科技部補助專題研究計畫成果報告
期末報告

克雷白氏肺炎桿菌毒殺細胞的分子機制探討

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 103-2320-B-040-019-
執 行 期 間 ： 103年08月01日至104年07月31日
執 行 單 位 ： 中山醫學大學醫學系

計 畫 主 持 人： 賴怡琪

計畫參與人員： 碩士班研究生-兼任助理人員：程國洲

處 理 方 式 ：
1.公開資訊：本計畫涉及專利或其他智慧財產權，2年後可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：否
3.「本報告」是否建議提供政府單位施政參考：否

中 華 民 國 104 年 10 月 28 日

中 文 摘 要 ： Klebsiella pneumoniae（克雷白氏肺炎桿菌）是世界性分布的致病菌，它的感
染可引起多樣化的臨床表現。 自1980 年代開始，一種由單一致病菌引起的化
膿性肝膿瘍被發現與K. pneumoniae 的感染由關。 儘管這個由K. pneumoniae 引起的肝膿瘍 (K. pneumoniae liver abscess, KLA) 在臨床上的重要性已被許多流行病學的報告確立，目前對該菌感染肝臟的分子機轉方面的瞭解卻仍相當有限。我們運用比較基因體學分析，結果顯示在克雷白氏肺炎桿菌的基因體中帶有兩組第六型分泌系統的基因組，命名為T6SS (I)與T6SS (III)。將T6SS (I)的重要基因剔除後的突變菌株明顯喪失對小鼠的致病以及毒殺細胞的能力。因此我們在本研究計畫中探討T6SS (I)參與K. pneumoniae 毒殺細胞的分子機轉以及該分泌系統表現的調控作用。過去這一年研究的結果顯示克雷白氏肺炎桿菌CG43在小鼠模式引起肝膿瘍的過程中與紅血球形成緊密的結合，而這個能力對該菌進行系統性感染是必要的，剔除T6SS (I)基因組中的重要基因的突變菌株喪失引發小鼠肝膿瘍的能力。 在體外實驗中，我們也發現T6SS (I)參與在與紅血球的交互作用上，該分泌系統可藉由contact引起後續的溶血反應。 我們目前正針對該系統的表現調控進行後續分析。

中 文 關 鍵 詞 ： 克雷白氏肺炎桿菌，第六型分泌系統，細胞毒殺性

英 文 摘 要 ： Klebsiella pneumoniae is a worldwide spread pathogen responsible for a broad spectrum of clinical syndromes. Since 1980s, a single pathogen-induced pyogenic liver abscess has been noticed to be predominantly mediated by the primary infection of K. pneumoniae. Despite the clinical significance of K. pneumoniae liver abscess (KLA) has been established, our knowledge regarding the molecular basis of how this bacterium causes an infection in the liver is rather restricted. Based on comparative genomic analyses, two distinguishable and conserved genetic loci T6SS (I) and (III) were identified harboring genes encoding for putative T6SS core components in sequenced K. pneumoniae genomes. Mutants that had in?frame deletion on T6SS (I) genes significantly lost virulence in mice and cytotoxicity to cells. Therefore, we aim in this project to explore the molecular basis regarding the mechanism by which K. pneumoniae
T6SS (I) causes cytotoxicity and how T6SS (I) expression is regulated. Our results demonstrated that K. pneumoniae CG43 firmly associated with erythrocytes during its development of liver abscesses in the murine model and this association was required for its virulence. Knock-out of essential genes in T6SS (I) significantly attenuated the ability of CG43 to establish systemic infections in the murine model. In vitro experiments also demonstrated the involvement of T6SS (I) in the interaction between CG43 and erythrocytes.

Through T6SS (I)-dependent contact, K. pneumoniae CG43 induced hemolysis of erythrocytes. We are now analyzing the regulatory circuit of T6SS (I).

英文關鍵詞：Klebsiella pneumoniae, T6SS, cytotoxicity

Background and significance

Klebsiella pneumoniae is a Gram-negative enterobacterium ubiquitous in nature. As behaving like a saprophyte resided in nasopharyngeal and intestinal mucosa of humans, *K. pneumoniae* frequently involves in a wide range of clinical illnesses, such as pneumonia, urinary tract infections, suppurative infections, bacteremia, meningitis, and sometimes life-threatening septicemia. Without immediate treatments, infections caused by this bacterium have a significantly high rate of mortality (*1*). During 1990s, *K. pneumoniae* has been noticed as the primary pathogen responsible for community-acquired pyogenic liver abscess in Taiwan (*2*). Despite *K. pneumoniae*-caused liver abscess (KLA) was considered a disease of regional distribution; it has now been continually reported from other countries in Southeast Asian, including Korea, Singapore, Japan, and Thailand, and is an emerging infectious disease in the United States and worldwide (*3-10*). Distinct from *Escherichia coli*-associated liver abscess, KLA is generically cryptogenic without underlying hepatobiliary disorders and is frequently complicated in up to 20% of cases with septic metastatic lesions to other organs (*2, 11-14*). Of the metastatic infections of KLA, meningitis and endophthalmitis are most devastating that may lead to neurological sequelae, such as hearing loss, vision loss, and cognitive delay, and cause a high mortality rate even with the use of antibiotics therapy (*2*). By virtue of its primary and invasive nature, KLA represents one of the most severe *K. pneumoniae* infections (*6, 15-17*). In 1990s, a cytotoxin was identified from clinical strains of *K. oxytoca* isolated from patients with antibiotic-associated hemorrhagic colitis. This cytotoxin was demonstrated to cause cell death in cultured Hep2, Vero, CHO-K1, and HeLa cell lines and also in an isolated intestinal-loop model (*18-21*). Human infections with *K. pneumoniae* share many characteristics with those with *K. oxytoca*. Severe tissue damages in lungs, soft tissues, and liver are common symptoms observed in patients with *K. pneumoniae* infections. The understanding of molecular events regarding how *K. pneumoniae* cause tissue damage may provide insights into its pathogenesis.

