Discovery of Non-antibiotic Lead Compound to Suppress Virulence in Methicillin Resistant *Staphylococcus aureus* (MRSA)

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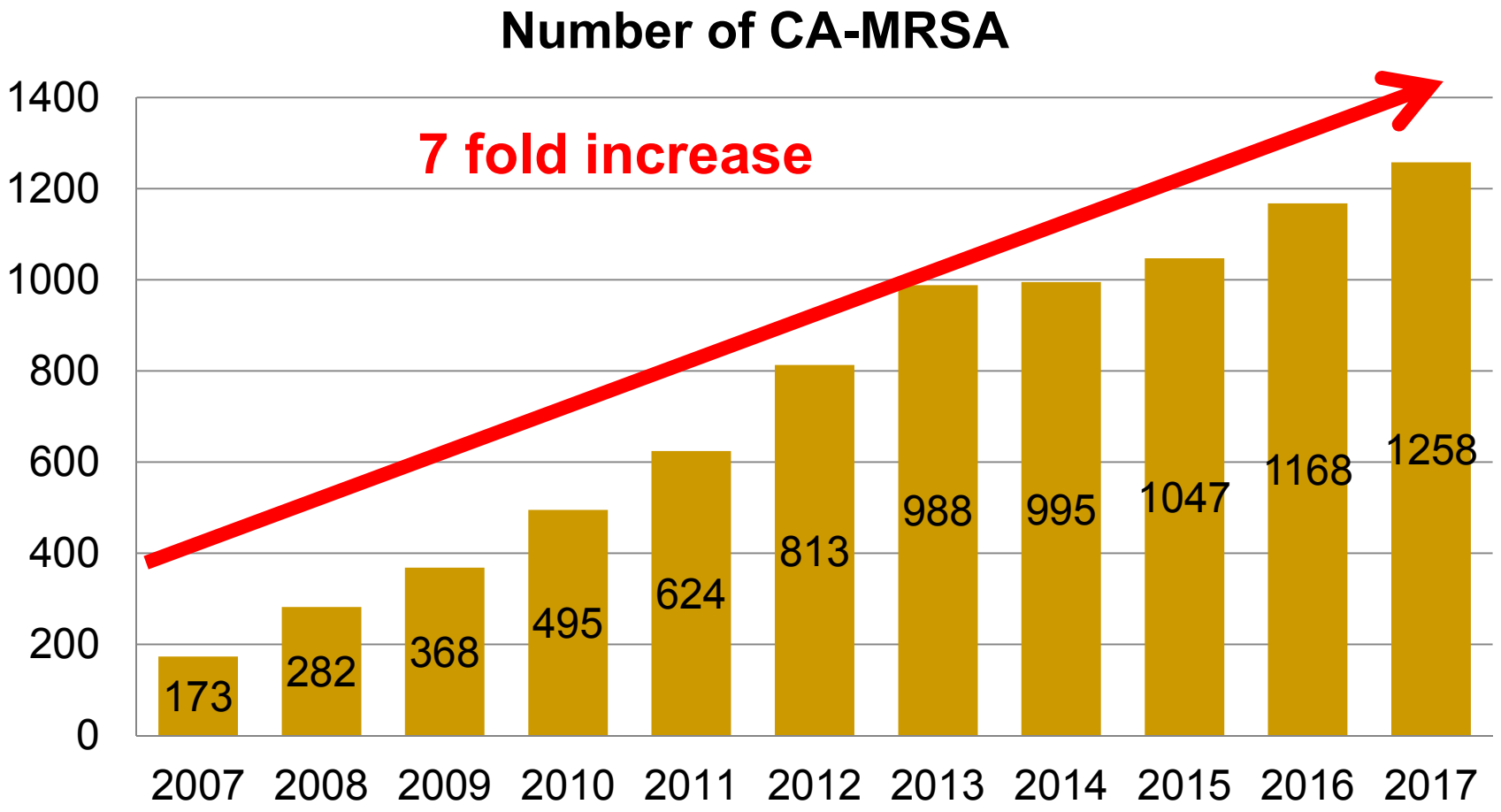
Background

- The emergence of multidrug resistant (MDR) bacteria including methicillin resistant *Staphylococcus aureus* (MRSA) is the inevitable outcome of indiscriminate use of antibiotics by mankind.

- Recently, community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA), known for their ability to cause infections in healthy individuals living in the community due to the intrinsic hyper-virulent properties of CA-MRSA, has elicited much concern in the society.

- As the World Health Organization (WHO) has declared antimicrobial resistance (AMR) one of the biggest threats to global health, alternate therapeutic strategies to fight against MDR bacteria including MRSA and CA-MRSA are urgently needed.

Community-associated MRSA, Hong Kong



Affected persons without healthcare risks; new types of MRSA with unique Genotypic features (e.g. PVL positive)

A notifiable disease in Hong Kong since 2007

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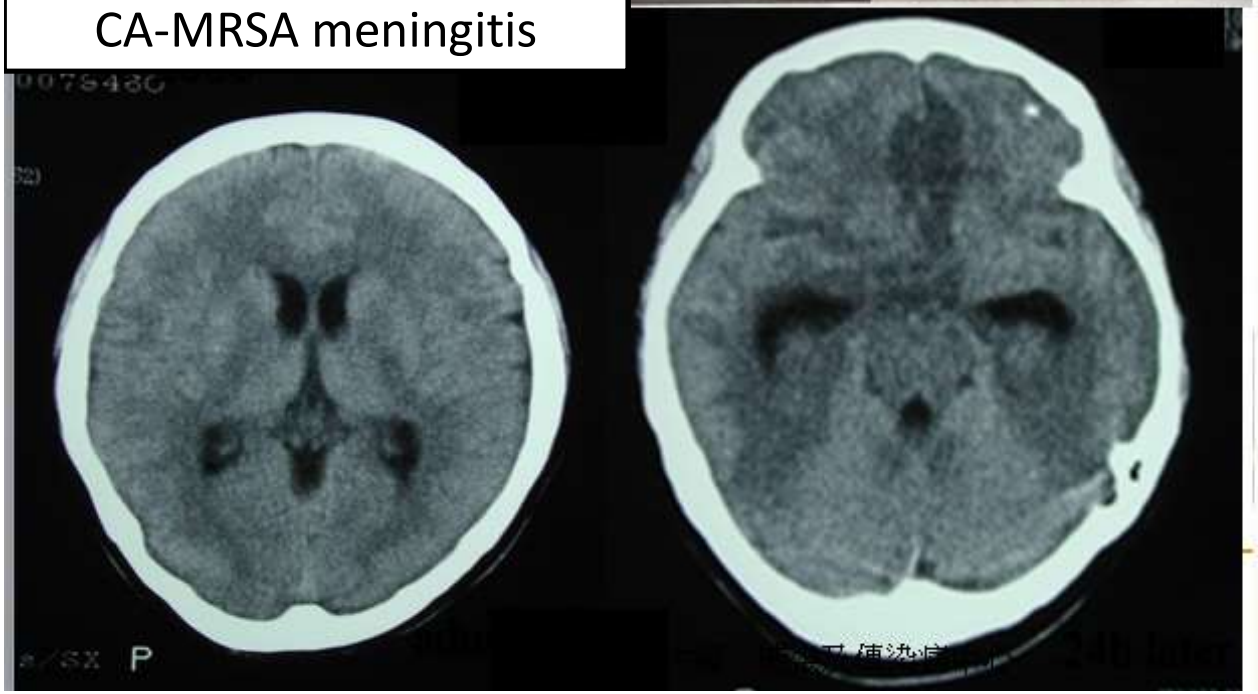
CA-MRSA

