Impact Objectives

- Distinguish drug-resistant bacterial species in fresh foods, and identify drug resistant plasmids and drug resistance genes
- Update an antibacterial method of limiting and killing drug-resistant bacteria using the antibacterial antisense RNA and bacteriophage
- Explore various phages that can infect drug resistant bacteria through different types of sex pili

Novel antimicrobial method to tackle drug-resistant bacteria

***Associate Professor Mitsuoki Kawano** has spent his career looking at the rising levels of antimicrobial resistance and is currently leading a team working on finding a controllable treatment for drug-resistant bacteria*

Firstly, can you talk about your background in antibiotic resistance research?

I first started research in this field during my time in the doctoral programme at graduate school. Since then, I have been performing physiological function analysis and biological information analysis using molecular, biological and genetic methods for non-coding RNA and functional small RNA. I was part of a team where we were able to identify multiple antisense RNAs that regulate the expression of toxic peptides after transcription by comprehensive transcriptional sequencing analysis.

As a result of functional analysis, it was found that these are bacterial growth control genes classified in the toxin-antitoxin system and act as biological defence factors that respond when cells are exposed to environmental stress. Moreover, our team constructed a system that can induce the expression of synthetic antisense RNA from a plasmid and developed a new antibacterial method by artificially suppressing the expression of the antitoxin gene and inducing the expression of the toxin gene.

Have you used any state-of-the-art methods or tools in your research?

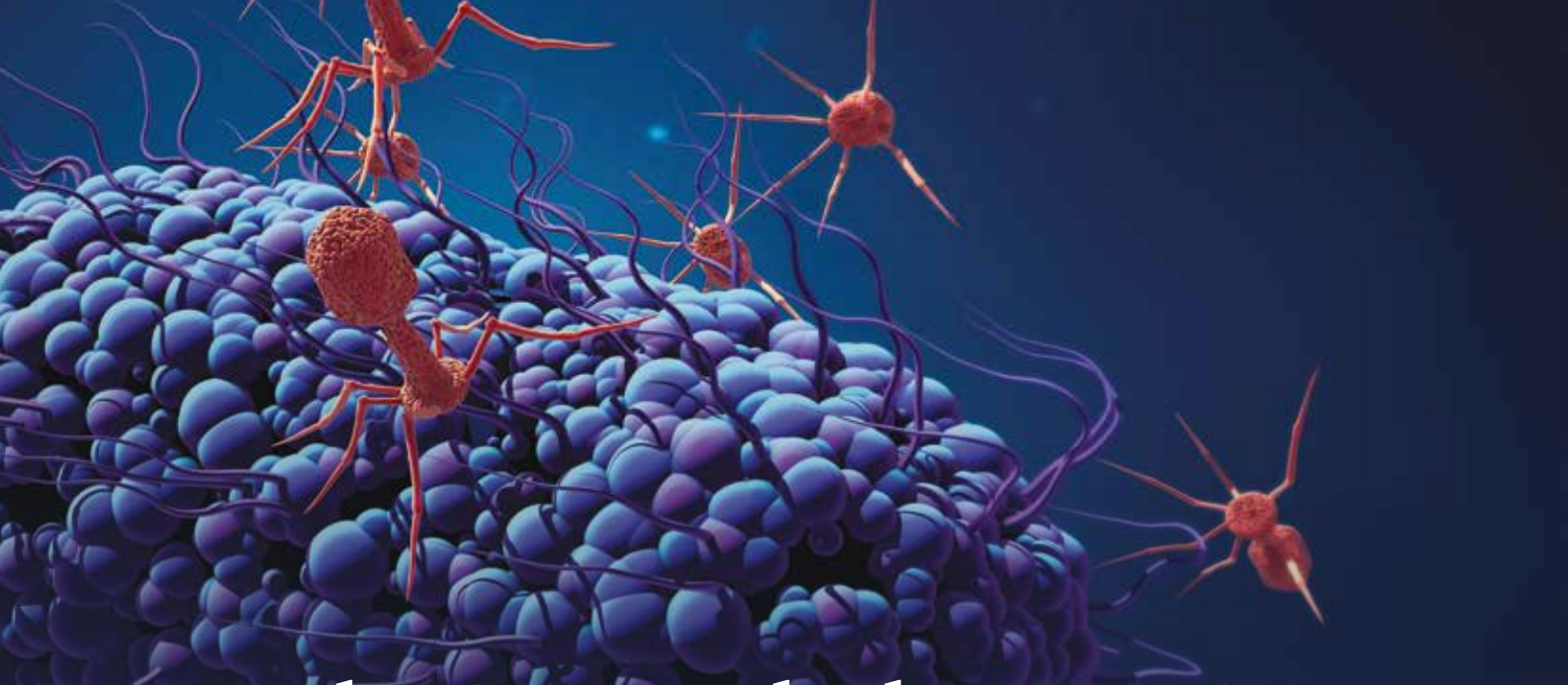
We have developed the world's first method to fight drug-resistant bacteria that produce F-type pili using antibacterial antisense RNA-expressing phage. Since the antisense RNA nucleotide sequence that identifies the target gene can be easily designed, various gene expressions can be controlled. We are also constructing a system that can simultaneously control the expression of multiple genes using polycistronic antisense RNA sequences.

