Department of Microbiology
Li Ka Shing Faculty of Medicine, HKU

HKU Discovers Novel Non-antibiotic Drugs to Treat Methicillin Resistant Staphylococcus Aureus (MRSA) Infections

Press Conference
September 7, 2017

Speakers

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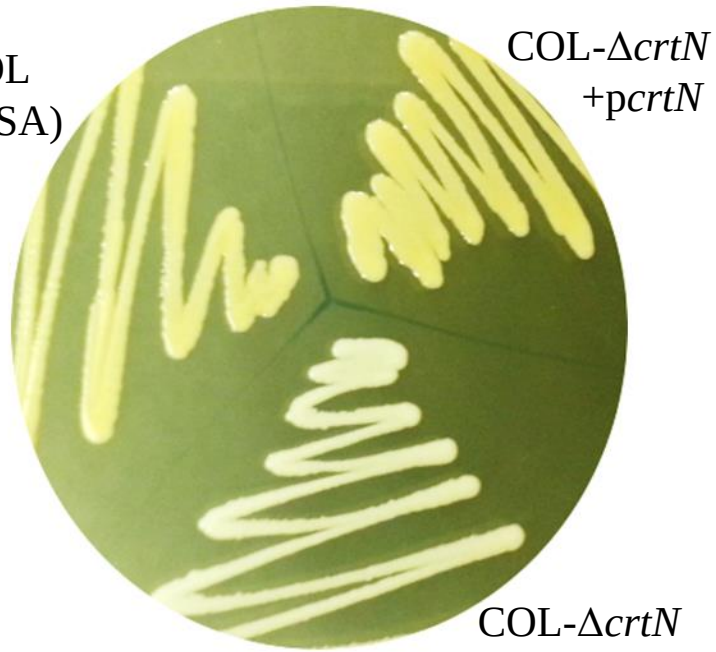
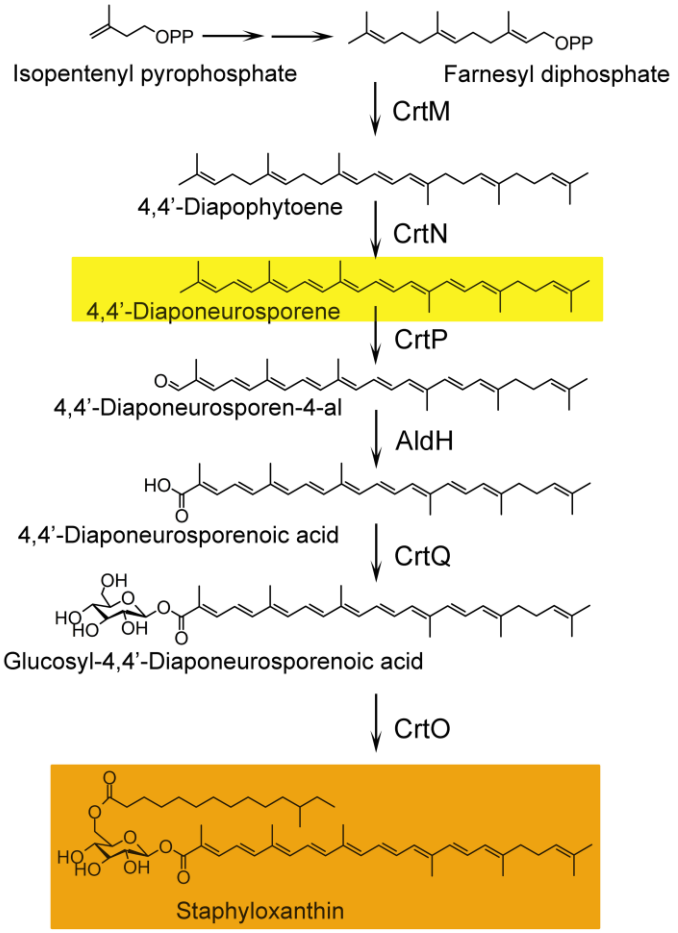
Background

- The indiscriminately use of antibiotics has led to the rapid emergence of multidrug resistant (MDR) bacteria including methicillin resistant *Staphylococcus aureus* (MRSA).
- Treatment by killing bacteria using antibiotics seems not to be an effective and sustainable way of controlling infections.
- Alternative strategies for treating bacterial infections without incubating the emergence of drug resistant bacteria are highly valued.