CA-MRSA meningitis



惡菌入腦殺37歲女子

抗藥性金黃葡萄球菌 恐在社區生根

【本報訊】一名三十七歲原本健康正常女子上月底因頭痛及頭痛進入廣華醫院治療，證實感染社區性抗藥性金黃葡萄球菌，一般抗生素無力對付，惡菌入侵其腦部造成腦膜炎，患者於上月三十一日死亡，這是本港有報告以來首宗社區感染該菌的死亡個案，專家警告這種社區性惡菌已在港「落地生根」，市民無分老弱均可受感染，呼籲市民注意個人衛生及避免濫用抗生素。

記者：梁淑瑜

衞生防護中心昨日接獲醫院管理局通知，證實一宗涉及一名三十七歲女士的社區性「抗藥性金黃葡萄球菌」死亡個案。病人於上月二十四日，因嚴重腹痛及頭痛入廣華醫院治療，出現腦膜炎症狀，經抗生素、其他藥物及支援性治療後，於十二月三十一日不治。

港首宗感染死亡個案

醫院化驗證實死者染上抗藥性金黃葡萄球菌，細菌具有社區性的抗藥性金黃葡萄球菌基因特徵，患者病發前健康正常，過去一年沒有入過醫院，故患者是在社區感染這種惡菌，初步顯示與她同住的四名家人無出現病徵，該中心正調查其旅遊紀錄及接觸史，這是本港首宗感染該菌的死亡個案。

香港醫學微生物學會副教授何栢良表示，抗藥性金黃葡萄球菌是金黃葡萄球菌的其中一種，對盤尼西林及頭孢子等 beta-lactam 抗生素呈抗藥性，以往常見於醫院內感染，但近年在歐美甚至全球開始在社區出現個案，故被稱為社區性抗藥性金黃葡萄球菌。

但這宗個案患者感染後出現腦膜炎，即惡菌入侵器官，故是本港有報告以來，首宗入侵性個案，也是首宗社區感染該菌的死亡個案，加上患者是一名健康正常的青年人，故他認為此宗個案已敲響警號，顯示社區性抗金黃葡萄球在本港社區落地生根，並有重症個案出現。

惡菌入血令器官衰竭

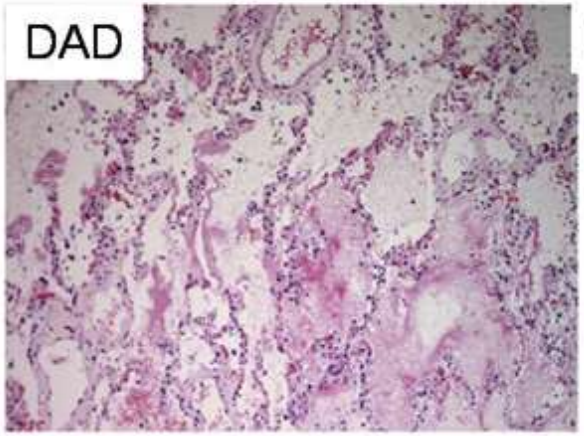
感染這種惡菌後，一般病徵包括皮膚長出膿瘡，或會發燒，但惡菌如入侵血液，即可在極短時間內造成器官衰竭，因為此惡菌其中一個特性，是會釋放很多有毒因子攻擊人體，例如會造成壞死仁肺炎等，入侵性個案死亡率可高達一成，必須以威力較強的高古霉素治療。

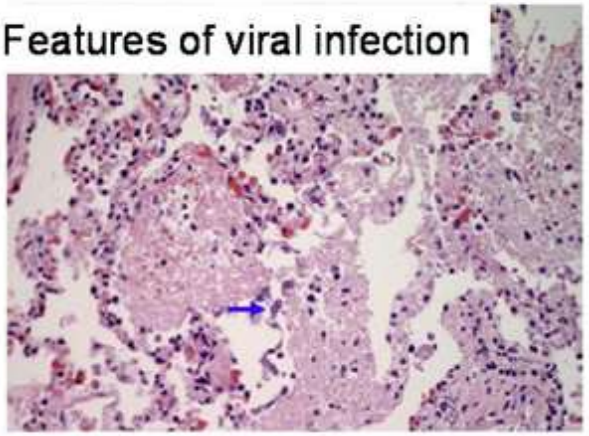
何栢良強調，入侵性個案仍屬罕見，市民不要過於恐慌，由於這種惡菌主要存在於皮膚上及鼻黏內，故市民必須注意個人衛生，每天洗澡及不要與他人共用毛巾及內衣等貼身衣物，並不要濫用抗生素。

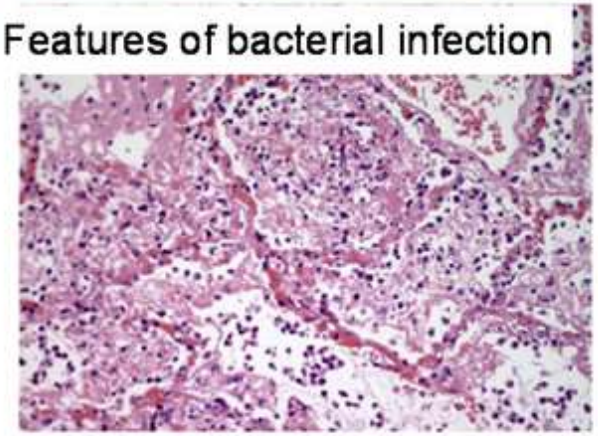
社區感染抗藥性金黃葡萄球菌簡介

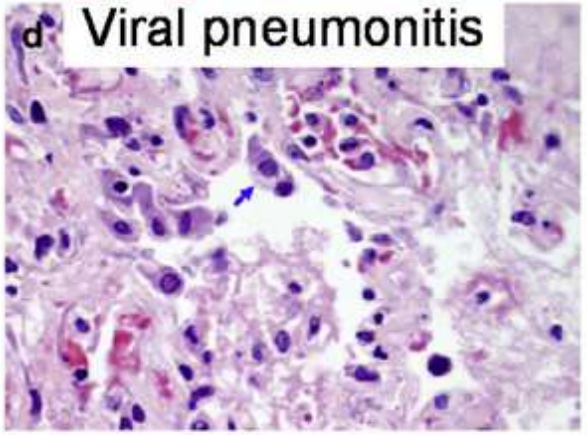
特性	具有對抗盤尼西林及頭孢子類抗生素能力，須改用其他威力更大的抗生素
感染途徑	病菌存在於環境或黏附在鼻黏，皮膚表面上，可經皮膚毛囊或傷口入侵體內
病徵	皮膚表面尤其是在大腿內側、下肢，臀部等位置長出膿瘡，發燒，若病菌入侵血液可致敗血及令器官衰竭
預防方法	注意個人衛生，多洗手，每天一定要洗澡，內衣須每天更換清洗，不與他人共用毛巾及貼身衣物 不要亂用抗生素
資料來源	港大微生物學系副教授何栢良

Fatal co-infection with swine origin influenza virus A/H1N1









Fatal co-infection with swine origin influenza virus A/H1N1 and community-acquired methicillin-resistant *Staphylococcus aureus*