Based on our preliminary results, among 35 KLA strains tested, *K. pneumoniae* 1158 constantly showed 30-60% cytotoxicity to cells. To further understand why this strain was so cytotoxic to eukaryotic cells, we started collaborating with Dr. Chen Ying-Tsong (Institute of Genomics and Bioinformatics, Natl. Chung-Hsing Univ.). By using 454 GS Junior (Roche), Dr. Chen's team completed the whole genome sequence of *K. pneumoniae* 1158. Based on comparative genome analysis, several genomic loci were identified with a

potential to have a role in related to bacterial cytotoxic activities. On this list gene clusters responsible for type IV secretion system (T6SS) were noted. In Gram-negative bacteria, protein secretion is a complex process that is carried out by proteinaceous machineries, known as protein secretion systems, in a controlled and efficient manner. At least six types of protein secretion system (T1SS-T6SS) have been identified. Among these, T6SS is the most recently characterized secretion system (*22, 23*). As analogous to the T3SS and T4SS, T6SS appears to constitute needle-like structures that span the bacterial cell wall and have the potential to translocate bacterial effector proteins directly into the target eukaryotic or bacterial cell. T6SS is widely distributed that has been predicted in > 25% of all sequenced Gram-negative bacteria.

Encoded within large gene clusters on bacterial chromosomes, the core T6SS machinery consists of 13 essential components. The T6SS-core essential and conserved genes are now named *tssA*-*tssM*. A molecular machinery extending from the bacterial cytoplasm, across the inner membrane, periplasm and outer membrane, and ultimately into a target cell was formed by the 13 T6SS-core components. Current structural models of T6SS indicate that the apparatus is composed of at least two complexes, a dynamic bacteriophage-like structure and a cell-envelope-spanning membrane-associated assembly. T6SS is a contractile injection system and driven by a mechanism similar to that of tailed bacteriophage (*24*). Secretion of effector proteins by T6SSs is in a one-step manner, independent of the Sec or Tat export machineries, and no secretion signals have yet been identified. Hcp (TssD) and VgrG (TssI) are extracellular components of T6SS that form a needle-like injection device resembling the tail of bacteriophage. Hcp forms hexameric rings stacking into a tube-like structure, which has an outer diameter of 90 A and a central channel with a width of 40 A. A trimer of VgrG sits at the distal tip of the Hcp tube, together forming a tail spike-like device to puncture target membranes (*25, 26*). VipA (TssB) and VipB (TssC) form tubular complexes similar to viral tail sheath proteins and have a role to eject Hcp-VgrG upon contraction. The syringe-like T6SS machinery is positioned and anchored to the bacterial cell envelope, to couple the energy from VipA/VipB contraction to transport cargos across the inner and outer membranes of Gram-negative bacteria.

Numerous reports implicate T6SS in virulence and/or interaction with host cells in multiple bacterial species, ranging from plat pathogens, animal pathogens, to human pathogens, such as *V. cholerae*, *Burkholderia* spp. and *P. aeruginosa* (*27*). More recently, T6SS has been found to have anti-bacterial activity in *P. aeruginosa*

and *V. cholerae* (*28, 29*). Now, based on its function, T6SS can be separated into four categories: (1) bacterial cell targeting, (2) eukaryotic cell targeting, (3) bacterial and eukaryotic cell targeting, and (4) other (such as conjugation, gene regulation, and cellular adhesion). Some of T6SS-bearing bacteria have multiple, distinct T6SSs, up to six in *Yersinia pestis* and *B. pseudomallei* (*30-32*). As predicted by P. F. Sarris *et al.* in 2011 (*33*), three distinguishable and conserved genetic loci, named T6SS locus I, II, and III, were identified harboring genes encoding for putative T6SS core components and effectors in sequenced *K. pneumoniae* genomes. *K. pneumoniae* 1158 has T6SS (I) and T6SS (III) in its genome. T6SS (I) contains all of the 13 T6SS-core genes which are organized in 2 segments, the *vgrG* gene cluster and the *icmF* gene cluster. To determine the role of T6SS in the physiology and virulence of *K. pneumoniae*, in-frame deletion mutants for *hcp* (now named as *tssD*), *clpV* (*tssH*), *vgrG* (*tssI*), and *icmF* (*tssM*) of T6SS (I) were generated. Our previous study found that the loss of *tssM* (*icmF*) significantly attenuated *K. pneumoniae* virulence in mice and cytotoxicity to erythrocytes. We therefore aim in this project to explore the molecular basis regarding the mechanism by which *K. pneumoniae* T6SS (I) causes cytotoxicity and how T6SS (I) expression is regulated.