Background

The golden-coloured pigment of *S. aureus*, staphyloxanthin, contributes to the resistance to reactive oxygen species (ROS) and host neutrophil-based killing.

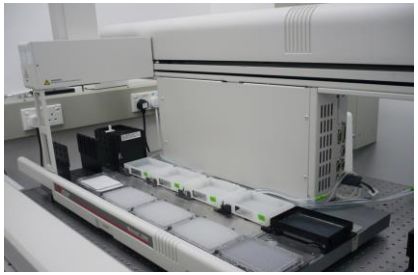




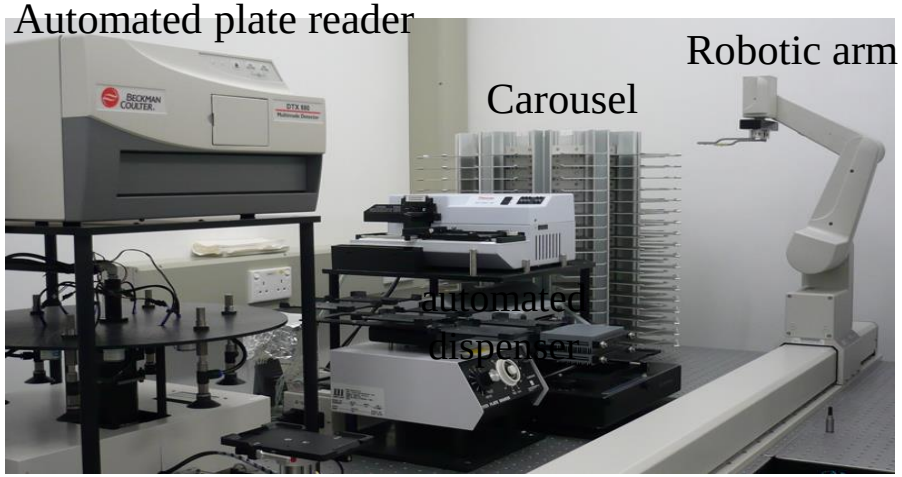
Biosynthesis pathway of staphyloxanthin.

Automated High-throughput Screening platform from Chemical Genetics Unit, Research Center of Infection and Immunity, LKS Faculty of Medicine, HKU

- Fully automated robotics screening platform
- Optimized for cell-based screening assays
- The only 384-well formatted chemical genetics screening facility in Hong Kong



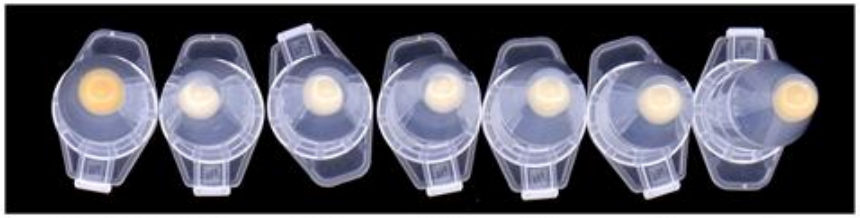
Liquid handling system



Incubator with carousel inside

Research Findings

A



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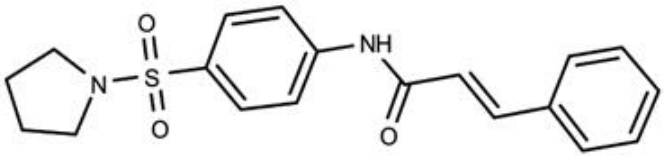