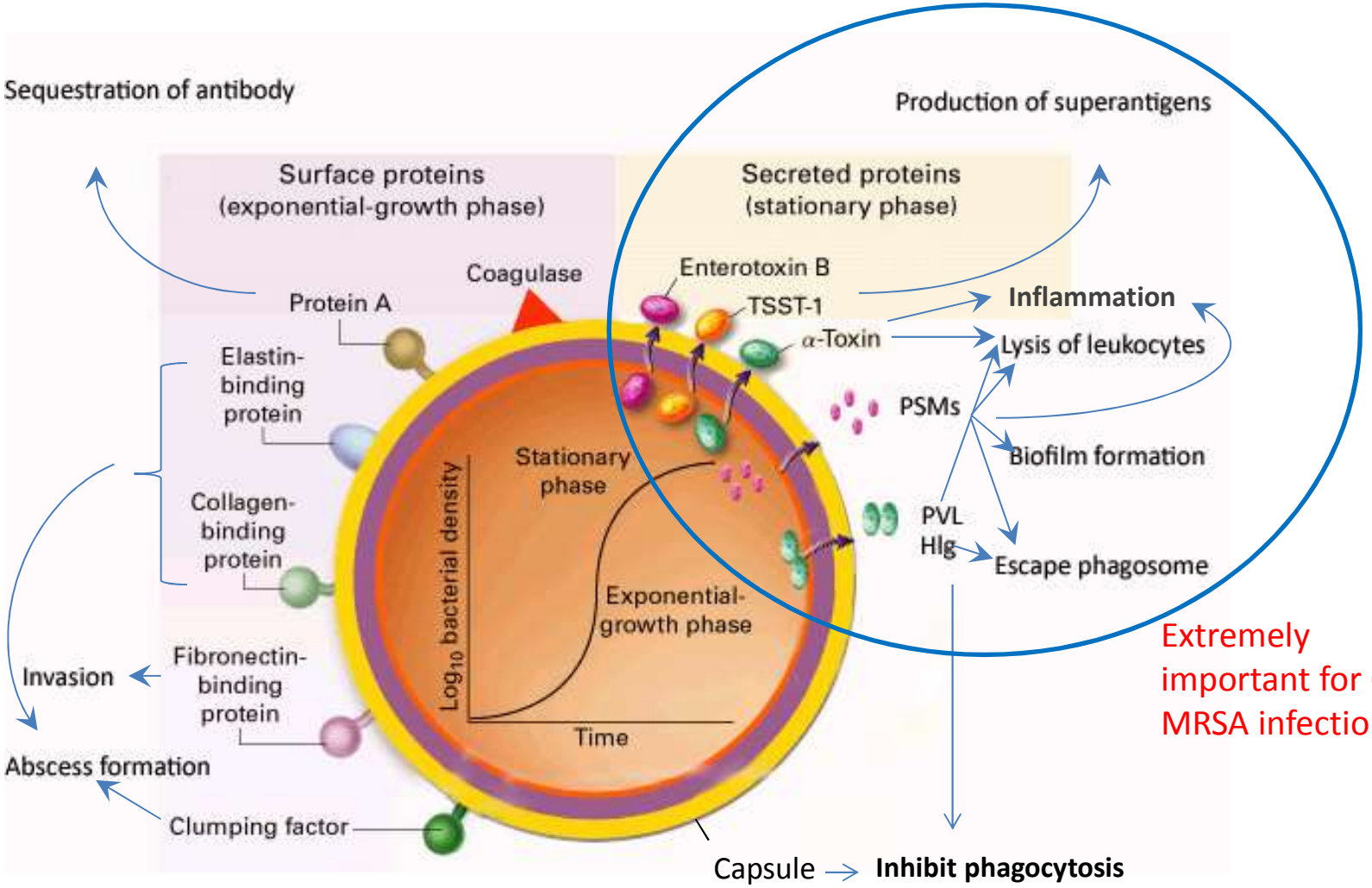
M/42y, ill for 5 days
Died 48h after admission despite aggressive treatment (Tazocin, vancomycin, Klacid)

J of Infect 2009

Important virulence factors of MRSA
耐藥性金黃葡萄球菌重要的毒力因子

Virulence factor 致病因子	Potential effect 致病因子的攻擊範圍
PVL toxin 殺白細胞素	Lysis of white blood cells 溶解白血球
Spa protein 金黃葡萄球菌蛋白	Prevent phagocytosis and contribute to apoptotic death of B cells 阻礙白血球吞噬細菌及引致免疫細胞死亡
Psm cytolytic peptides 溶細胞肽	Lysis of white blood cells 溶解白血球
Hla toxin 金黃葡萄球菌毒素	Damage red blood cell membranes and endothelium of small blood vessels 破壞 紅血球和小血管內壁
agr regulator 調節因子	Regulate virulence factor production in bacteria 調節毒力因子的生產
ClpP protease 蛋白酶	Regulate virulence factor production in bacteria 調節毒力因子的生產

Virulence factors contributing to *Staphylococcus aureus* pathogenesis



Extremely important for CA-MRSA infection

Modified from report Rachel J. Gordon, et al. Clin Infect Dis. (2008)

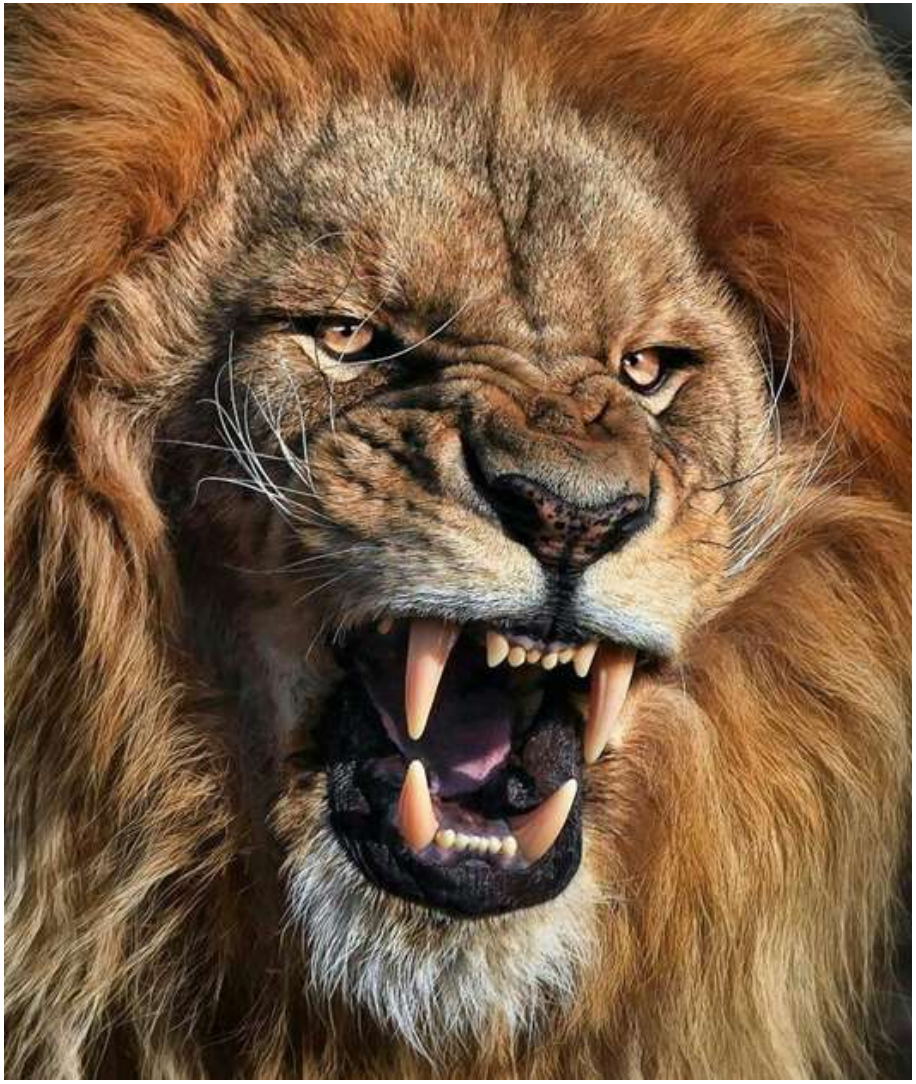
The Greek mythology of Hercules and the Nemean Lion

(Adopted from Wikipedia)

The Nemean lion was a vicious monster in Greek mythology that lived in Nemea. Its claws were sharper than mortals' swords and could cut through any armor and its golden fur was impervious to attack by mortals' weapons.

In the Greek myth, Hercules with his divine power won the fight and killed the Nemean Lion.

The MRSA today is in some way similar to the mythical creature Nemean lion – vicious and difficult to kill. The problem is that MRSA is not a mythical creature but a real pathogen and we are not Hercules but humans.



Virulent state of MRSA

Avirulent state of MRSA

The big question is: Can this beast (MRSA) be tamed?

