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HKU scientists reveal silver-based antimicrobials

Can be utilised as antibiotic adjuvants to combat antibiotic resistant *Staphylococcus aureus*

A research team led by Professor Hongzhe SUN, Norman & Cecilia Yip Professor in Bioinorganic Chemistry and Chair Professor from Research Divison for Chemistry and Department of Chemistry, Faculty of Science, in collaboration with Dr Richard Yi-Ysun KAO, Associate Professor from the Department of Microbiology, Li Ka Shing Faculty of Medicine, and Dr Aixin YAN, Associate Professor from School of Biological Sciences, The University of Hong Kong (HKU), discovers that silver (Ag)-based antimicrobials can effectively combat antibiotic resistant *Staphylococcus aureus* by targeting multiple biological pathways via functional disruption of key proteins and can be further exploited to enhance the efficacy of conventional antibiotics as well as to resensitize methicillin-resistant *Staphylococcus aureus* (MRSA) to antibiotics.

The study resolves the long-standing question of the molecular targets of silver in *Staphylococcus aureus* and offers insights into the sustainable bacterial susceptibility of silver, providing a new approach for combating antimicrobial resistance. The ground-breaking findings are now published in a leading multidisciplinary science journal, *Nature Communications*.

Background

Antibiotics are medicines designed to kill bacteria and treat bacterial infections. Antibiotic resistance occurs when bacteria adjust in response to the misuse or overuse of these medicines, and it has become one of the biggest public health challenges in this era. At least 2.8 million people get an antibiotic-resistant infection annually in the US, and more than 35,000 people die from it.

Staphylococcus aureus, a round-shaped Gram-positive bacterium, is a dangerous and versatile pathogen for humans and is estimated that approximately 30% of the human population are asymptomatic nasal and long-term carriers. *Staphylococcus* is the causative agent of a variety of diseases, such as skin infection, food poisoning, bone/joint infection, and bacteremia, ranging from subacute superficial skin infection to life-threatening septicemia. The rise in incidence has been accompanied by an increase in antibiotic-resistant strains, especially MRSA. Moreover, the outbreak of the Coronavirus Disease 2019 (COVID-19) pandemic may further increase antimicrobial resistance due to the heavy use of antibiotics to treat patients infected with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-COV-2). Given the rapid emergence of drug-resistant *Staphylococcus aureus* but a lack of antibiotic-development pipeline, alternative strategies are urgently needed to combat antibiotic-resistant *Staphylococcus aureus*.

Key findings

Metal ions have been historically used as antimicrobial agents owing to their inherent broad-spectrum antimicrobial properties and less chance of resistance. There is a growing interest in revitalizing metal-based compounds as promising alternatives to tackle the antimicrobial resistance crisis. Silver ions (Ag^+) and silver nanoparticles (AgNPs) have been used as antimicrobial agents for centuries and are still being widely used in the healthcare and food industry. Previously, the team has built a technical platform named LC-GE-ICP-MS to systematically identify Ag^+-proteome in *Escherichia coli* and developed a strategy named metabolome reprogramming to enhance the efficacy of antibacterial metallodrugs (*PLoS Biol.*, **2019**, *17*, e3000292; *Chem. Sci.*, **2019**, *10*, 7193-7199; *Chem. Sci.*, **2020**, *11*, 11714-11719).

In this study, using the customized approach of LC-GE-ICP-MS, the team successfully separated and identified 38 authentic Ag^+-binding proteins (Ag^+-proteome) in *Staphylococcus aureus* at the whole-cell scale. In combination with bioinformatics analysis and systematic biochemical characterization, they demonstrate that Ag^+ exploits a shotgun action through targeting multiple proteins, thus interfering with multiple pathways, including glycolysis, oxidative pentose phosphate pathway (oxPPP), and reactive oxygen species (ROS) stress defence system, to exert its bactericidal effect against *Staphylococcus aureus*. Further studies unveiled that oxPPP served as a vital pathway targeted by Ag^+ in *Staphylococcus aureus*, with 6PGDH identified as the key enzyme involved in the inhibitory effects of Ag^+ against *Staphylococcus aureus*. They resolved the first crystal structures of 6PGDH from *Staphylococcus aureus* both in substrate-bound and Ag-bound forms and revealed that Ag^+ abolished the enzymatic activity of 6PGDH through targeting Histidine 185 in the active site and morphing its catalytic pocket. This study resolves the long-standing question on the molecular targets and mode of action of silver against *Staphylococcus aureus*. Such a unique mode of action of silver via targeting multiple pathways confers the inability to select silver-resistant *Staphylococcu aureus* and endows it with the sustainable efficacy against *Staphylococcus aureus*.

Based on the uncovered molecular mechanism, they further demonstrate that Ag^+/AgNP can potentiate the efficacy of a broad range of antibiotics, resensitize MRSA to antibiotics, and slow down the evolution of antibiotic resistance in *Staphylococcus aureus*. Therefore, a combination of antibiotics with silver or other metal-based compounds or nanomaterials could serve as a promising strategy to suppress the selection effects of antibiotics, thus preventing the occurrence of primary antibiotic resistance and extending the lifespan of conventional antibiotics to relieve the current crisis of antibiotic resistance.

About the research team

The research was conducted by a team led by Professor Hongzhe SUN, Norman & Cecilia Yip Professor in Bioinorganic Chemistry and Chair Professor of Department of Chemistry. Dr Haibo WANG is the first author. Other scientists contributing to the research include Dr Richard Yi-Ysun KAO, Associate Professor (Microbiology), Dr Aixin YAN, Associate Professor (Biological Sciences), Dr Hongyan LI, Senior Research Assistant (Chemistry), Dr Minji WANG, Associate Professor (Chemistry, East China Normal University), Dr Xiaohan XU, PhD student (Chemistry), Dr Peng GAO, Research Assistant Professor (Microbiology), Dr Zeling XU, Associate Professor (South China Agricultural University), and Dr Qi ZHANG (Chemistry).