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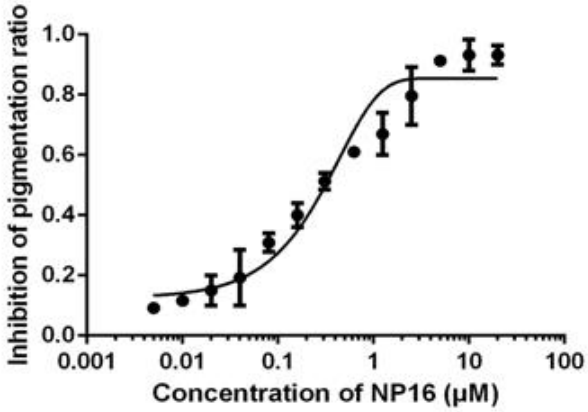

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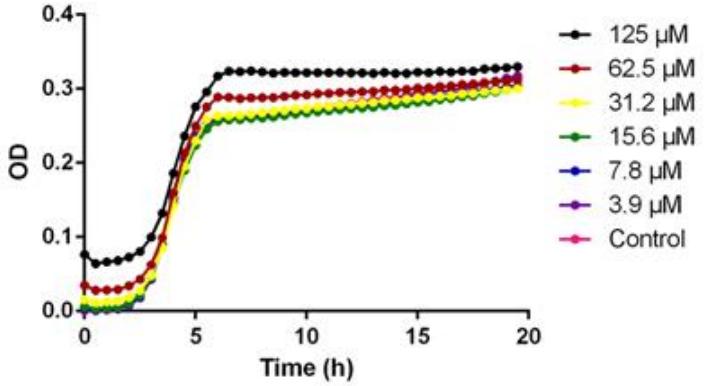


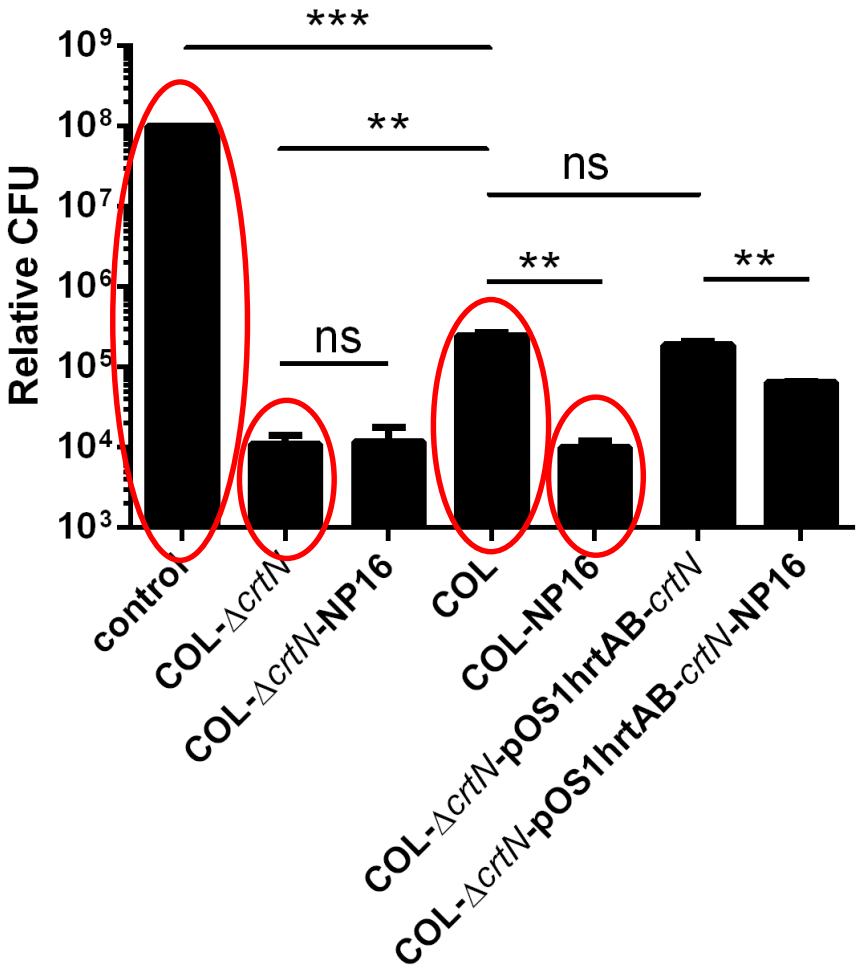
A. Inhibition of *S. aureus* pigmentation using increasing concentrations of NP16.