Specific aims

1 Determine whether *K. pneumoniae* T6SS (I)-mediated hemolysis requires intimate bacteria-RBC membrane contact.

2 Determine whether *K. pneumoniae* T6SS (I) contributes to the insertion of translocation pores into the RBC membrane.

3 Examine whether T6SS (I)-mediated association with erythrocytes contributes to systemic *K. pneumoniae* infections in mice.

Results and discussion

1. **Association of *K. pneumoniae* with erythrocytes.** *K. pneumoniae* was never considered a hemolytic bacterium by using a conventional test with sheep erythrocytes. However, about three years ago, through the collaboration with Dr. Han-Chen Ho (Department of Anatomy, Tzu-Chi University, Hualien), we observed a significant amount of *K. pneumoniae* tightly associated with erythrocytes which were distributed inside the liver of *K. pneumoniae*-infected mice (Fig. 1A and B). This phenomenon suggested that the interaction with erythrocytes might assist the systemic

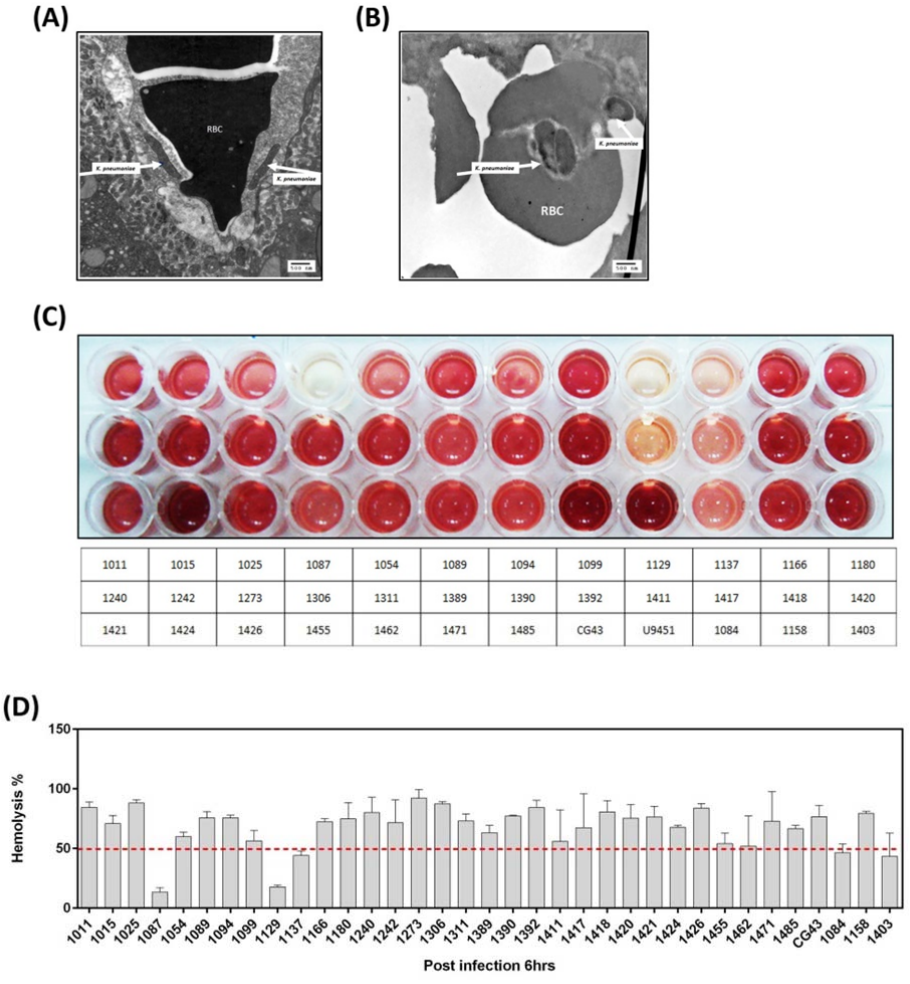

Figure 1. Association of *K. pneumoniae* with erythrocytes. *K. pneumoniae* CG43S was orally inoculated into 8-wk-old male BALB/c mice. At 72 hours post-inoculation, liver was retrieved and fixed for TEM analysis. Representative images are shown in **(A)** and **(B)**. Each of the 35 *K. pneumoniae* KLA strains was respectively incubated with horse erythrocytes for 6 hours **(C)**. Absorbance values detected at 420nm were normalized with the value of total lysis of erythrocytes and presented in average percentage ± SEM **(D)**. Red line stands for 50% of hemolysis.