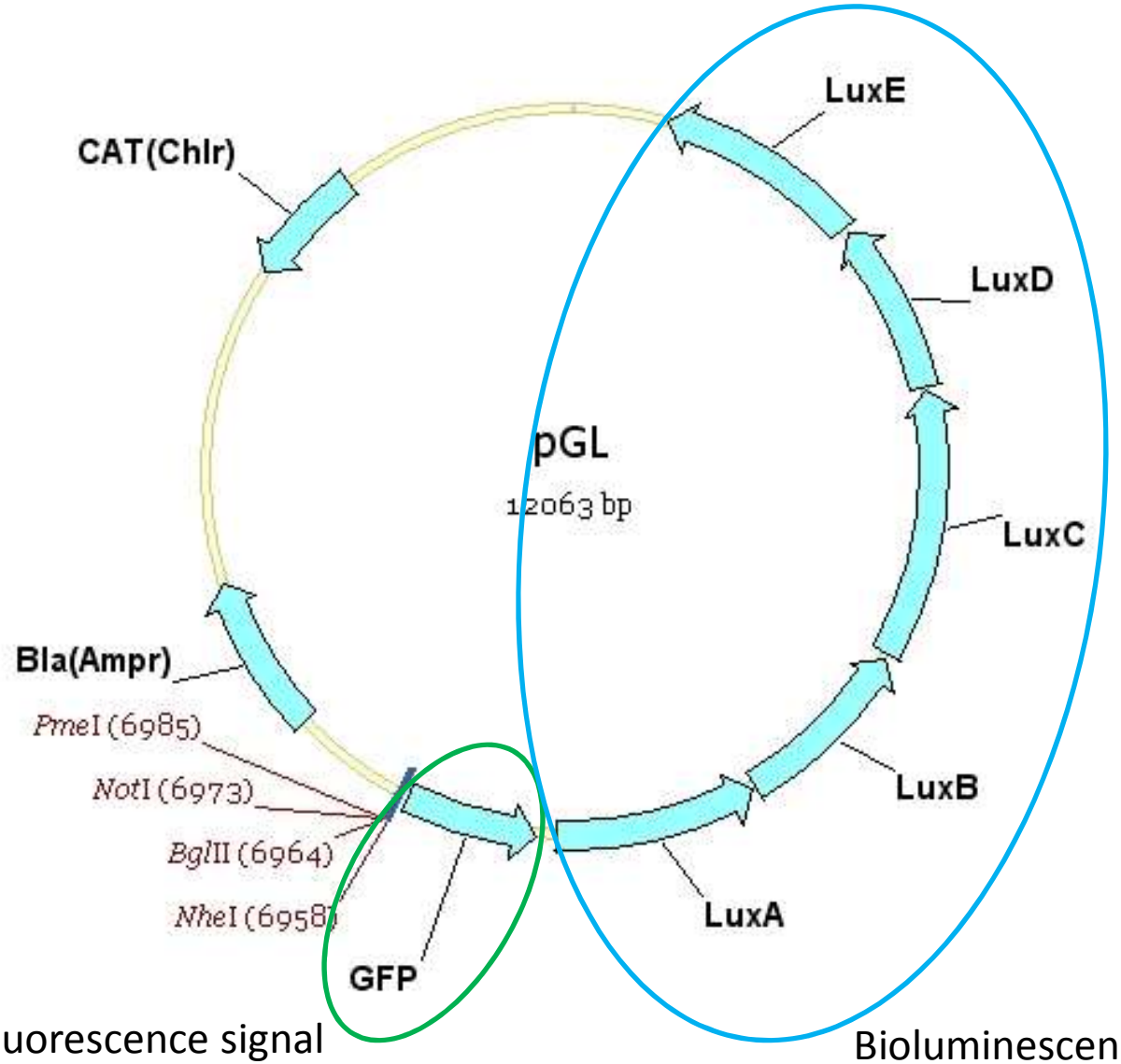
We believe that as it is difficult to abolish MRSA virulence by targeting any single virulence factors or regulators. The simultaneous suppression of multiple virulence genes may offer promising therapeutic potentials.

If a non-antibiotic compound can be identified to suppress all or most of the important virulence factors of MRSA, it will offer promise in the treatment of staphylococcal infections.

