

Novel methods to assess the effectiveness of individual disinfectants

Disinfectants are crucial in the fight against infectious diseases. However, it is often unclear which is the best disinfectant for the job. Professor Daisuke Sano of Tohoku University, Japan has developed culture-independent methods to evaluate the effectiveness of an individual disinfectant, olanexidine gluconate. Prof Sano and his research team suggest that olanexidine gluconate is effective against a wide range of viruses, including norovirus, and works on both human skin and environmental surfaces. This suggests that olanexidine gluconate has the potential to play a key role in infection control.

The importance of keeping surfaces and hands clean to minimise transmission of infectious diseases is well known. Disinfectants – substances that destroy pathogens such as bacteria, viruses, and fungi – are often used to help control the spread of infectious diseases. Different disinfectants are used for different purposes and the choice of chemical depends on the type of pathogen that is being targeted. For example, disinfectants can be based on a variety of chemicals such as alcohols, aldehydes, phenols, acids, iodine, or chlorine. Some disinfectants may not be chemical based, such as ultraviolet light (the same light emitted by the sun) or heat treatments. In homes, chlorine bleach tends to be the most used disinfectant.

However, less is known about the effectiveness of these individual

disinfectants. In order to test how well a disinfectant works, an appropriate evaluation system is required – for example, by growing bacteria in a lab which the disinfectant can be tested against.

Professor Daisuke Sano and his team at Tohoku University have built several evaluation systems to inform decisions about which disinfectants should be used for certain pathogens. One example of this was for human norovirus, a gut bug which causes vomiting and diarrhoea. There is currently no therapeutic agent or vaccine that can treat or prevent human norovirus infection; therefore, most emphasis is placed on disrupting routes of viral infection, such as transmission via contaminated surfaces or human hands. Despite this, no hand antiseptics have yet proven completely effective against norovirus.

A NOVEL ANTISEPTIC AGAINST HUMAN NOROVIRUS

Olanexidine gluconate (OLG) is a novel compound used in the field of medical surgery in Japan, suggesting that it may also have potential for use as a hand antiseptic against bacteria and viruses. OLG is considered to work by disrupting membrane and denaturing proteins; the protective cell membrane and outer proteins are disrupted, meaning that viruses possibly lose their infectivity.

Prof Sano and colleagues proposed that a hand antiseptic based on OLG



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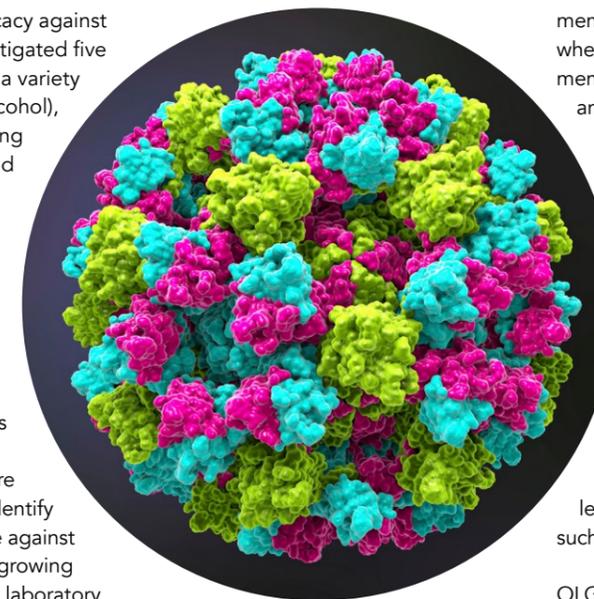
would have the potential to destroy even robust proteins in human noroviruses. They developed an OLG-based hand antiseptic and tested its efficacy against human norovirus. They investigated five different materials based on a variety of different OLG, ethanol (alcohol), and pH formulations, including several hand rubs and a liquid that could be used to clean skin before surgery.

Prof Sano also explained that there is not a simple culture system that can be used for norovirus, making it difficult to evaluate suitable disinfectants. The researchers developed a system that did not rely on an ability to culture the virus, allowing them to identify a disinfectant that is effective against human norovirus. Instead of growing the virus in a container in the laboratory and adding the disinfectant to this, the researchers used a culture-independent method.