dissemination of *K. pneumoniae.* In the bloodstream highway, erythrocytes probably serve as a free rider for the tightly-associated *K. pneumoniae*, and the subsequent lysis of some erythrocytes releases nutrients and iron, which nursing the *in vivo* proliferation of *K. pneumoniae*. To test this hypothesis, we examined the interaction of *K. pneumoniae* CG43S with erythrocytes isolated from various mammal species and human volunteers with different blood types. Preliminary results indicated that *K. pneumoniae* CG43S exhibited a significant *in vitro* hemolytic activity when interacted with O-type human blood and also with horse erythrocytes. We therefore investigated the hemolytic activity to horse erythrocytes for a total of 35 clinical strains isolated from cases of *K. pneumoniae* liver abscess (KLA). As shown in Fig. 1C and 1D, most of the KLA strains caused lysis for more than 50% of erythrocytes except four super-mucoid strains (1087, 1129, 1137, and 1403).

2. **Type VI secretion system locus 1 (T6SS_I) was required for the *K. pneumoniae* CG43 hemolytic activity.** T6SS is originally described for *Vibrio cholerae* and *Pseudomonas aeruginosa* in 2006 (*22, 23*). As analogous to the T3SS and T4SS, T6SS is composed of needle-like structures that span the bacterial cell wall and protrude from the cell surface. T6SS is widely distributed that has been predicted in > 25% of all sequenced Gram-negative bacteria. Encoded within large gene clusters on bacterial chromosomes, the core T6SS nanomachinery consists of 13 essential components. Some of T6SS-bearing bacteria have multiple, distinct T6SSs, up to six in *Yersinia pestis* and *Burkholderia pseudomallei* (*30-32*). As predicted by P. F. Sarris *et al.* in 2011 (*33*), three distinguishable and conserved genetic loci, named T6SS locus I, II, and III, were identified harboring genes encoding for putative T6SS core components and effectors in sequenced *K. pneumoniae* genomes. Similar to *K. pneumoniae* NTUH-K2044, *K. pneumoniae* CG43 lost the locus II. Only T6SS_I and T6SS (III) were found in its genome. As shown in Fig. 2A, the T6SS-core genes which are organized in 2 segments, the *icmF* gene cluster (*icmF*/*impG*/*impH*/*sciN*/*impF*), and the *vgrG* gene cluster (*vgrG*/*clpV*/*hcp*/*ompA*/*dotU*/*impJ*/*impC*/*impB*). Increasing evidence indicates roles of T6SS in bacterial virulence and/or host cell interaction. Thereby, T6SSs can functionally be classified into two types: the anti-eukaryotic T6SS and the anti-bacterial T6SS. Among the *K. pneumoniae* T6SS_I-core components, Hcp (Hemolysin co-regulated protein) and VgrG (valine-glycine repeat protein G) are the main extracellular components forming a needle-like injection device closely resembling the bacteriophage

tail. ClpV and IcmF are ATPase in the cytoplasm that energizes the action of T6SS. Therefore, in-frame deletion mutants for *hcp* (now named as *tssD*), *clpV* (*tssH*), *vgrG* (*tssI*), and *icmF* (*tssM*) of T6SS_I were generated (Fig. 2A) for the determination of whether T6SS_I was required for the *K. pneumoniae* hemolytic activity. After 6-h incubation, the hemolytic activity of the T6SS_I mutants, ΔtssM1, ΔtssD, ΔtssDM1, and ΔtssDHI, were significantly attenuated when compared with that of the wild type strain (Fig. 2B and C). Time dependency was also noted for the *K. pneumoniae* caused hemolysis (Fig. 2D). The result suggested that the integrity of T6SS_I structure was required for the *K. pneumoniae* cytotoxic activity toward red blood cells.