The work was supported by the Research Grants Council of Hong Kong, Health and Medical Research Fund and Seed Fund for Basic Research.

About Professor Hongzhe SUN

Professor Hongzhe SUN is the Norman & Cecilia Yip Professor in Bioinorganic Chemistry and Chair Professor from Research Division for Chemistry and Department of Chemistry at HKU. His research focuses on metalloproteins, the discovery of antimicrobial agents, and inorganic chemical biology. Professor Sun has published a series of well-recognized work in overcoming antimicrobial resistance against superbugs, developing anti-coronavirus agent, and understanding the functions of metalloproteins.

More information about Professor Hongzhe SUN and his research group can be found from their group's webpage: http://www.bioinorg-chem.hku.hk

About the research paper: https://www.nature.com/articles/s41467-021-23659-y

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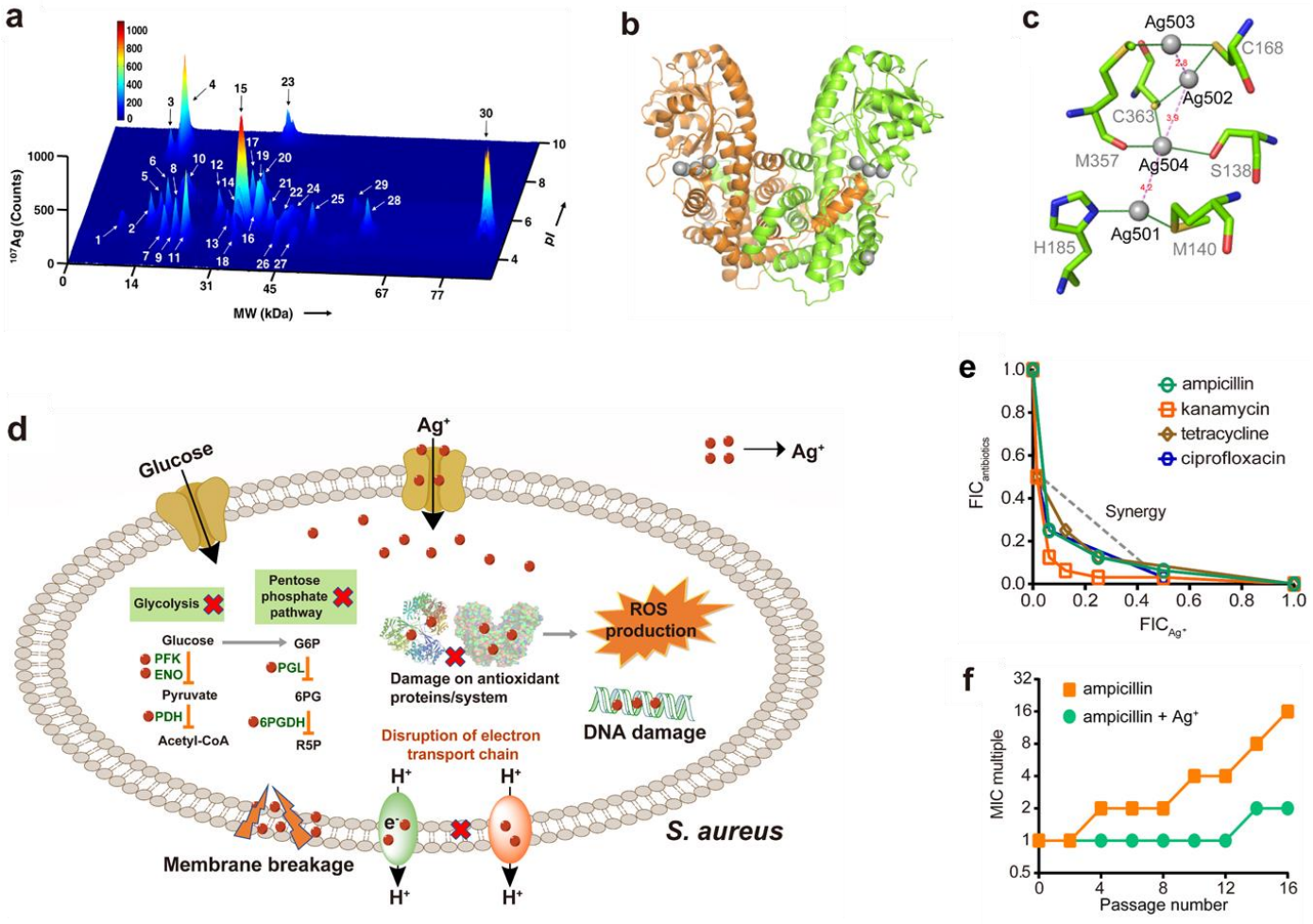

Image 1. (**a**) Exploration of Ag^+-binding proteins in the soluble fraction of *S. aureus* with LC-GE-ICP-MS. (**b**) The overall structure of Ag-bound 6PGDH. (**c**) Silver coordination sites in 6PGDH and silver cluster composed of four adjacent Ag ions. (**d**) Diagram showing that Ag^+ kills *S. aureus* by targeting multiple essential pathways. (**e**) Isobolograms of the combination of conventional antibiotics and Ag^+ against *S. aureus Newman*. (**f**) Resistance acquisition curves during serial passage with the subinhibitory concentration of ampicillin or combination of ampicillin and Ag^+ against *S. aureus Newman*.