(Revised 07/09)

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(please insert ref. above)

NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Characterizing Prevalent Clones of Multi-drug Resistant, Community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in Mainland China and Hong Kong: Resistance Mechanisms and Virulence Factors Distribution.

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Margaret IP	Prof. Shen Xuzhuang
Post	Professor	Professor
Unit / Department / Institution	Dept of Microbiology, Chinese University of Hong Kong	Dept of Paediatrics, Capital Medical University
Co-investigator(s) *(with title)*	-	-

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01.01.2011		
Project Completion date	31.12.2012		
Duration *(in month)*	24		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Characterization of molecular types, resistance mechanisms, and their distribution in prevalent clones of Community-associated methicillin- and multidrug-resistant (MDR) Staphylococcus aureus (CA-MRSA) in mainland China and Hong Kong;
2. Study of the acquisition, transferability of resistant genes, the relationships of multiple resistance determinants within common mobile genetic element, inc. plasmids and transposons and its potential to spread; and
3. Study of the presence of major virulence factors, variable genes encoding toxins, and their distribution within mobile genetic elements; and invasiveness of strain types in an infection murine model.

5.2 Revised Objectives

Date of approval from the RGC: -NA-

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome

(maximum 1 page; please make reference to Part C where necessary)

A collection of over 480 non-duplicate MRSA isolates from blood cultures and were saved from six major hospitals from Hong Kong during the period 2009 to 2011 for the study. An additional 72 strains with confirmed CA-MRSA infections notified to the Centre for Health Protection, Hong Kong Department of Health, during the same period were also included. The latter strains were mainly from pus and wound sites from skin and soft tissue infections. Isolates were characterized by a combination of spa types, ST types, and SCCmec types. The most prevalent MRSA clones from blood cultures identified in Hong Kong included ST45-IV/VII-t1081, ST1774-IV-t1081 and ST239-III-t037 and these types were responsible for more than 80% of MRSA bacteremia. A decline in ST239-III-t037, previously the predominant clone associated with MRSA bacteremia in 1990s and before 2000, in Hong Kong was observed. Among strains from confirmed CA-MRSA skin and soft tissue infections, ST30-IV-t019, ST59-IV/VII-t437 and ST338-IV/VII-t437 were identified to be the 3 predominant CA-MRSA clones [section 9 (ref 1), section 8 (ref 2)]. Overall, a diverse range of MRSA ST /SCCmec/spa types were identified causing bacteremia, or skin and soft tissue infections in Hong Kong during this period. Representatives of these strains were further characterized in terms of their antibiotic resistance determinants and virulence genes profiles, fitness of the strains and invasiveness in animal studies.

In mainland China, the predominant clone in Chinese pediatric community acquired infections was shown to belong to clonal complex CC59 [see report from Mainland PI]. These isolates were further analyzed by MLST, SCC*mec*, spa typing, PFGE and antibiotics susceptibility testing. The distribution of virulence gene profiles in CA-MRSA was characterized. The molecular characteristics, expression of virulence factors, carriage of plasmids of CC59 strains, and the clinical spectrum of infections from children in mainland China were described in detail [section 8 (ref 1): PLOS One 2013]. It was concluded that ST59-t437-IVa was the dominant clone among CC59 MRSA strains. The carriage rate of *pvl* gene was high, at 55.5%. CC59 MRSA strains were present in both community-associated (CA-) and healthcare-associated (HA-) infections. The majority of MRSA infection in children were in infants, with a median age of 4.8 months [section 8 (ref 1): PLOS One 2013].

Overall, clonal type CC59 was identified as the commonest CA-MRSA clones from Mainland China and the second commonest clone among notified CA-MRSA infections in Hong Kong. These strains belong to ST59, and possess either Scc*mec* types Iva or V. Interestingly from animal studies, strains of SCC*mec* type V (+/- PVL) were highly invasive with 100% mortality in murine infection model at 5 x 10^8cfu/ml, and followed in descending order of invasiveness by ST59-IV (+/- PVL), and other common ST types (ST30-IV > ST8-IV > ST239-III > and laboratory strain, ATCC700698, this latter strain with 0% mortality). Competition studies also revealed the fitness for survival of these ST59-IVa/V strain types, in contrast to the other commonly isolated ST types from this locality and from laboratory strains.