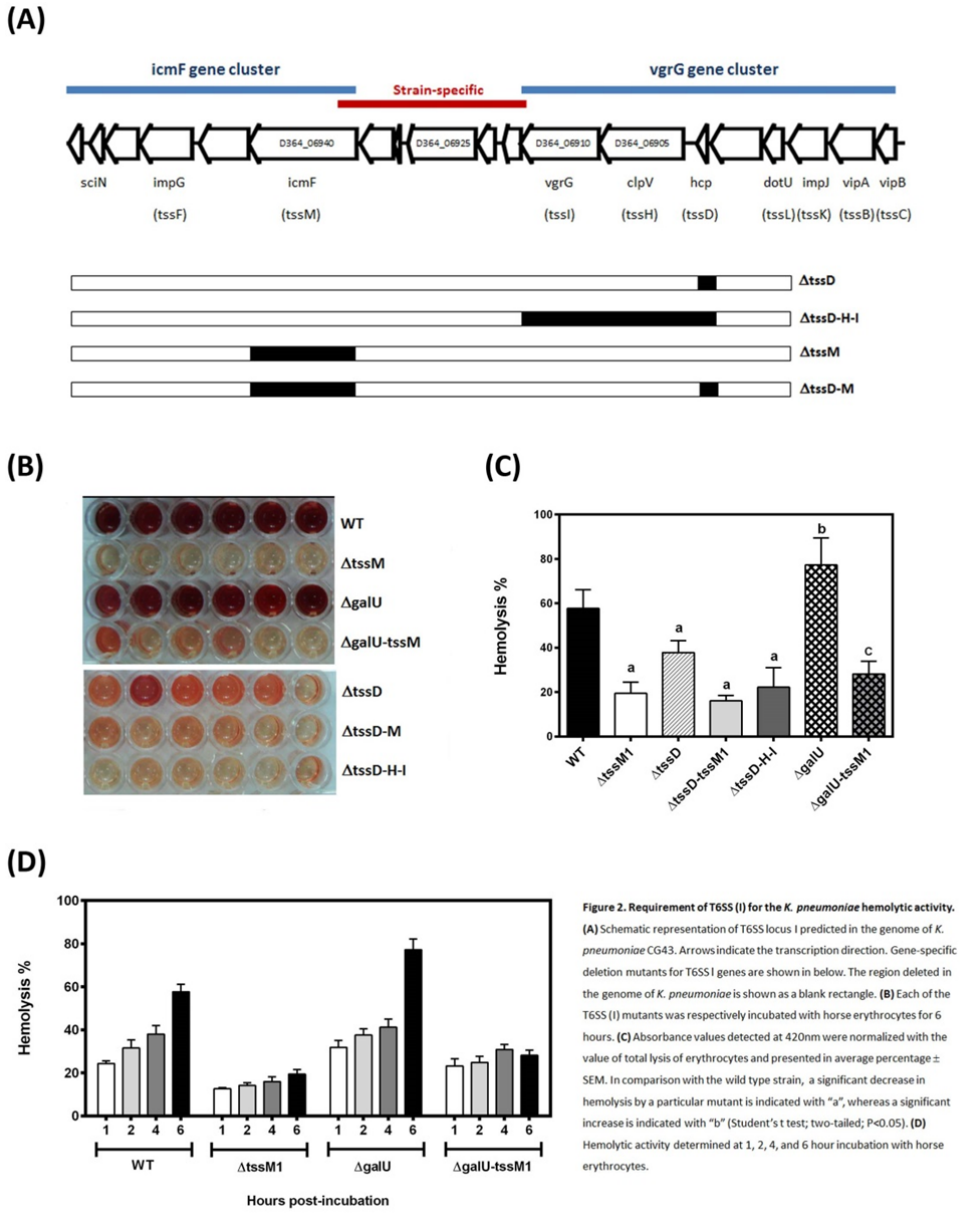

Figure 2. Requirement of T6SS (I) for the *K. pneumoniae* hemolytic activity. **(A)** Schematic representation of T6SS locus I predicted in the genome of *K. pneumoniae* CG43. Arrows indicate the transcription direction. Gene-specific deletion mutants for T6SS I genes are shown in below. The region deleted in the genome of *K. pneumoniae* is shown as a blank rectangle. **(B)** Each of the T6SS (I) mutants was respectively incubated with horse erythrocytes for 6 hours. **(C)** Absorbance values detected at 420nm were normalized with the value of total lysis of erythrocytes and presented in average percentage ± SEM. In comparison with the wild type strain, a significant decrease in hemolysis by a particular mutant is indicated with "a", whereas a significant increase is indicated with "b" (Student's t test; two-tailed; P<0.05). **(D)** Hemolytic activity determined at 1, 2, 4, and 6 hour incubation with horse erythrocytes.

3. **Requirement of T6SS_I for *K. pneumoniae* virulence.** Groups of BALB/c mice were intraperitoneally inoculated with 10^4 CFU of wild type strain or with the same inoculum of a particular T6SS_I mutant, including ΔtssD (*hcp* deletion mutant), ΔtssM1 (*icmF1* deletion mutant), ΔtssD-M1 (*hcp* and *icmF1* double deletion mutant), and ΔtssDHI (*hcp*, *vgrG*, and *clpV* triple deletion mutant). Survival of the infected mice was monitored for two weeks. The majority of mice which were infected with ΔtssM1, ΔtssD-M1, or ΔtssDHI, survived the experimental period (purple-, brown-, and green-colored lines shown in Fig. 3A), which was significantly higher than the survival of wild type-infected mice (black-colored, Fig. 3A). All of the ΔtssD-infected mice died at day 6 (red-colored line, Fig. 3A). The attenuation on virulence was not related to growth defects, given the *in vitro* growth capacity for all the mutants were comparable to that of the wild type strain. This result suggested requirement of *tssM1*, *tssH*, and *tssI* for *K. pneumoniae* virulence. In contrast to tssM1, tssM3, the *icmF* gene in T6SS locus III was dispensable for *K. pneumoniae* virulence. To further determine the role of T6SS_I in the pathogenesis of *K. pneumoniae*, ΔtssM was selected for subsequent studies. The colonization and dissemination ability of *K.*