You are based at the Department of Human Nutrition, Faculty of Contemporary Life Science at Chugokugakuen University in Japan. What type of research are you involved with?

The ingestion of foods carrying drug-resistant bacteria is one of the main causes for the human transmission of drug-resistant bacteria. Even if drug-resistant bacteria carried in the body are non-pathogenic, the horizontal gene transmission of drug-resistant plasmids may contribute to the acquisition of drug resistance of pathogens that have invaded the body. Therefore, it is important to eradicate drug-resistant bacteria that are attached to any foods. In the future, we will investigate the detection of drug-resistant bacteria in commercially-available fresh foods, try to distinguish the bacterial species and identify drug resistant plasmids and drug resistance genes. Following this, we will update a method of controlling bacterial gene expression using small antisense RNA and a new antibacterial method of limiting and killing drug-resistant bacteria using the antibacterial antisense RNA and bacteriophage. In the near future, I would like to undertake some research aimed at eradicating multi-drug resistant bacteria from the body.

How are you planning to progress your work?

We are currently conducting experiments on infected animals and investigating with our collaborators whether the method developed at this point has a therapeutic effect. We are also searching for various phages that can be used in this study, which can infect drug resistant bacteria through different types of sex pili. Moreover, we design and prepare antisense RNA-expressing phage that can be used for the treatment of multidrug-resistant bacterial infections in humans and conduct clinical trials, and I want to develop an antibacterial drug with a new action by phage and antibacterial RNA. Collaborators are critical to our work and I am still looking for more collaborators to help sustain and accelerate this research.



Combating multidrug-resistant infections and saving lives

*A team based within the **Department of Human Nutrition** at **Chugokugakuen University** is working on developing a novel method of controlling bacterial gene expression to help clinicians overcome the problems associated with the increase in drug-resistant bacteria*

The COVID-19 outbreak and subsequent pandemic has helped to emphasise the need for effective treatments for different conditions. Researchers from around the world have come together to show the importance of scientific studies through the development and approval of vaccines that can effectively prevent millions of people from being infected by COVID-19. It is difficult to predict how many people's lives will be saved by this work, but it would surely run into the tens of millions at least.

In a similar, though far more important way, the discovery, development and creation of antibiotics has saved hundreds of millions of lives and enabled the effective treatment of bacterial infections, as well as facilitating surgical procedures. Without antibiotics a simple cut could quite easily become life-threatening, many more women would die from childbirth and countless diseases would ravage human life. However, in recent years antibiotic resistance is on the rise, meaning that drug-resistant bacteria are less likely to respond to antibiotics. This means that finding new treatments and therapies is critical.