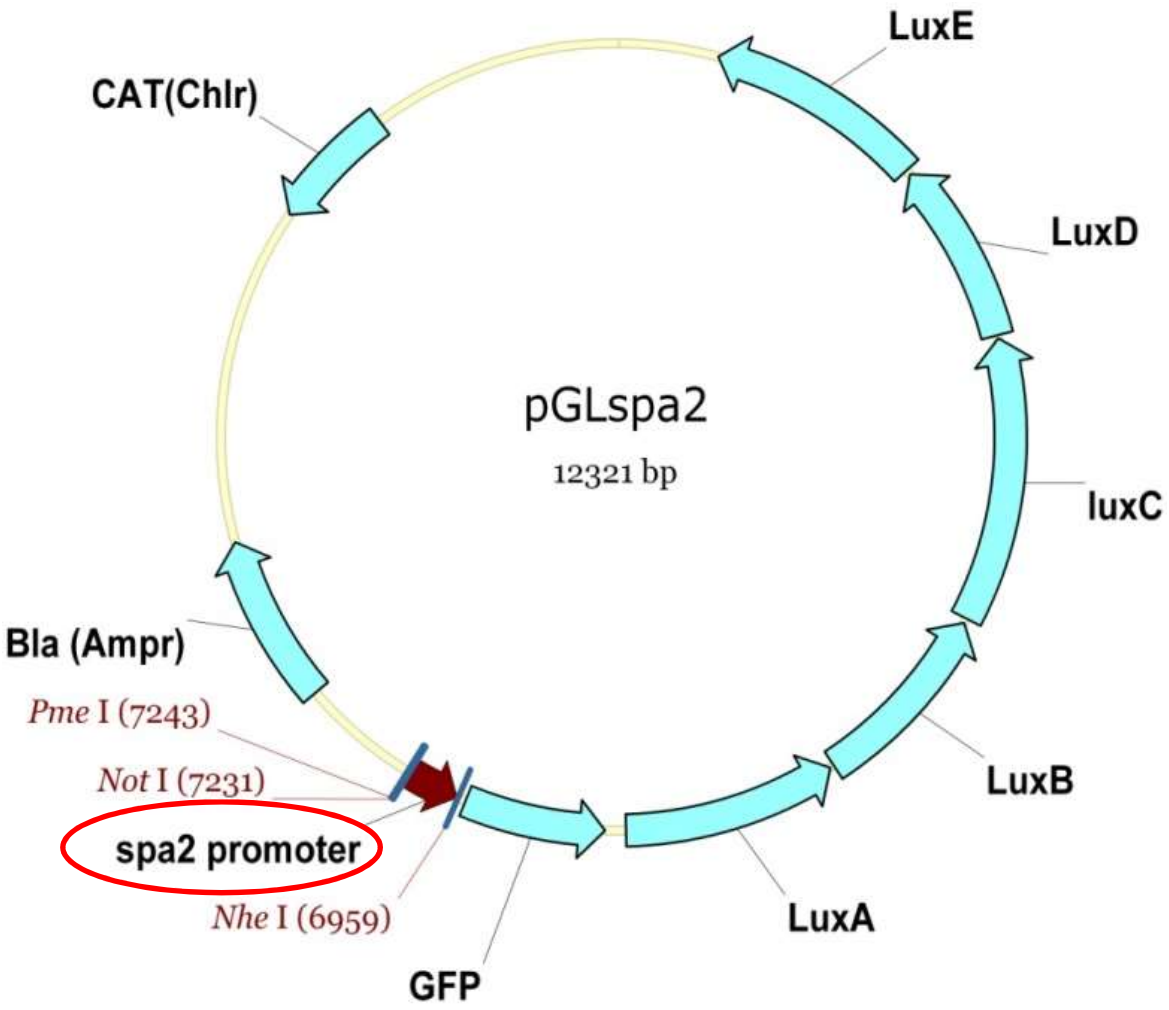
Virulence-associated gene promoters

No.	Gene	Product	Type	Role in virulence
1	*spa*	Protein A	Surface adhesions	Inhibits opsonophagocytosis
2	*map*	methionine aminoprptidase	Post translational modification	
3	*hla*	α-toxin	Exotoxins	Cell lysis
4	*lukF-PV, lukS-PV*	Panton-Valentine leukotoxin	Exotoxins	
5	*Psms* *Psmf*	phenol-soluble modulins	Exotoxins	
6	*Cap5* *Cap8*	capsular polysaccharide	Exopolysaccharidees	Inhibit opsonophagocytosis
7	*agr*	Accessory gene regulator	Regulator	Quorum sensing
8	*RNAIII* */hld*	regulator/ δ-hymosin	Regulator /Exotoxins	Quorum sensing / cell lysis
9	*sarA*	Staphylococcal accessory regulator	Regulator	Regulation agr and extracellar and surface-associated virulence factor
10	*SaeP1* *Sae P3*	S. aureus exoprotein expression	Regulator	Regulation of exotoxins
11	*Ami*	aminopterin resistance operon	Control	
12	*sigBR*	sigB regulator	Regulator	Regulation of exotoxins
13	*sigB*	RNA polymerase sigma B	Regulator	Regulation of exotoxins
14	*EAP*	Extracellular Adhesion Protein	Adhesion	
15	*rot*	repressor of toxin	Regulator	
16	*fnbA*	fibronectin binding protein A	Adhesion	Adhesion: fibrinogen
17	*fnbB*	fibronectin binding protein B	Adhesion	
18	*coa*	coagulase	Enzyme	Adhesion: collagen
19	*sarS*	Staphylococcal accessory regulator	Regulator	regulate spa
20	*clfA*	clumping factors A	Clumping factors A	Adhesion: fibrinogen, Nasal colonization, Evasion of phagocytosis

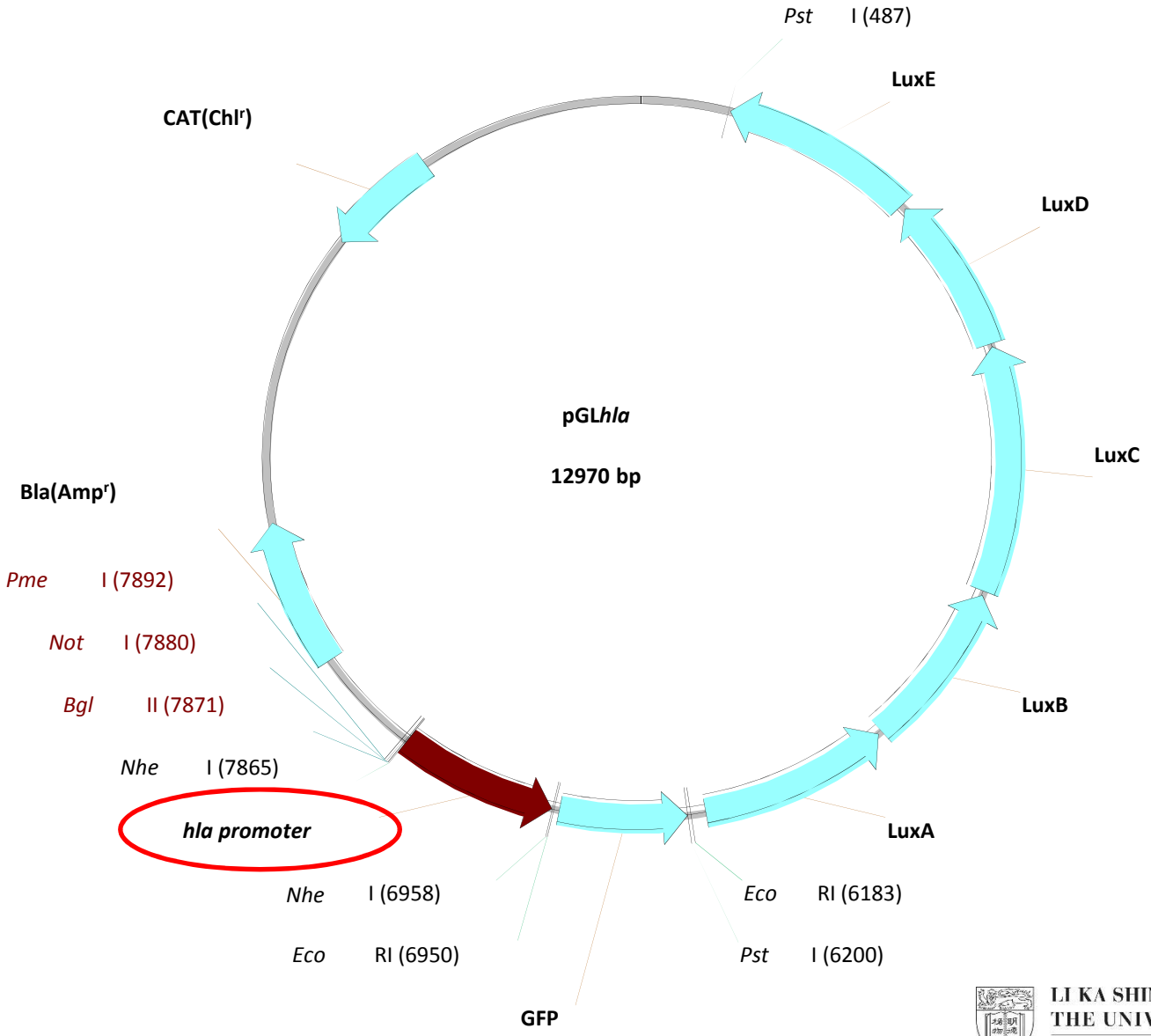
Construction by genetic engineering of a GFP-Lux dual reporter system monitoring the virulence genes expression in *S. aureus*



Insertion of virulence gene *spa2* promoter into the GFP-Lux dual reporter system