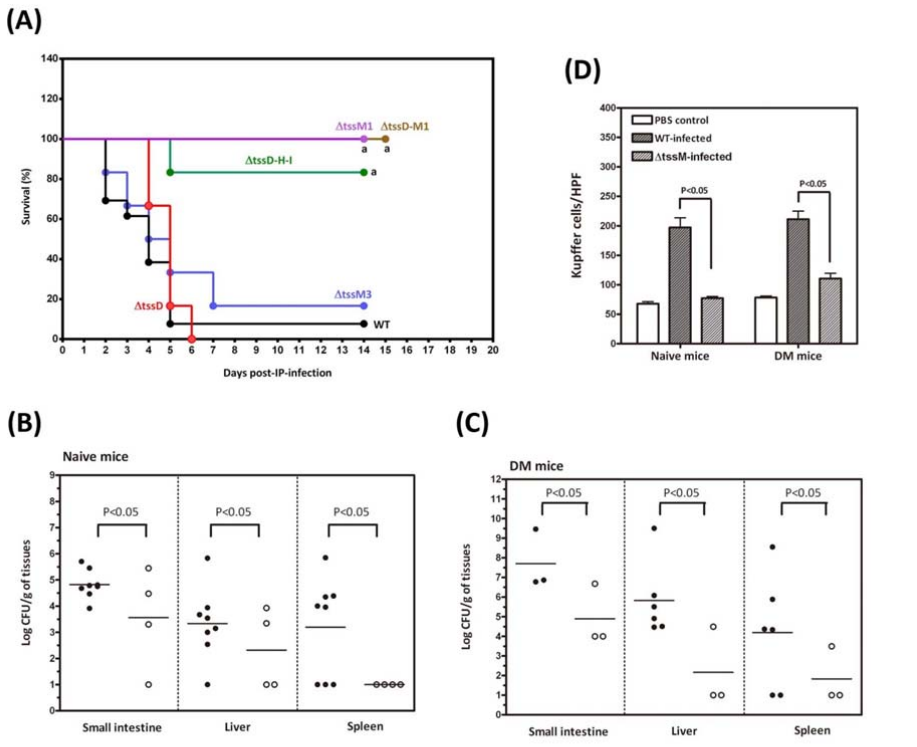

Figure 3. ***In vivo*** **virulence assessment for T6SS mutants. (A)** Survival rate of mice which were intraperitoneally infected with 10^4 CFU of *K. pneumoniae* CG43S (black) or with T6SS mutants, including ΔtssD (red), ΔtssD-M1 (brown), ΔtssM1 (purple), ΔtssDHI (green), and ΔtssM3 (blue). Bacterial loads of wild type (solid circles) or ΔtssM (empty circles) in different mouse tissues at 48 hours post-oral-infection with an inoculums of 10^8 CFU in naïve BALB/c mice **(B)** or in DM mice **(C)**. **(D)** Average number of Kupffer cells in the liver retrieved from mice which were infected with wild type *K. pneumoniae* or with ΔtssM.

pneumoniae to extra-intestinal organs was also affected by the loss of *tssM1*. As shown in Fig. 3B, at 2 days after an oral inoculation of 10^8 CFU of ΔtssM1 or wild type strain, a decrease of $1\times\log_{10}$ in the average bacterial loads of small intestines was profound in the Δ*tssM1*-infected group as compared to that of wild type-infected mice. When the wild type-infected mice had a bacterial burden approaching $10^{3\text{-}4}$ CFU in the liver and spleen, Δ*tssM1* was undetectable at these extra-intestinal organs for most of the mice infected. The virulence attenuation of ΔtssM was more profound in mice with diabetes (DM mice; Fig. 3C). Besides, the number of Kupffer cells activated upon the hepatic infection of *K. pneumoniae* was significantly reduced by the loss of *tssM1*. These results suggested an involvement of T6SS_I in several stages of *K. pneumoniae* pathogenesis, including the development of systemic infection, extraintestinal dissemination, and inflammatory activation.

4. **Expression of *K. pneumoniae* T6SS_I was activated upon contact with erythrocytes but impeded by capsular polysaccharides (CPS).** Many bacterial pathogens employ protein secretion systems to facilitate their survival in hosts. The expression of T6SS genes have been demonstrated to be mostly induced *in vivo*. To analyze the expression of *K. pneumoniae* T6SS_I genes, polyclonal anti-serum against the HCP protein, which is the major component of T6SS needle structure, was generated. The expression of HCP was hardly detected in conventional LB cultures (Fig. 4A) except for the *K. pneumoniae* lysates which were collected after 6h-incubation with erythrocytes. As shown in Fig. 4B, a significant amount of HCP was present in the wild type *K. pneumoniae*, Δ*galU*, Δ*tssM3*, and Δ*galU*-Δ*tssM3*, but absent in ΔtssM1, ΔtssD, and Δ*galU*-Δ*tssM1*. In addition, when the direct contact with erythrocytes was blocked with transwell membrane inserts, the degree of hemolysis was decreased to the background level (Fig. 4C), suggesting that the T6SS_I-mediated hemolysis was contact-dependent. Because the T6SS structure is predicted as an inverted bacteriophage, the distance between bacterial surface and erythrocyte membrane is probably a determinant for this needle-like structure to deliver effectors into cells to cause hemolysis. If so, the thickness of capsule may become a negative factor for the *K. pneumoniae* hemolytic activity. As shown in Fig. 2C, the loss of *galU*, which impairs the synthesis of CPS and lipopolysaccharides, elevated the degree of T6SS_I-mediated hemolysis from 58% to 77%. This result suggested that capsule of *K. pneumoniae* CG43S impeded T6SS_I-mediated hemolysis. The interference of capsule on hemolysis might explain the result

shown in Fig. 1C that some clinical strains with a super-mucoid capsule exhibited lower hemolytic activity than others.