CULTURE-INDEPENDENT METHOD FOR EVALUATING DISINFECTANTS

This culture-independent technique involved a process using RNase A and photo-reactive DNA-binding dye before quantitative reverse transcription polymerase chain reaction (RT-qPCR). This acts as a measure of

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Human noroviruses are a type of non-enveloped virus. They are highly contagious and can cause gastroenteritis. Kateryna Kon/Shutterstock.com

viability; in other words, the amount of undamaged, and likely infectious, virus in the sample. In order to correlate the results of the RT-qPCR assay with viral infectivity levels, the researchers used a mouse norovirus which could be cultivated to create a standard curve

that linked the RT-qPCR results with the level of virus in the sample.

The researchers tested the formulations against 11 different strains of human norovirus using culture-independent methods and found that the OLG hand rub had the strongest virucidal efficacy out of the five different formulations, whether a 30 second or a 60 second contact time was considered.

Interestingly, the virucidal effects of the antiseptic varied according to the strain of norovirus. Prof Sano reports that more work still needs to be done to fully understand the mechanisms of antiseptic resistance in noroviruses. Despite this, an OLG-based hand rub has potential for use as a novel antiseptic against human norovirus infection.

OLG TARGETS A WIDE RANGE OF VIRUSES

Viruses can be split into two main categories. Enveloped viruses are contained within a lipid

membrane derived from the host cell, whereas non-enveloped viruses lack this membrane. The presence or absence of an enveloping membrane influences how the virus can enter host cells and can also affect how well a disinfectant will work. Generally, enveloped viruses are more susceptible to substances like detergents and disinfectants, as they are very vulnerable if their lipid envelope is damaged. On the other hand, non-enveloped viruses lack protection from a membrane. Instead, they have a robust outer shell protein which may make them less susceptible to other stressors, such as heat and chemicals.

OLG is already used in Japan to disinfect the skin of patients before operations. The researchers' culture-independent method showed that OLG hand rub is effective against human noroviruses, one type of non-enveloped virus. However, the disinfecting abilities of the chemical against many viruses have not yet been fully explored. To evaluate the effectiveness of OLG as an environmental disinfectant, the researchers have constructed a testing system that replicates real-life scenarios, such as

OLG is a promising antiviral disinfectant candidate, because it is effective on both skin and surfaces.

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<https://en.wikipedia.org/wiki/Disinfectant>

wiping surfaces, and used it to evaluate effectiveness against enveloped viruses.

In this study, the research group tested how well OLG worked as an environmental disinfectant on a non-porous surface, as well as its effectiveness against different strains of enveloped viruses. To begin with, they tested how well the disinfectant performed under laboratory conditions, where it was added into a liquid containing enveloped viruses.

OLG was tested against five different enveloped viruses grown in cells in the laboratory: human coronavirus, feline coronavirus, influenza virus, herpes virus and respiratory syncytial virus. A small amount of the virus suspension was mixed with either OLG or a control substance with no viricidal properties, and incubated at room temperature. After this, a variety of techniques were used to determine how many of the virus particles had survived this incubation phase.

The results showed that OLG was indeed effective against the variety of enveloped viruses that it was tested against.

The researchers then tested OLG on a stainless-steel surface and evaluated

the effectiveness of OLG against feline coronavirus at 3 time points (1, 5 and 10 minutes) after application and 3 time points after the surface had been wiped. They also looked at how long the antimicrobial abilities of OLG lasts by allowing the disinfectant to dry out on a surface before coming into contact with the virus.

Prof Sano explains that using a variety of different exposure methods is likely to be a more accurate representation of how

that the virucidal activity of OLG increases gradually over time once it has been applied to a surface.

CONCLUSION

Through various test systems such as non-culture evaluation systems and stainless-steel surface tests, Prof Sano has also shown that the antimicrobial, antiseptic molecule, OLG, is effective against enveloped and non-enveloped viruses, such as human noroviruses. Research has demonstrated that OLG is a

Through novel evaluation systems, Prof Sano has shown that the antiseptic molecule, OLG, is effective against enveloped and non-enveloped viruses.

well OLG works, as it has greater similarity with how the disinfectant would be used in a real-life scenario.