Insertion of virulence gene *hla* promoter into the GFP-Lux dual reporter system



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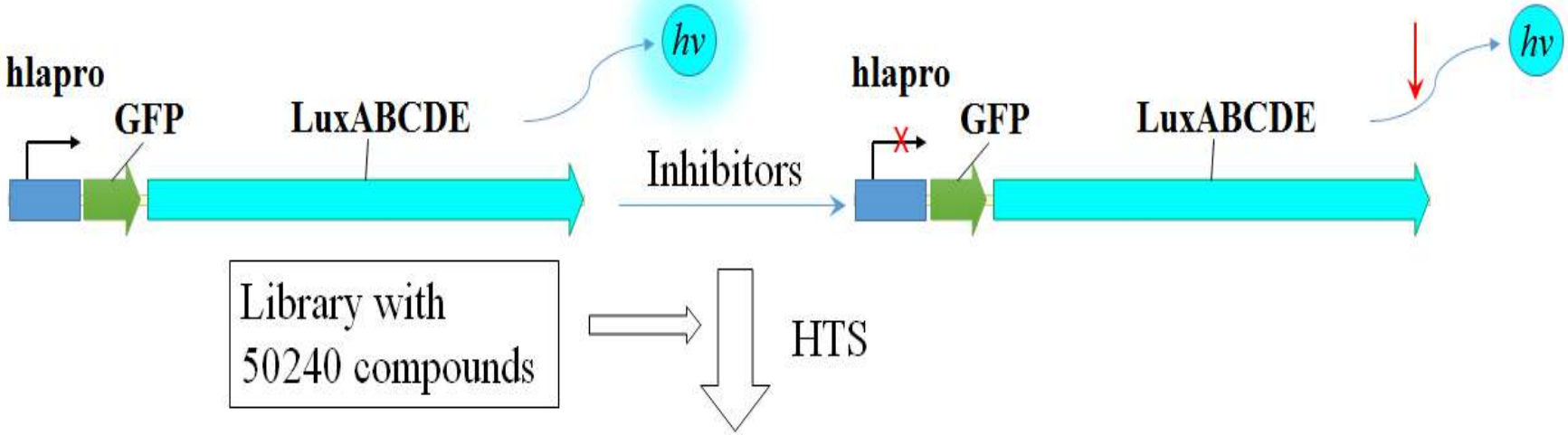
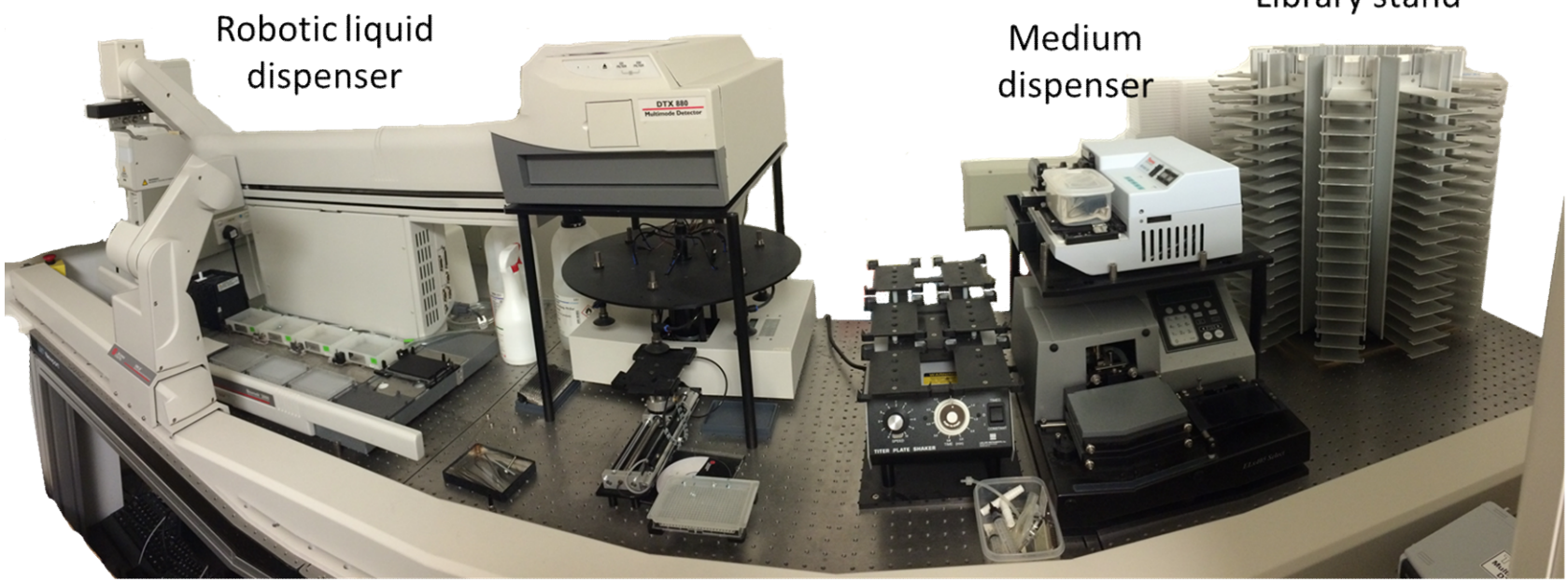
Identification of inhibitors of virulence expression by HTS