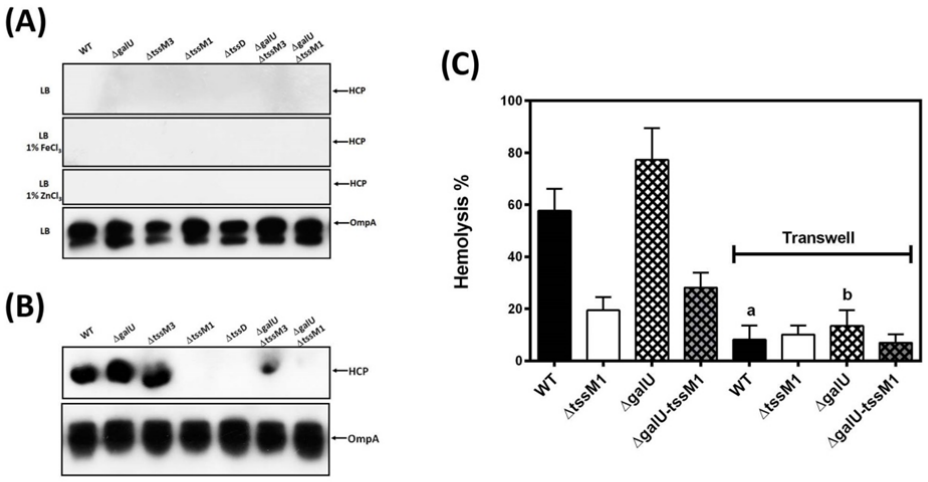

Figure 4. Expression of T6SS (I) in *K. pneumoniae* was induced upon contact with erythrocytes. Total proteins were extracted from wild type or a particular mutant of *K. pneumoniae* which were grown in LB medium, supplemented with $FeCl_3$ or $ZnCl_2$ **(A)**, or were incubated with erythrocytes for 6 hours **(B)**, and were subjected to Western blot analysis with HCP- or OmpA-specific antisera. **(C)** The hemolytic activity of wild type or a particular mutant of *K. pneumoniae* was determined at 6-h incubation with or without the Transwell inserts. In comparison with its parental strain, a significant decrease in hemolysis is indicated with "a" or "b" (Student's t test; two-tailed; P<0.05).

References

1. R. Podschun, U. Ullmann, Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* **11**, 589 (1998).
2. J. H. Wang *et al.*, Primary liver abscess due to Klebsiella pneumoniae in Taiwan. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **26**, 1434 (Jun, 1998).
3. D. R. Chung *et al.*, Emerging invasive liver abscess caused by K1 serotype Klebsiella pneumoniae in Korea. *J Infect* **54**, 578 (Jun, 2007).
4. E. M. Karama *et al.*, Endogenous endophthalmitis complicating Klebsiella pneumoniae liver abscess in Europe: case report. *Int Ophthalmol* **28**, 111 (Apr, 2008).
5. Y. Keynan, J. A. Karlowsky, T. Walus, E. Rubinstein, Pyogenic liver abscess caused by hypermucoviscous Klebsiella pneumoniae. *Scand J Infect Dis* **39**, 828 (2007).
6. K. A. Nadasy, R. Domiati-Saad, M. A. Tribble, Invasive Klebsiella pneumoniae syndrome in North America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **45**, e25 (Aug 1, 2007).

