce in the intestine, and detoxified LT toxin and an adjuvant



The most common causes of traveller's diarrhoea are the so-called diarrhoea coliform bacteria, of which there are five different types. One of the most important is the enterotoxigenic Escherichia coli, or ETEC. It is found in about 20–40% of disease cases. Of the other bacteria, the Campylobacter, Salmonella and Shigella species, for example, may result in very severe symptoms. Their combined share is about 15%.

produced from it", says Anu Kantele.

Once the samples of the 800 test subjects who travelled to Benin have been analysed, it will be possible to evaluate, in particular, how an immune system trained by the vaccine works against the ETEC bacteria contracted on the trip and its LT toxin. Half of the subjects have received the vaccine and the other half a placebo.

What happens in the intestines?

Anu Kantele and her team study the microflora, pathogens and resistant bacteria in the human intestines from stool samples. The participants provide various samples for the study. The analysis of ETEC bacteria and other pathogens requires stool samples. Blood and saliva samples are used to study, for example, the immune response to the ETEC vaccine. Data is also collected on the possible adverse effects of the vaccine. Diarrhoea samples are collected and processed immediately on site in a laboratory in Benin. The number of samples obtained is essentially huge. One gram of human faeces can contain up to one million bacteria.

The researchers compare cultivation-based and molecular laboratory methods used to identify ETEC and other causes of intestinal infections from the stool samples. They analyse the antibodies and genes involved in immune defence.

Anu Kantele has been studying diarrhoeal diseases for a long time.

"I am interested in knowing how new bacteria that arrive in the intestines manage to settle into the ecosystem formed by the native intestinal bacteria and how antibiotic treatment affects this", says Kantele.

Antibiotic-resistant strains of bacteria are most likely to develop in the poor countries of the world. The reason is the excessive use of antibiotics. If the sanitary conditions are inadequate, resistant bacteria spread easily and even from one country to another. According to the WHO, for example, the bacterial strain resistant to fluoroquinolone, which is commonly used to treat urinary tract infections caused by coliform bacteria, is widespread.

"The Benin test subjects will provide a lot of data that can be used to analyse the efficacy of the vaccine. In addition,

Anu Kantele at the laboratory in Benin. Photo: Else Kyhälä.

the intention is to use genetic engineering techniques to analyse the microbes in the stool samples and to investigate the presence of antibiotic-resistant strains. The amount of data is enormous but, by combining data, it is possible to gain new insight into, for example, the spread of antibiotic resistance."

According to Kantele, the majority of the antibiotics used by tourists are taken for traveller's diarrhoea. The antibiotic shortens the duration of the disease, but it would almost always go away by itself also without antibiotics. Kantele emphasises that the symptoms can be alleviated with drugs that affect the functioning of the intestines without increasing the risk of contracting resistant bacteria.

"Antibiotics facilitate the settlement of resistant bacteria into the intestines, and so one of the best ways to avoid such colonisation is to not take antibiotics. Nowadays, antibiotic treatment is usually recommended only for severe diarrhoea; less severe cases are treated with fluid therapy and possibly drugs that affect the functioning of the intestines. The carriers of antibiotic-resistant bacteria may carry the bacteria to their home country and possibly spread them further there. To reduce the flow of resistant bacteria into the home country, antibiotics should be used with caution in the treatment of traveller's diarrhoea."

Antibiotic-resistant strains can now be quickly identified through genetic engineering techniques, particularly polymerase chain reaction (PCR). Sequencing can be used for even more accurate analysis. The rapid identification of the infection-causing bacteria prior to drug selection is one way to control the use of antibiotics.

One of the studies conducted under Kantele's leadership demonstrated that 80% of the tourists to high-risk areas who fell ill with diarrhoea and took antibiotics brought the ESBL super bacteria with them. ESBL (Extended Spectrum Beta-lactamases) is a special enzyme that breaks down antibiotics and makes the bacteria resistant to many common antibiotics.

The diarrhoea-causing ETEC coliform bacteria may also have the ESBL characteristic.

The premise of Anu Kantele is that we can learn from travel. What interests her is how the microbial activity of human intestines changes while travelling.

"I would like to find out what happens in the intestines of the 800 test subjects during the trip. We are talking about an ecosystem where the strongest bacteria win. It is exciting to combine the data on the changes in the microflora to how the body responds to them, what genes are activated, etc. We learn more about the intestines every day."

Sequencing and intestinal metagenomics

Diarrhoea-causing bacteria can be detected in a stool sample through cultivation or PCR examination, or a combination thereof. PCR, or polymerase chain reaction, is one of the most important techniques used in molecular biology. It can be used, for example, to amplify a single gene or any segment of DNA multiple times. PCR is performed outside of living cells in a laboratory (in vitro) using a special PCR device. With PCR, a very small amount of DNA can be amplified to produce a billion times the amount of the same DNA in a few hours.

PCR technology is used for many purposes, including finding hereditary diseases, identifying individuals using genetic fingerprints, diagnosing infectious diseases and cloning genes. Microbial DNA is isolated from stool samples and amplified. By amplifying different gene areas, it is possible to quickly and efficiently identify pathogens

400 of the study participants will receive a vaccine containing inactivated ETEC bacteria. The remaining 400 will receive a placebo vaccine. The vaccination trip was arranged with the collaboration of the cultural center Villa Karo.

from the stool sample. Microbes are identified by the base pair sequence. Cultivation is necessary alongside PCR because it allows the detection of antibiotic sensitivity.

The majority of the microbes in humans are located in the intestines. More than a thousand different species of bacteria live in the intestines of an adult human. The microflora in the intestines has up to a hundred times more genes than the human genome. 99% of intestinal bacteria are anaerobic, meaning that they grow in the absence of oxygen. Of the remainder, the most common are E. coli bacteria.

The intestinal microbes form their own ecosystem. Microbes have traditionally been studied and cultivated in laboratories. Now, with metagenomics, it is possible to also study them better in their natural habitat, be it soil or the intestines. DNA sequencing is used to try and ascertain the genome of an entire ecosystem. The human genome includes 20,000 genes. However, in addition to these genes, the intestinal bacteria of a single human being encode up to a million genes that affect the regulation of bodily functions. Almost 10 million genes originating from various bacteria have been identified in samples from human intestines. There is great genetic diversity and the amount of data is enormous. Knowledge of the functions of the genes of the intestinal microflora is still in its infancy.

The metagenomics service of EMBL-EBI is an automated data transfer service (EBI Metagenomics Pipeline) for the analysis and archiving of metagenomic data. There are samples from the human digestive system, soil, water, animals and plants. The data to be studied can be submitted to the service for analysis and comparison.

The service can be used to gain additional information on the evolutionary history of different microbial species as well as the functioning and metabolism of microbes. The data archived by EMBL-EBI is publicly available. EMBL-EBI is part of the ELIXIR infrastructure.

Ari Turunen (7.5.2018)

FURTHER INFORMATION:

https://www.ebi.ac.uk/metagenomics/

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