BACTERIAL GENE EXPRESSION

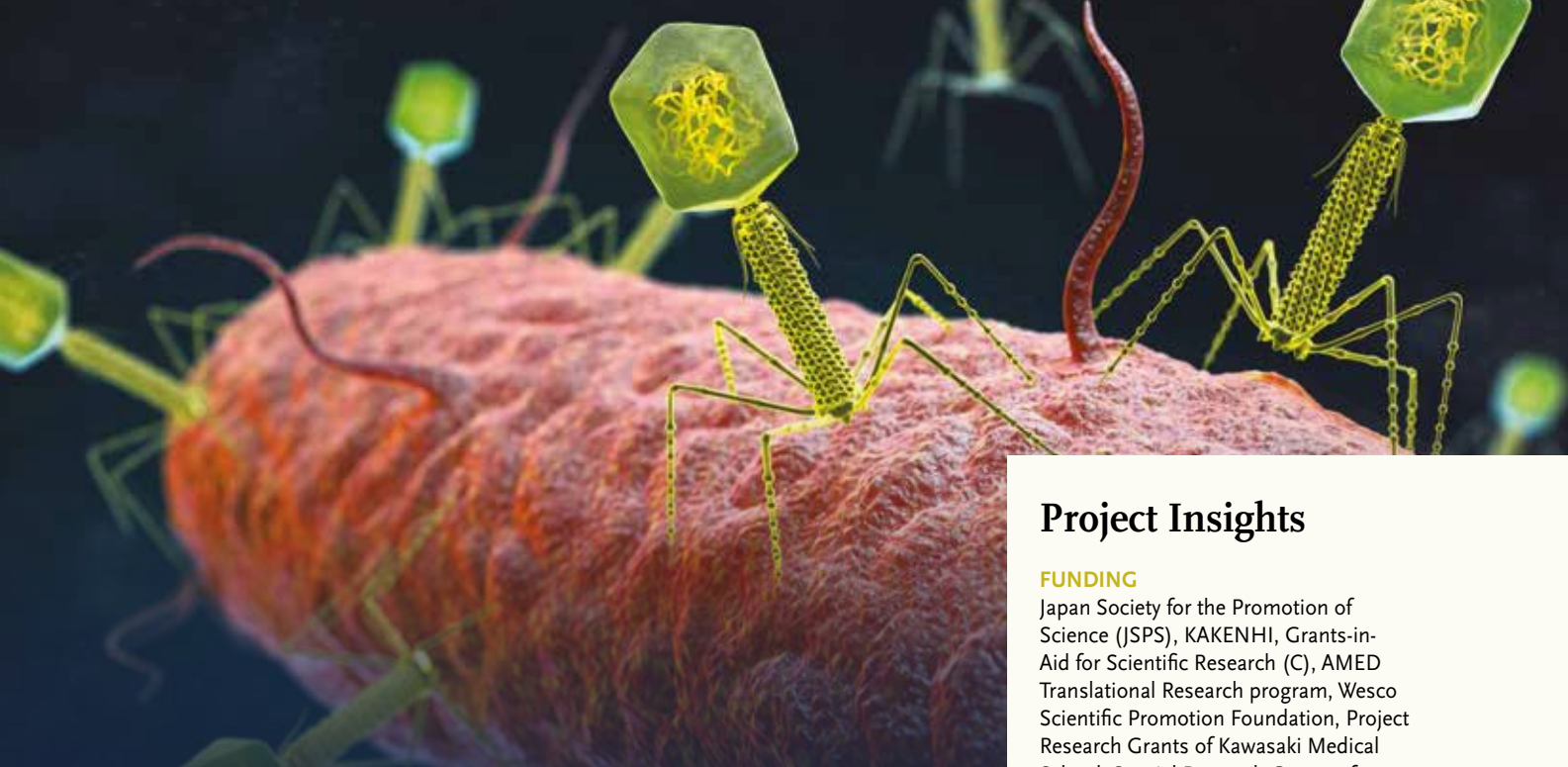
Associate Professor Mitsuoki Kawano, who is based at the Department of Human Nutrition, Chugokugakuen University in Japan, is working on a project that seeks to develop a novel antimicrobial method to tackle the problems associated with drug-resistant bacteria, also known as antimicrobial resistance (AMR). Kawano is acutely aware of the challenges associated with AMR. 'The increase in drug-resistant bacteria has been expanding globally in recent years and the World Health Organization has positioned it as "the most important issue that humankind should tackle jointly",' observes Kawano. 'It is said that about 700,000 people died from AMR infections worldwide in 2013 and about 10 million people will die in 2050 if no effective action is taken, which exceeds the number of cancer deaths. About 5 million of the estimated deaths are said to be in the Asian region.'