7. H. Okano *et al.*, Clinicopathological analysis of liver abscess in Japan. *Int J Mol Med* **10**, 627 (Nov, 2002).
8. Y. M. Tan, S. P. Chee, K. C. Soo, P. Chow, Ocular manifestations and complications of pyogenic liver abscess. *World J Surg* **28**, 38 (Jan, 2004).
9. V. Wiwanitkit, Causative agents of liver abscess in HIV-seropositive patients: a 10-year case series in Thai hospitalized patients. *Trop Doct* **35**, 115 (Apr, 2005).
10. K. G. Yeoh *et al.*, Tropical liver abscess. *Postgrad Med J* **73**, 89 (Feb, 1997).
11. S. Ohmori *et al.*, Septic endophthalmitis and meningitis associated with Klebsiella pneumoniae liver abscess. *Hepatol Res* **22**, 307 (Apr, 2002).
12. B. S. Hu, Y. J. Lau, Z. Y. Shi, Y. H. Lin, Necrotizing fasciitis associated with Klebsiella pneumoniae liver abscess. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **29**, 1360 (Nov, 1999).
13. S. T. Lindstrom, P. R. Healey, S. C. Chen, Metastatic septic endophthalmitis complicating pyogenic liver abscess caused by Klebsiella pneumoniae. *Aust N Z J Med* **27**, 77 (Feb, 1997).
14. C. H. Lee, H. S. Leu, T. S. Wu, L. H. Su, J. W. Liu, Risk factors for spontaneous rupture of liver abscess caused by Klebsiella pneumoniae. *Diagn Microbiol Infect Dis* **52**, 79 (Jun, 2005).
15. C. T. Fang, Y. P. Chuang, C. T. Shun, S. C. Chang, J. T. Wang, A novel virulence gene in Klebsiella pneumoniae strains causing primary liver abscess and septic metastatic complications. *J Exp Med* **199**, 697 (Mar 1, 2004).
16. W. C. Ko *et al.*, Community-acquired Klebsiella pneumoniae bacteremia: global differences in clinical patterns. *Emerg Infect Dis* **8**, 160 (Feb, 2002).
17. W. L. Yu *et al.*, Association between rmpA and magA genes and clinical syndromes caused by Klebsiella pneumoniae in Taiwan. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **42**, 1351 (May 15, 2006).
18. J. Minami, S. Saito, T. Yoshida, T. Uemura, A. Okabe, Biological activities and chemical composition of a cytotoxin of Klebsiella oxytoca. *Journal of general microbiology* **138**, 1921 (Sep, 1992).
19. M. Higaki, T. Chida, H. Takano, R. Nakaya, Cytotoxic component(s) of Klebsiella oxytoca on HEp-2 cells. *Microbiology and immunology* **34**, 147 (1990).
20. J. Minami, S. Katayama, O. Matsushita, H. Sakamoto, A. Okabe, Enterotoxic activity of Klebsiella oxytoca cytotoxin in rabbit intestinal loops. *Infection and immunity* **62**, 172 (Jan, 1994).
21. J. Minami, A. Okabe, J. Shiode, H. Hayashi, Production of a unique cytotoxin by Klebsiella oxytoca. *Microbial pathogenesis* **7**, 203 (Sep, 1989).

22. J. D. Mougous *et al.*, A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. *Science* **312**, 1526 (Jun 9, 2006).
23. S. Pukatzki *et al.*, Identification of a conserved bacterial protein secretion system in Vibrio cholerae using the Dictyostelium host model system. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 1528 (Jan 31, 2006).
24. P. G. Leiman *et al.*, Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 4154 (Mar 17, 2009).
25. N. Kapitein, A. Mogk, Deadly syringes: type VI secretion system activities in pathogenicity and interbacterial competition. *Current opinion in microbiology* **16**, 52 (Feb, 2013).
26. J. M. Silverman, Y. R. Brunet, E. Cascales, J. D. Mougous, Structure and regulation of the type VI secretion system. *Annual review of microbiology* **66**, 453 (2012).
27. S. J. Coulthurst, The Type VI secretion system - a widespread and versatile cell targeting system. *Research in microbiology* **164**, 640 (Jul-Aug, 2013).
28. R. D. Hood *et al.*, A type VI secretion system of Pseudomonas aeruginosa targets a toxin to bacteria. *Cell host & microbe* **7**, 25 (Jan 21, 2010).
29. M. Basler, B. T. Ho, J. J. Mekalanos, Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions. *Cell* **152**, 884 (Feb 14, 2013).
30. L. E. Bingle, C. M. Bailey, M. J. Pallen, Type VI secretion: a beginner's guide. *Current opinion in microbiology* **11**, 3 (Feb, 2008).
31. C. J. Blondel, J. C. Jimenez, I. Contreras, C. A. Santiviago, Comparative genomic analysis uncovers 3 novel loci encoding type six secretion systems differentially distributed in Salmonella serotypes. *BMC genomics* **10**, 354 (2009).
32. F. Boyer, G. Fichant, J. Berthod, Y. Vandenbrouck, I. Attree, Dissecting the bacterial type VI secretion system by a genome wide in silico analysis: what can be learned from available microbial genomic resources? *BMC genomics* **10**, 104 (2009).
33. P. F. Sarris, C. Zoumadakis, N. J. Panopoulos, E. V. Scoulica, Distribution of the putative type VI secretion system core genes in Klebsiella spp. *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases* **11**, 157 (Jan, 2011).

科技部補助計畫衍生研發成果推廣資料表

日期:2015/10/28

科技部補助計畫	計畫名稱: 克雷白氏肺炎桿菌毒殺細胞的分子機制探討
	計畫主持人: 賴怡琪
	計畫編號: 103-2320-B-040-019- 學門領域: 微生物及免疫學

無研發成果推廣資料

103年度專題研究計畫研究成果彙整表

計畫主持人：賴怡琪	**計畫編號：**103-2320-B-040-019-
計畫名稱：克雷白氏肺炎桿菌毒殺細胞的分子機制探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果（無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）		無					

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
      □實驗失敗
      □因故實驗中斷
      □其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形：
   論文：□已發表 □未發表之文稿 ■撰寫中 □無
   專利：□已獲得 □申請中 ■無
   技轉：□已技轉 □洽談中 ■無
   其他：（以100字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500字為限）