B. Pigment inhibition by NP16; the IC_{50} for pigment formation is ~300 nM.

C. The chemical structure of compound NP16.

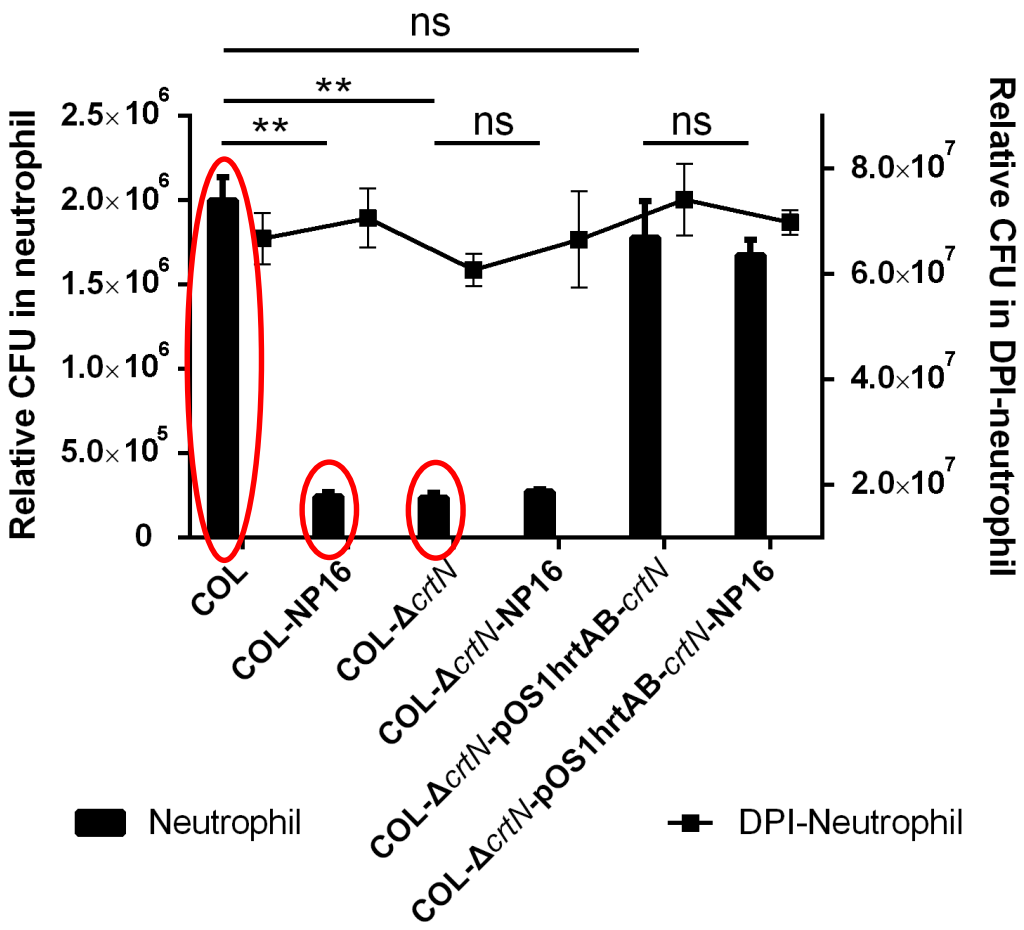
D. Growth curve of *S. aureus* COL in the presence of different concentrations of NP16.