This temporal relationship of invasiveness and fitness with different ST59 strains possessing various Scc*mec* cassettes are inconclusive and deserve further studies. These strains may possess other genetic makeup that predispose to their virulence and fitness. Future work should focus on the monitoring of these multidrug-resistant CA-MRSA clones of CC59 and further elucidation of their propensity for virulence and disease.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

12-17th June 2013 China PI and her postgraduate students, Ms Li Juan and Ms Ning Snow, made reciprocal visit to Hong Kong PI's laboratory to present research findings with Hong Kong PI and team.

Discussion was also made on future laboratory activities and directions on collaborations. The proposed plan was to continue monitor and further study the predominant multidrug-resistant clonal type(s) ST59 in Mainland China and Hong Kong using newer techniques of next generation high-throughput sequencing. The aims include elucidating the micro-evolutionary changes of these specific clones across Mainland China and Hong Kong, their relationships to those of neighboring countries; the relationship of varying SCCmec types of ST59 and fitness and virulence; and any alterations with implementation of new specific control measures. Further funding support will be applied both locally and at international level.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Community-associated MRSA (CA-MRSA) is an important cause of bacterial infections and has emerged in the community to become epidemic in many countries. The major MRSA types in Hong Kong and mainland China belong to a few clonal types that are distinct and unique, in that they are often multidrug-resistant, and thus significantly limiting the choice for treatment and increases clinical failure rates. Our study examined the molecular characteristics of these strains and provided a comprehensive representation and detailed snapshot of the genetic population structure of CA-MRSA in Mainland China and Hong Kong. A platform was established to compare and examine their detailed characteristics, insights to the resistance mechanisms and their mechanisms of spread, and virulence potential of these prevalent CA-MRSA clones. These clones possess variable characteristics that enhance their propensity for colonization, disease and dissemination. The study revealed major insights into the transmission of these clones; facilitates strategies and established methodologies for rapid identification and delineation of these strains critical in a prevention program for control of MRSA both at the hospital and community level. The study also contributes to the basis of future potential targets for treatment of staphylococcal disease.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.))

(Revised 07/09)

The Latest Status of Publications				Author(s) *(bold the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*					
2013				Juan Li[1]., Lijuan Wang1., **Margaret Ip[2]**, Mingjiao Sun[1], Jing Sun[1], Guoying Huang[3], Chuanqing Wang[3], Li Deng[4], Yuejie Zheng[5], Zhou Fu[6], Changcong Li[7], Yunxiao Shang[8], Changan Zhao[9], Sangjie Yu[1], Kaihu Yao[1], Yonghong Yang[1], **Xuzhuang Shen[1*]**	Molecular and Clinical Characteristics of Clonal Complex 59 Methicillin-resistant Staphylococcus aureus Infections in Mainland China. PLOS ONE, vol 8, e70602, p1-8. (ref 1)	2012	Yes	Yes
			2013	**Margaret Ip** et al.	Changing Trends of Prevalent Clones of Methicillin-resistant Staphylococcus aureus in Hong Kong. (ref 2)		No	

9. **Recognized International conference(s) in which paper(s) related to this research project was/were delivered** *(Please attach a copy of each delivered paper)*

(Revised 07/09)

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*
Mar/2011/ Hong Kong Eaton Hotel	MRSA – An Update.	The Hong Kong Society for Infectious Diseases Fifteenth Annual Scientific Meeting, 19th March 2011.	2011	No	Yes
Oct/2011/ School of Public Health, Chinese University of Hong Kong	Use of a DNA array for the delineation of CA- and HA-MRSA.	International Conference on Global Health and Public Health Education, 25-27th October 2011.	2011	No	Yes
Sept/2012/ Domaine de Rockefeller, University of Lyon, France.	Characterization of prevalent clones of Methicillin-resistant *Staphylococcus aureus* in Hong Kong.	15th International Symposium on Staphylococci and Staphylococcal Infections (15th ISSSI), 26th-30th August 2012. (ref 1)	2012	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Mr Chris K Y Wong	PhD	April 2011	Mar 2014
Dr Wang Zheng	PhD	August 2011	July 2014

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*