Somewhat unfortunately, the focus of new drug development has shifted to more profitable chronic disease treatments while the development of antibacterial drugs is declining sharply. Thus, finding a controllable treatment for drug resistant bacteria other than the existing antibacterial agents is important and urgent. It is this that forms the basis of Kawano's studies as his team has worked to develop a technology that can easily control bacterial gene expression using antisense RNA.

SUPPRESSING PROTEIN SYNTHESIS

There are many challenges associated with multidrug-resistant bacteria. 'Because existing antibacterial drugs also kill indigenous bacteria and upset the balance of the intestinal flora, it is important to develop a bactericidal method capable of killing multidrug-resistant bacteria carrying drug-resistance genes in a limited manner,' points out Kawano. 'In addition, when bacteria acquire a transmissible plasmid that carries a drug resistance gene, drug sensitive bacteria easily become drug-resistant across any genus and species.' Therefore, it is necessary not only to develop the idea of killing drug-resistant bacteria, but also to develop a method to prevent the transmissive plasmid from being transmitted to other bacteria.

The system the team have developed can inhibit cell growth by suppressing the synthesis of proteins essential for bacterial growth by the action of antisense RNA transcribed from phagemid nucleic acid introduced into bacteria by phage infection. 'When we targeted the S13 ribosomal protein gene, which is conserved in almost all bacteria, as an antisense RNA target, we were able to kill clinical isolates of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* with an efficiency rate of 99.99 per cent or higher,' says Kawano. ▶



'This method does not depend on the type of drug resistance gene, and since phages infect only bacteria carrying the F-type plasmid and show antibacterial activity, it can be applied to drug-resistant bacteria that will appear in the future.' Because the phage is non-lytic and non-self-propagating, the team expects that the development of a nucleic acid phage drug without side effects such as endotoxin shock will become a reality before long.

"The next step is to expand the research and investigate the status of multidrug-resistant bacteria contained in fresh foods sold in Japan"

However, despite these successes, the team's work is far from done. One of the major next steps is to translate the learnings from their research into real-world healthcare practices. Thus, Kawano and his colleagues are conducting experiments on infected mice using the antibacterial antisense RNA-expressing phage system. If there is a clear therapeutic effect, Kawano would like to connect it to clinical research in the future.

DETECTING DRUG-RESISTANT BACTERIA

In recent years, extended-spectrum β-lactamase (ESBL)-producing bacteria have been detected in various commercial foods in many countries. In particular, it has been frequently confirmed in edible chickens, which are attracting attention as a source of human infection. In addition, a large number of drug-resistant bacteria have been detected in fresh food by Kawano's investigation and, because these foods are generally not sterilised by heating before being eaten, it is believed that these resistant bacteria remain alive and invade the human body.

Having said this, if Kawano's research is successfully translated into real-world outcomes, the global population stands to benefit. The team's recent experiments have led to their isolating multidrug-resistant bacteria that frequently appears in raw vegetables, raw fish (sashimi), seawater and faeces. 'The next step is to expand the research and investigate the status of multidrug-resistant bacteria contained in fresh foods sold in Japan and work on research to eradicate the only drug-resistant bacteria in the environment and in the human body using natural phages and recombinant phages that they have developed,' confirms Kawano.

The team plans to create an environment where they can work consistently from basic research to application, inspection and sterilisation for the purpose of setting up a university-launched bio venture within the next 10 years, investigate multidrug-resistant bacteria possessed by each individual and eliminate only those bacteria with phages from their phage collection. Ultimately, Kawano wants to reduce the number of people who suffer from multidrug-resistant infections and save as many people as possible from multidrug-resistant infections in the future.

Project Insights

FUNDING

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BIO

Mitsuoki Kawano received a PhD from the Nara Institute of Science and Technology (NAIST) in 2002. He is currently an Associate Professor at Chugokugakuen University, where he is developing technologies to fight against multidrug-resistant bacteria using small antisense RNAs and bacteriophages.