Research Findings



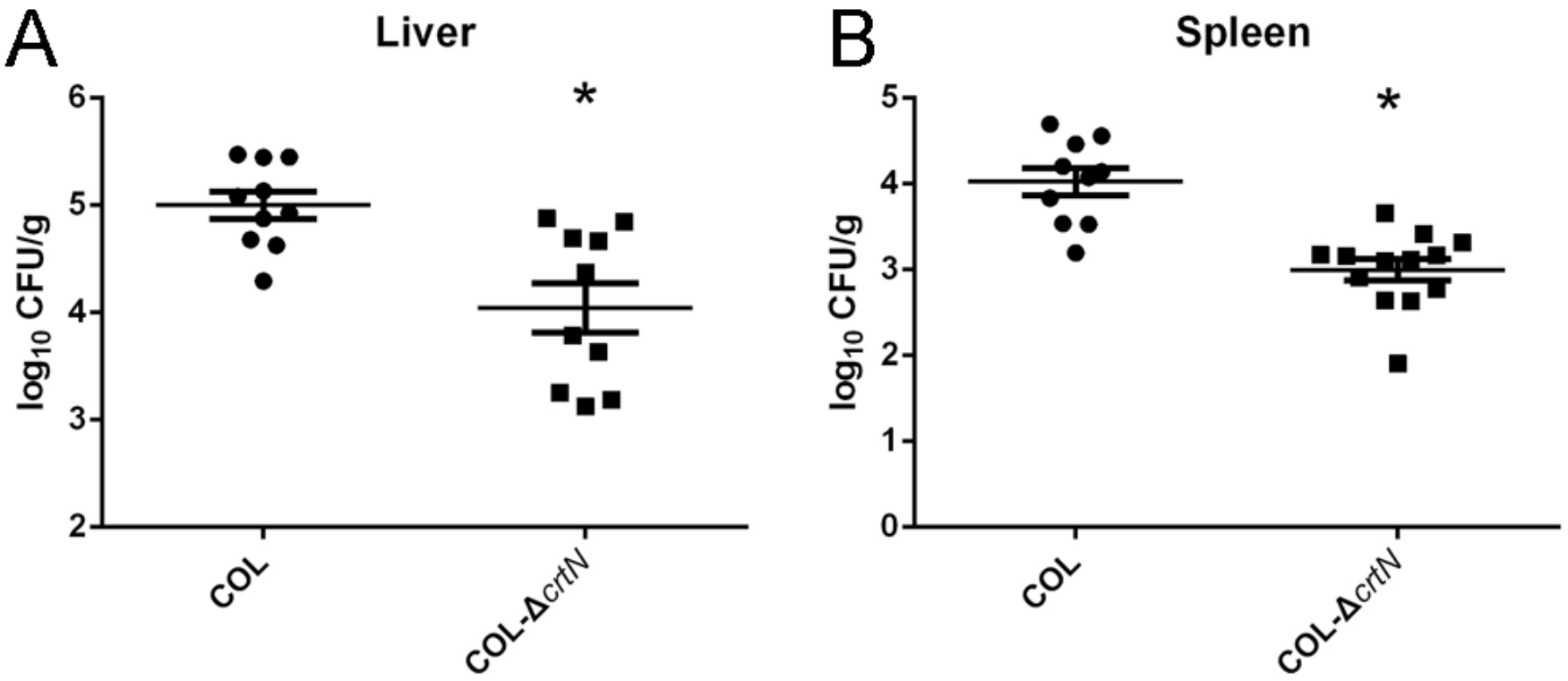
Increased susceptibility of the NP16-treated *S. aureus* COL strain to killing by hydrogen peroxide.