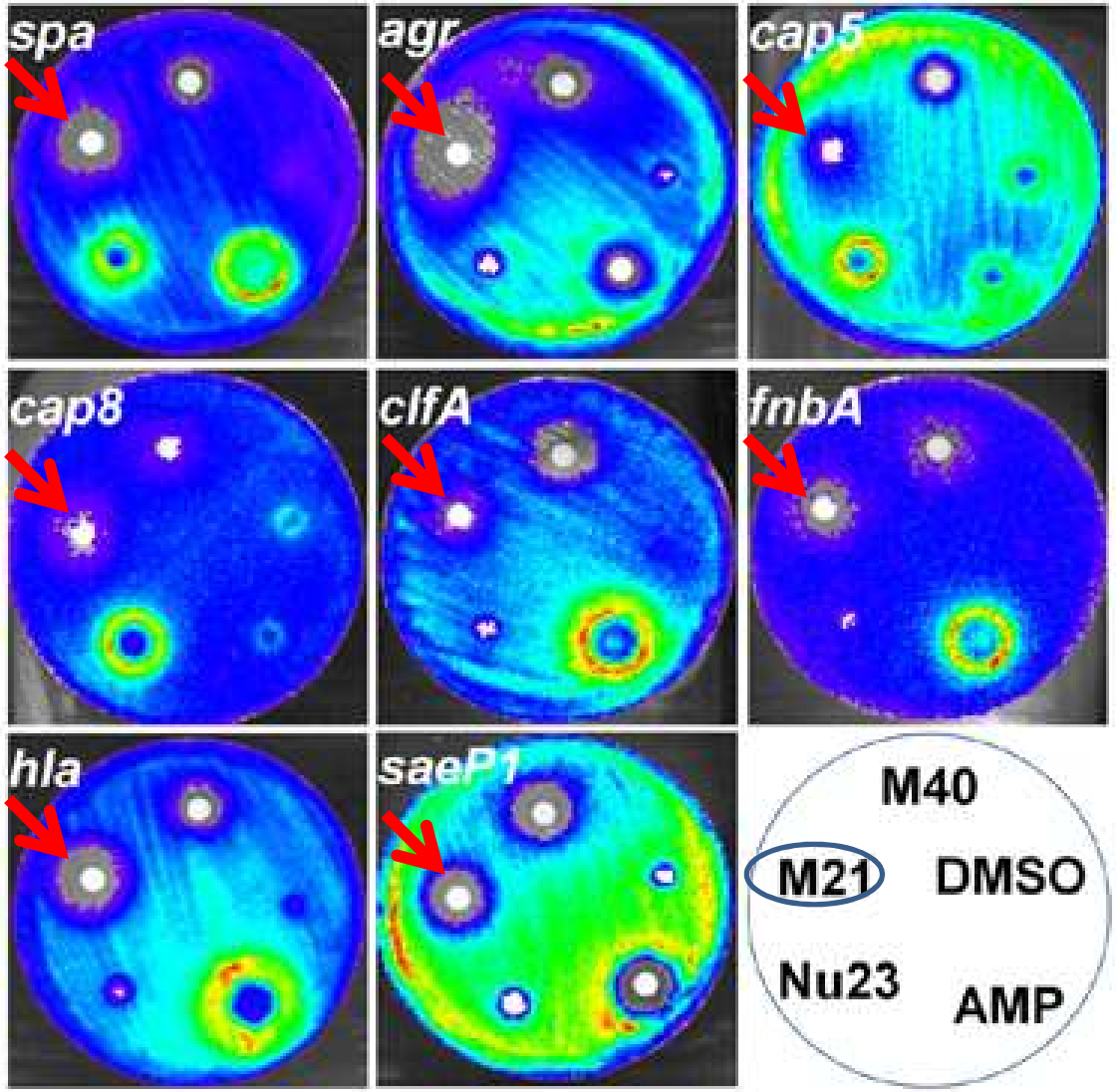


Robotic arm + Rail

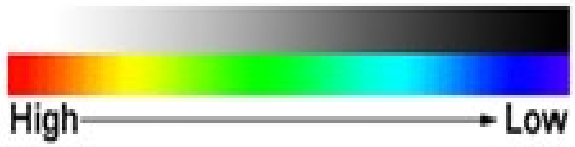
Plate rocker

Incubator

Promoter modulating effects of selected hit compounds

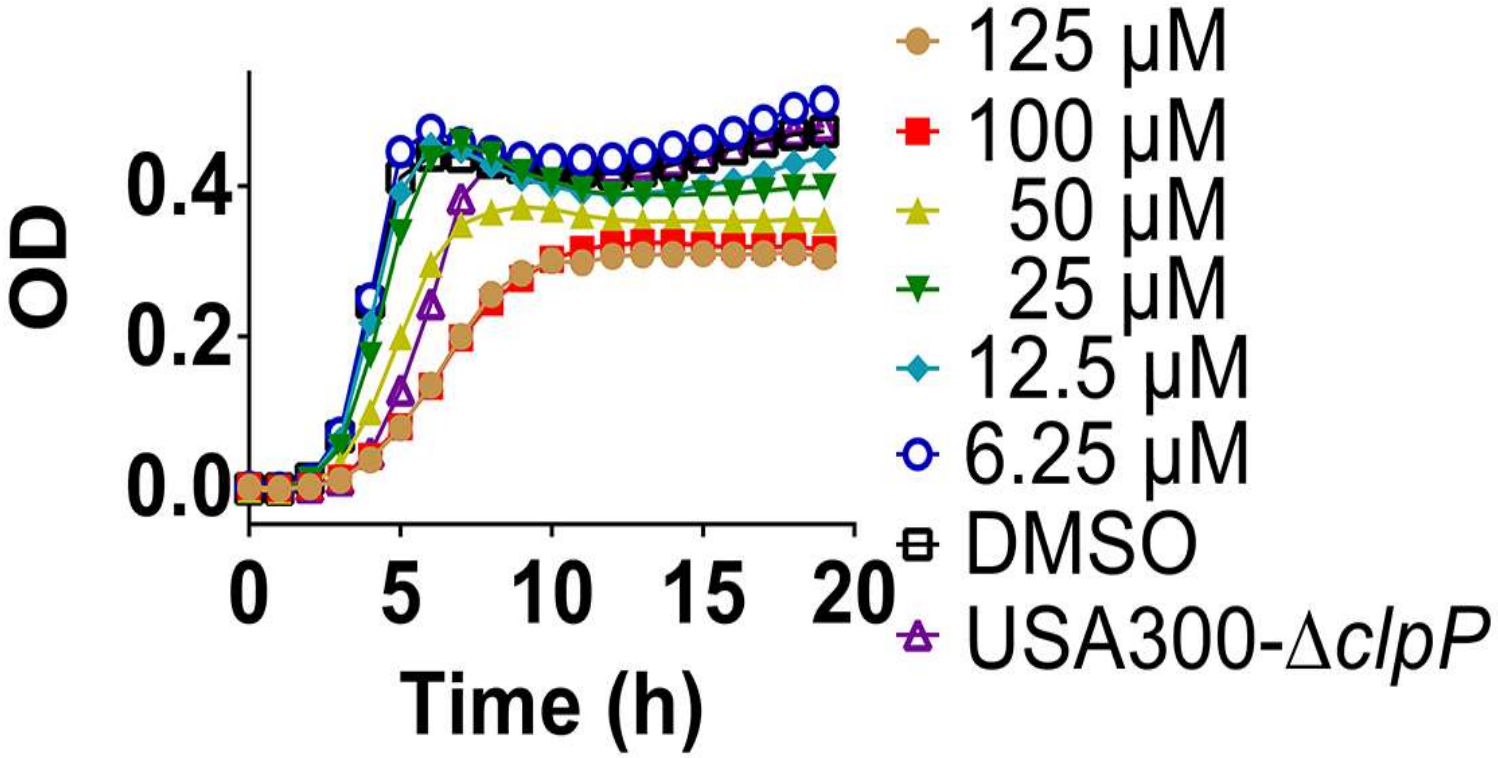


Enhanced promoter activity

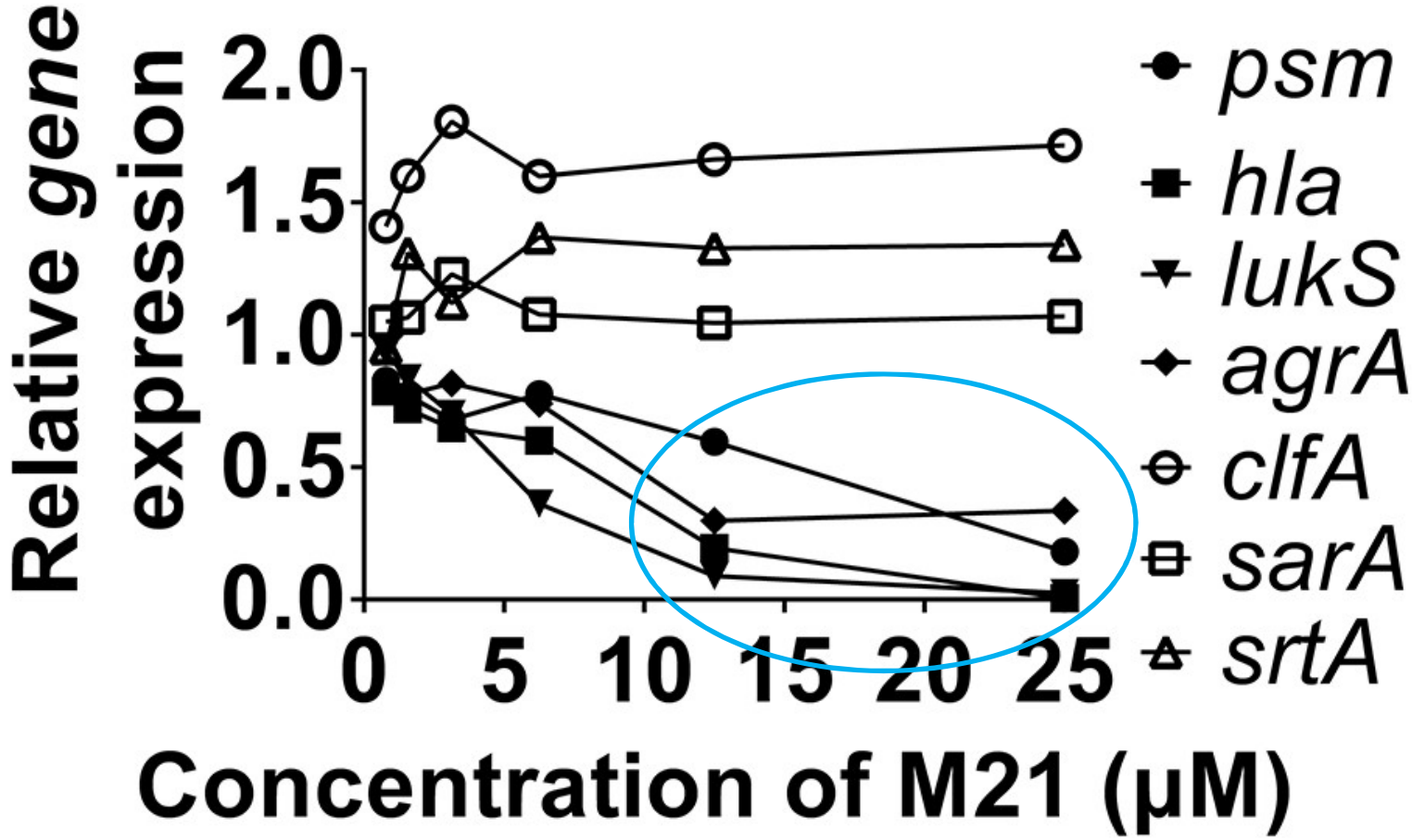


Repressed promoter activity

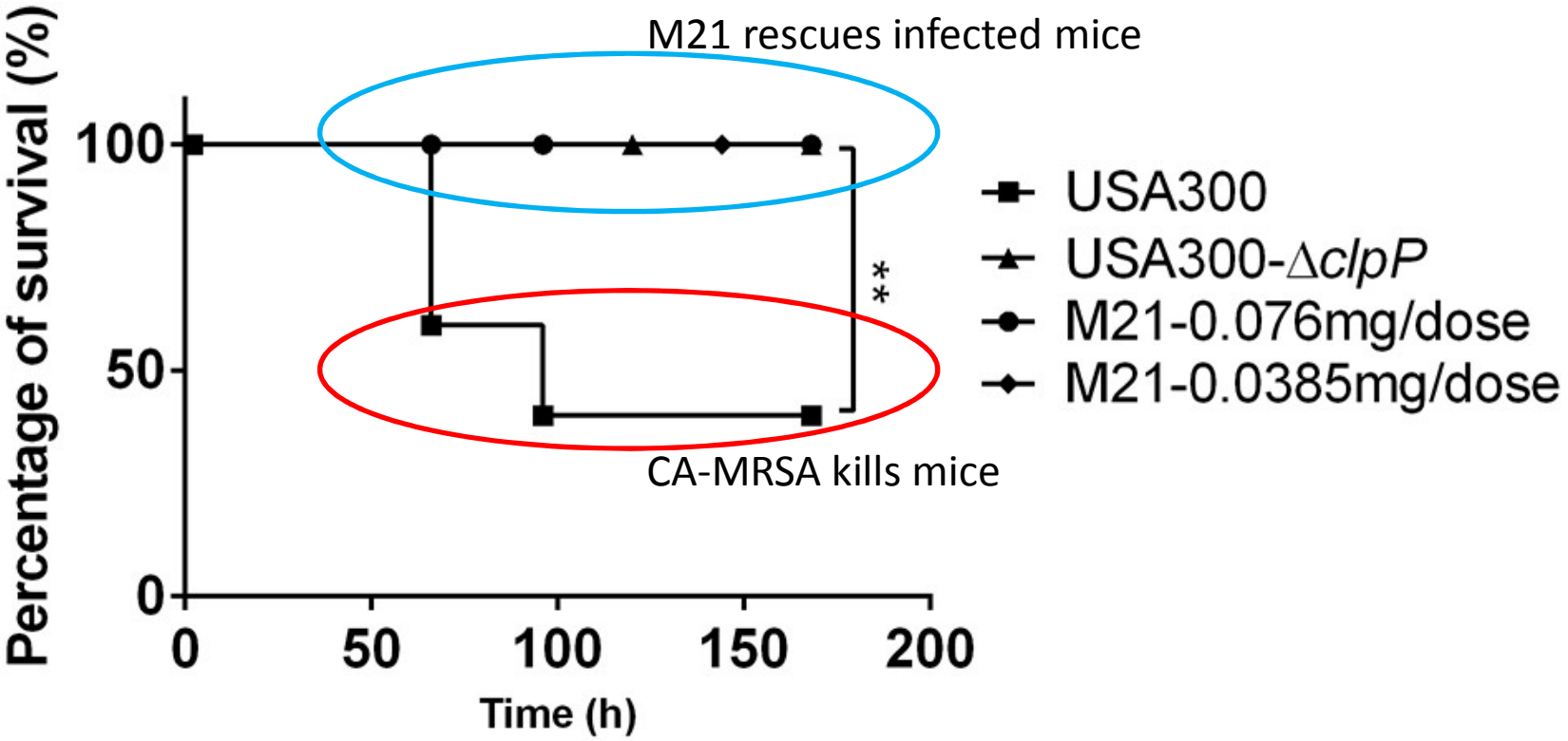
CA-MRSA USA300 can grow in the presence of M21



M21 selectively suppresses the expression of major secreted toxins in CA-MRSA

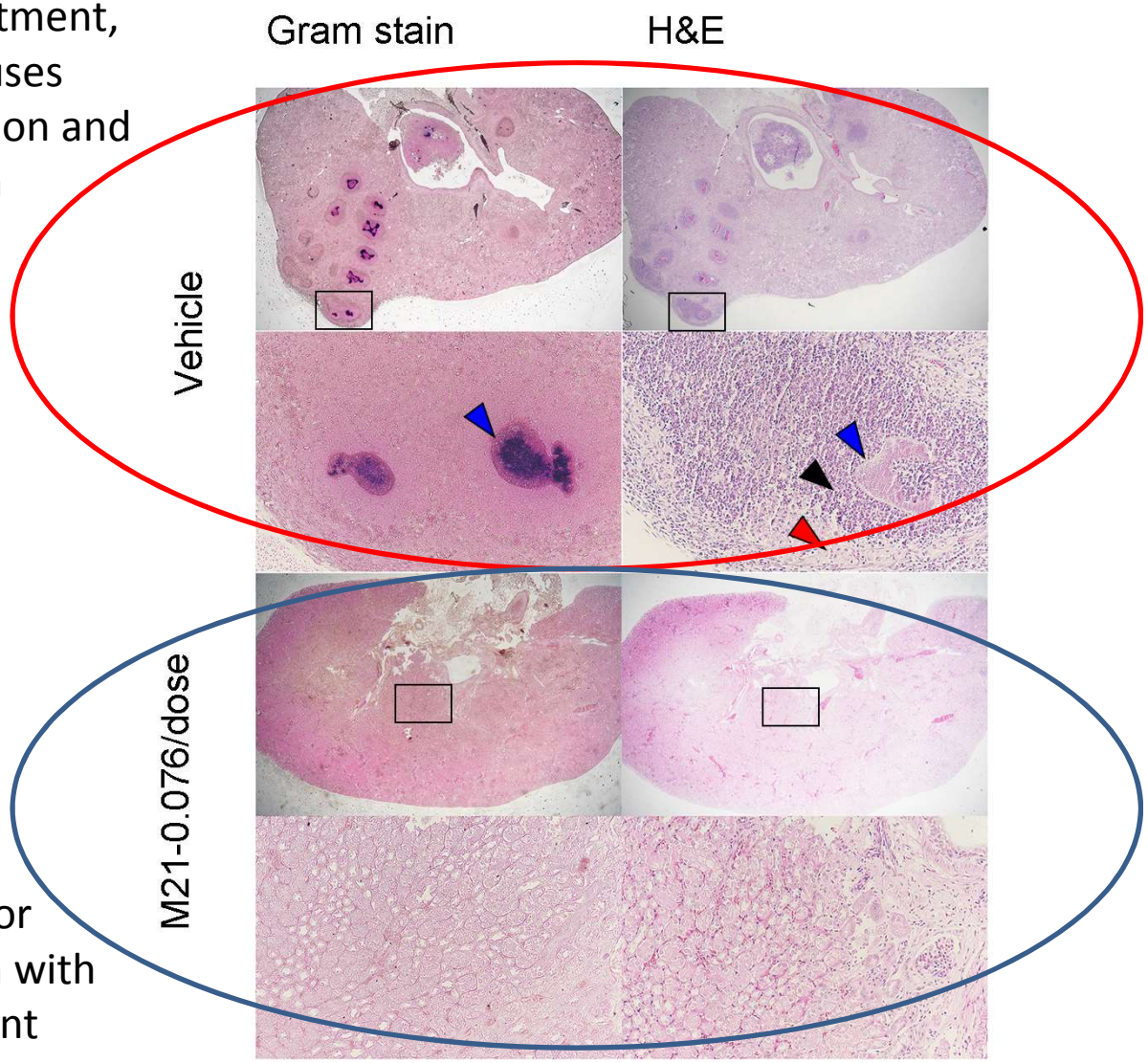


M21 rescues mice infected with CA-MRSA



M21 prevents kidney infections CA-MRSA in mice

Without treatment, CA-MRSA causes kidney infection and inflammation



No infection or inflammation with M21 treatment

The new era of anti-virulence drugs: Suppressing the virulence of MRSA without killing them

Can the beast be tamed?

YES!

Past and present

Future

Discovery of Non-antibiotic Lead Compound to Suppress Virulence in Methicillin Resistant *Staphylococcus aureus* (MRSA)

Acknowledgement

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Thank you!