The results showed that the anti-viral ability of the disinfectant was greater than using alcohol alone and it was also shown that OLG continued working, even once the surface onto which it had been applied had dried out. Indeed, Prof Sano and colleagues concluded

promising antiviral disinfectant candidate, as it is effective on both skin and surfaces. In addition, OLG appears to destroy a wide range of enveloped viruses.

Prof Sano emphasises that development of novel systems to test the effectiveness of disinfectants will contribute to the fight against infectious diseases, something that is particularly poignant during the global COVID-19 pandemic.



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Research Objectives

Professor Daisuke Sano uses culture-independent methods to assess the efficacy of olanexidine gluconate as an individual disinfectant.

Detail

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Dr Daisuke Sano, Professor of Environmental Engineering at Tohoku University, Japan, is managing a team undertaking water

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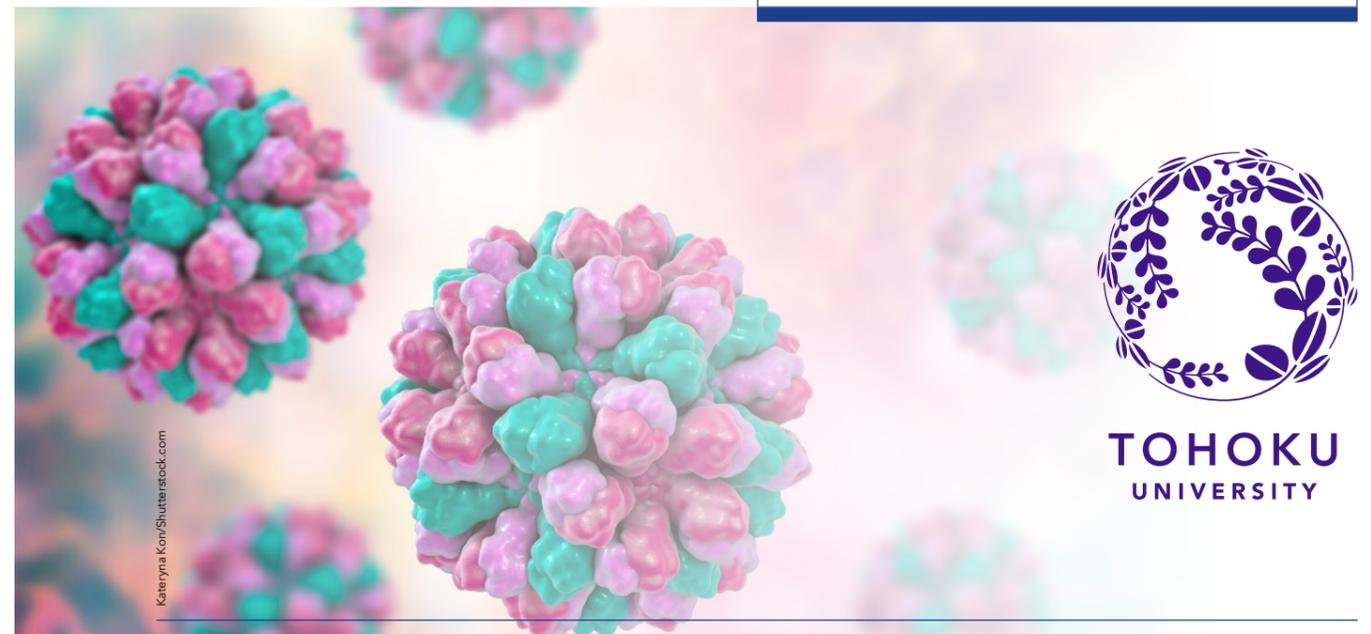
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Personal Response

What is the future research direction?

One of the important features of viruses, irrespective of enveloped and non-enveloped, is the high mutation rate, which contributes to the emergence of disinfectant-resistant mutants. Environmental viral strains are commonly more resistant to disinfectants than laboratory strains, probably because only persistent viral strains can survive under variety of inactivation pressure, including disinfection practices. It is very important to reveal the resistance mechanisms, which must lead to the development of novel disinfectants that can prevent the generation of resistant viral strains.



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