Research Findings



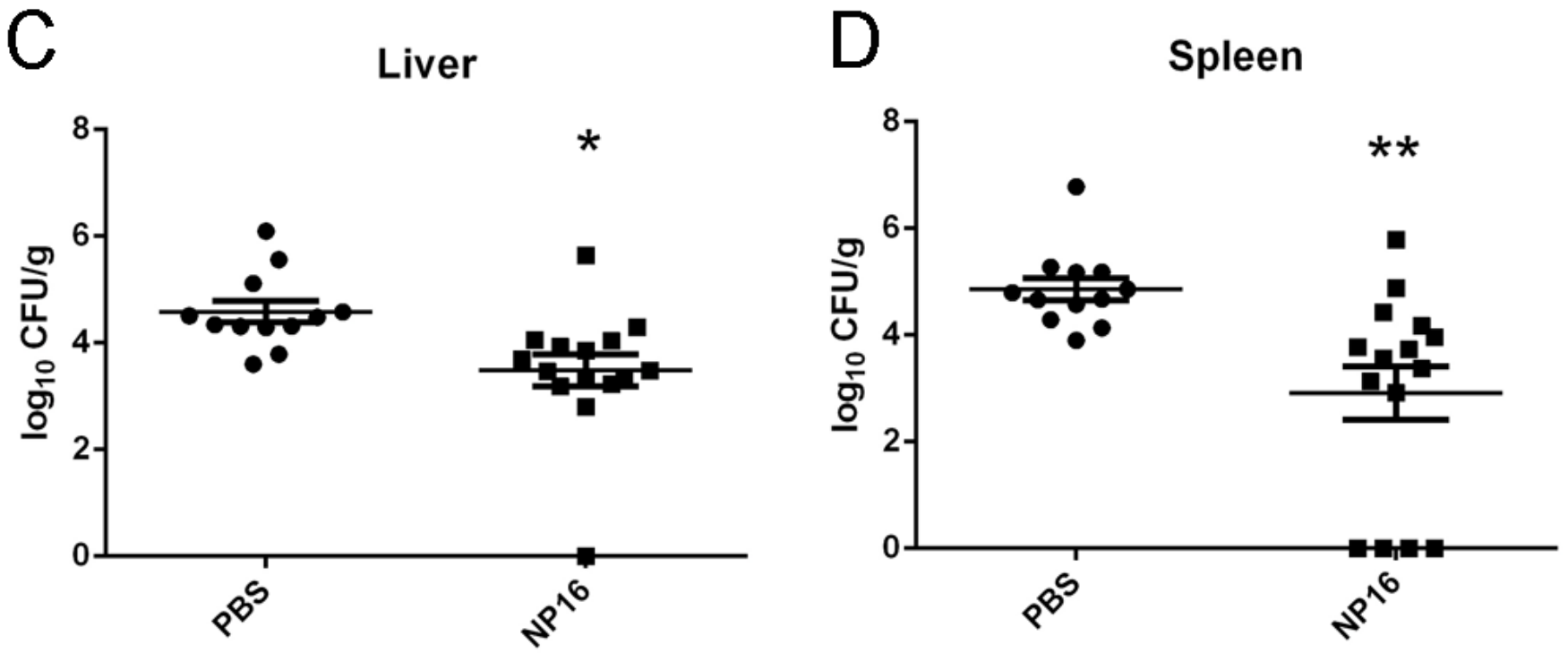
Increased susceptibility of the NP16-treated *S. aureus* COL to killing by neutrophils.

Research Findings



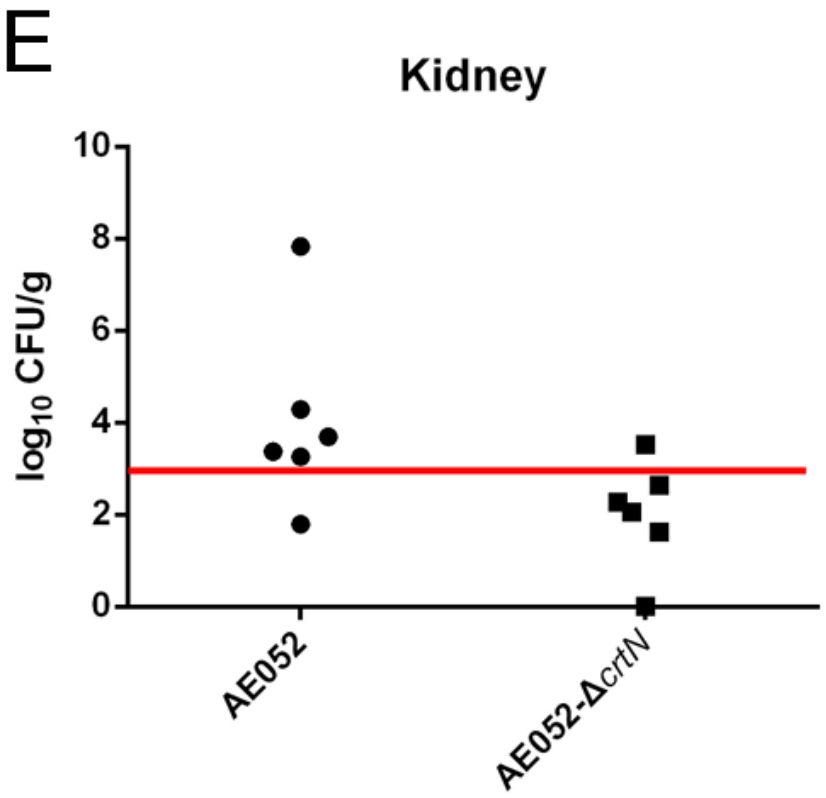
Bacteria recovered from the spleens and livers of mice infected with the wild-type COL or COL-Δ*crtN* strains.

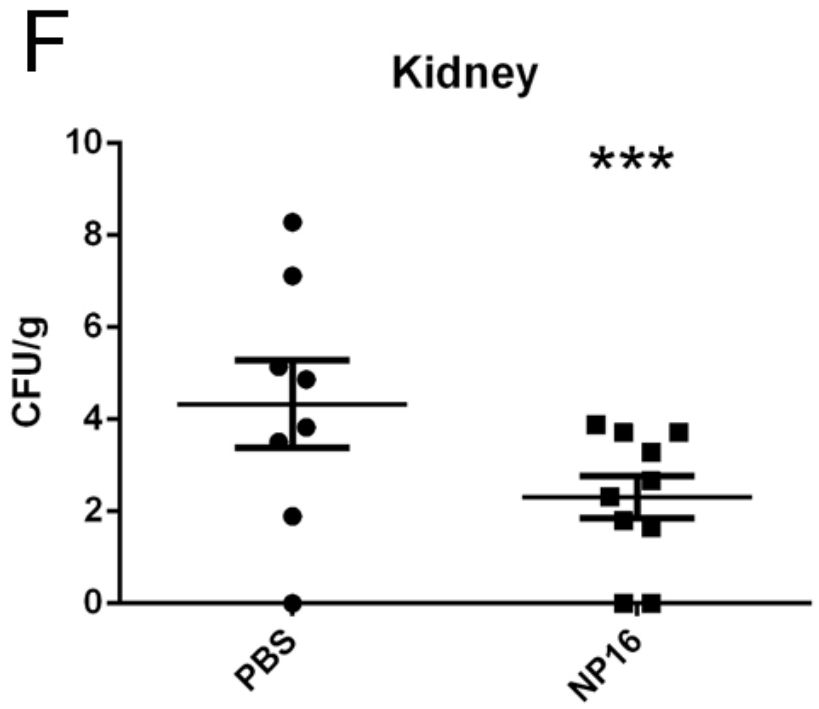
Research Findings



Bacteria recovered from the spleens and livers of mice infected with the COL strain, with or without compound NP16 treatment.

Research Findings





E. Bacteria recovered from the kidneys of mice infected with clinical isolate AE052 or AE052-Δ*crtN*.

F. Bacteria recovered from the kidneys of mice infected with clinical isolate AE052, with or without compound NP16 treatment.

Conclusion

- CrtN is a novel drug target for the virulence factor-based therapy against *S. aureus*.

- Compound NP16, as a potent CrtN inhibitor without direct bactericidal properties, renders the pathogen susceptible to normal host innate immune clearance on one hand, reduces the emergence of drug resistance on the other.

- Our approach of employing non-antibiotic drugs to treat MRSA infection by disarming the defensive shield of invading pathogens has offered new hope and new strategies for the treatment of bacterial infections related to multidrug resistant pathogens.

Conclusion

- This is the world's first study to apply a cutting-edge technology – chemical genetics to tackle MRSA infection.

- This groundbreaking concept and discovery has been highly praised in the 4th International Conference on Prevention & Infection Control (ICPIC 2017) in Geneva, Switzerland at which the Hong Kong team for the first time has won the 1st Prize of the Innovation Academy Award. The findings were recently published in ***mBio,*** the top international scientific journal in the field of Microbiology in September, 2017.

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RESEARCH ARTICLE

Dehydrosqualene Desaturase as a Novel Target for Anti-Virulence Therapy against *Staphylococcus aureus*

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ABSTRACT *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), is a life-threatening pathogen in hospital- and community-acquired infections. The golden-colored carotenoid pigment of *S. aureus*, staphyloxanthin, contributes to the resistance to reactive oxygen species (ROS) and host neutrophil-based killing. Here, we describe a novel inhibitor (NP16) of *S. aureus* pigment production that reduces the survival of *S. aureus* under oxidative stress conditions. Carotenoid components analysis, enzyme inhibition, and *crtN* mutational studies indicated that the molecular target of NP16 is dehydrosqualene desaturase (CrtN). *S. aureus* treated with NP16 showed increased susceptibility to human neutrophil killing and to innate immune clearance in a mouse infection model. Our study validates CrtN as a novel druggable target in *S. aureus* and presents a potent and effective lead compound for the development of virulence factor-based therapy against *S. aureus*.

IMPORTANCE *S. aureus* staphyloxanthin contributes substantially to pathogenesis by interfering with host immune clearance mechanisms, but it has little impact on *ex vivo* survival of the bacterium. Agents blocking staphyloxanthin production may discourage the establishment and maintenance of bacterial infection without exerting selective pressure for antimicrobial resistance. Our newly discovered CrtN inhibitor, NP16, may offer an effective strategy for combating *S. aureus* infections.

KEYWORDS MRSA, anti-virulence, bacterial infection, staphyloxanthin

Received 13 July 2017 **Accepted** 7 August 2017 **Published** 5 September 2017

Citation Gao P, Davies J, Kao RYT. 2017. Dehydrosqualene desaturase as a novel target for anti-virulence therapy against *Staphylococcus aureus*. mBio 8:e01224-17. https://doi.org/10.1128/mBio.01224-17.

Editor Victor J. Torres, New York University School of Medicine

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Staphyloxanthin has proven to be an important factor in promoting bacterial invasion (1). Five genes, *crtOPQMN*, located in an operon are responsible for the biosynthesis of the pigment. The transcription of the operon is driven by a σ^B-dependent promoter upstream of *crtO* and ends with a terminator downstream of *crtN* (2).

The pigments that endow *S. aureus* with a golden color (Fig. 1) also make it resistant to attack from reactive oxygen species (ROS) and neutrophils (3). Pigmented bacteria have increased resistance to the host's immune defenses (4).

In a mouse subcutaneous model of infection, animals infected with a wild-type strain of *S. aureus* had higher bacterial loads and larger visible lesions than those infected with nonpigmented bacteria (4). The reduced virulence of bacterial strains with defective carotenoid synthesis was also shown in a mouse systemic *S. aureus* infection model (3). *In vitro* and *in vivo* data suggest that blocking pigment synthesis may reduce pathogenicity.

Dehydrosqualene synthase (CrtM), which catalyzes the first step of the biosynthetic pathway, was shown to be a target for anti-infective therapy, based on virulence factor neutralization. A drug candidate already tested in humans in the context of cholesterol-lowering therapy provides a good lead, based on its structural similarity to human squalene synthase (SQS) (3). Because of common structural features, agents selective

Acknowledgement

The research project was supported by the Research Fund for the Control of Infectious Diseases (RFCID; HK-09-01-14 and HK-09-01-15) and Health and Medical Research Fund (HMRF; HKM-15-M11) under Food and Health Bureau, and Research Grant Committee (RGC; AoE/P-705/16) of the Hong Kong SAR.

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Q & A