LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*
FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Development of a cold chain free DNA based rapid test for the early detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical samples

Investigator: Dr. Kirnpal Kaur Banga Singh

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USM Short Term Grant

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Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. Nama Ketua Penyelidik: Dr. Kirnpal Kaur Banga Singh
Name of Research Leader

☐ Profesor Madya/ *Assoc. Prof.* ☒ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. Pusat Tanggungjawab (PTJ): School of Medical Sciences
School/Department

3. Nama Penyelidik Bersama:
Dr. Habsah Hasan (PPSP);
Prof. M. Ravichandran (AIMST University)

4. Tajuk Projek:
Title of Project

Development of a cold chain free DNA based rapid test for the early detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical samples

5. Ringkasan Penilaian/*Summary of Assessment*:	Tidak Mencukupi *Inadequate*		Boleh Diterima *Acceptable*	Sangat Baik *Very Good*	
	1	2	3	4	5
i) Pencapaian objektif projek: *Achievement of project objectives*	☐	☐	☐	☐	☒
ii) Kualiti output: *Quality of outputs*	☐	☐	☐	☐	☒
iii) Kualiti impak: *Quality of impacts*	☐	☐	☐	☐	☒
iv) Pemindahan teknologi/potensi pengkomersialan: *Technology transfer/commercialization potential*	☐	☐	☒	☐	☐
v) Kualiti dan usahasama : *Quality and intensity of collaboration*	☐	☐	☐	☐	☒
vi) Penilaian kepentingan secara keseluruhan: *Overall assessment of benefits*	☐	☐	☐	☐	☒

6. Abstrak Penyelidikan

(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris**. Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research

(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English).
This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Abstrak Penyelidikan

Kemunculan *Staphylococcus aureus* yang rintang Methicillin (MRSA) bertanggungjawab terhadap jangkitan nosokomia dan jangkitan pada komuniti. Prevalens pembawa MRSA nasal adalah amat berbeza di antara negara-negara yang berlainan dan juga di antara hospital-hospita di dalam negara yang sama. Pengenalpastian awal MRSA pada pesakit dan diikuti dengan langkah pencegahan penyebaran melalui pesakit kepada pesakit dipercayai merupakan langkah yang efektif dalam pengawalan jangkitan MRSA.Ujian kultur konvensional mengambil masa selama 2 hingga 5 hari untuk pengenalpastian organisma dan pola sensitiviti antibiotiknya. Penggunaan kaedah asid nukleik iaitu ujian reaksi berantai polimerasi berganda (PCR) semakin luas digunakan di dalam makmal untuk menggantikan kaedah rutin diagnostik seperti identifikasi biokimia dan ujian sensitiviti antimikrob Kirby-Bauer yang mengambil masa yang lama, memerlukan tenaga pekerja yang berlebihan dan kurang sensitif.

Oleh yang demikian, kajian ini memberi fokus kepada pembangunan asai multipleks PCR untuk pengesanan MRSA dalam masa yang singkat. Asai tersebut secara serentak boleh mengesan lima gen iaitu 16S rRNA (gen *Staphylococcus* genus), femA (*Staphylococcus aureus*), mecA yang mengekod gen rintangan metisi;in, lukS yang mengekod gen pengeluaran leukosidin Panton-Valentine (PVL), sitotoksin nekrotizing, dan satu gen kawalan dalaman. Pasangan primer unik dan spesifik telah direka untuk mengamplifikasi 5 gen dengan produk PCR pada julat 151 hingga 759 bp. Spesifisiti primer telah disahkan menggunakan turutan DNA produk multipleks dan analisa BLAST. Sensitiviti dan spesifisiti asai PCR multipleks telah dibandingkan dengan kaedah kultur konvensional. PCR multipleks telah ditermostabil dan ujian kestabilan pantas telah dinilai pada suhu bilik, 37°C dan 10°C. Sensitiviti analitikal asai didapati pada 10 ng pada peringkat DNA manakala spesifisiti telah dinilai dengan 34 rujukan stafilokoki dan strain bukan stafilokokal menunjukkan 100%. Campuran multipleks PCR yang termostabil yang disimpan pada 10°C adalah stabil sehingga dua setengah tahun menurut ujian kestabilan pantas.

Ketepatan diagnostik ditentukan dengan menggunakan sejumlah 231 swab nasal isolat klinikal dan menunjukkan keputusan 100% sensitiviti, spesifisiti, nilai jangkaan positif dan nilai jangkaan negatif. Gen *fem*A dalam ujian reaksi berantai polimerasi berganda ini mampu menentukan bukan *S. aureus* staphylococci. Kehadiran kawalan dalaman pada asai PCR multipleks membantu dalam menyingkirkan kes negatif palsu. Asai PCR multipleks adalah tegap dan boleh memberi maklumat mengenai 5 gen yang amat penting untuk mengenal pasti spesis stafilokokus dan pola rintangan Methicillin mereka. Kajian ini dipercayai adalah pertama dalam membangunkan kombinasi ujian molekular pantas untuk pengenalpastian dan pengecaman *Staphylococcus* genus daripada yang lain, diskriminasi serentak dalam mengenalpasti methicillin-resistant daripada sensitiviti strain staphylococcal, *S. aureus* daripada CoNS, dan pengesanan gen PVL. Asai PCR yang dibangunkan dalam kajian ini boleh digunakan sebagai alat yang berkesan untuk penyaringan dan diagnosis pembawa nasal MRSA di hospital dan pada komuniti.

Abstract of Research

Methicillin-resistant *Staphylococcus aureus* strains are an increasing infection control problem in hospitals worldwide. Infection with MRSA strains is usually preceded by nasal colonization. Although some patients are admitted with MRSA colonization, others acquire MRSA during their hospital stay. The prevalence of MRSA nasal carrier differs widely among different countries including from one hospital to another in the same country. Early identification of patients colonized with MRSA and subsequent prevention of patient-to-patient spread through infection control measures are believed to be potent interventions to control MRSA.

Nucleic acid-based tests using PCR are increasingly being used in laboratories to replace time-consuming, labor-intensive and less sensitive conventional diagnostic methods such as biochemical identification and Kirby-Bauer antimicrobial susceptibility tests. The multiplex PCR assay was successfully used with nasal swab specimen to detect staphylococcal genus, species, PVL toxin and methicillin resistant genotypes within one day, thus permit the same day identification and isolation of MRSA nasal carrier subjects. Due to lack of a rapid test which gives quick info on the detection of MRSA clinical isolates currently, the thermostabilized multiplex PCR assay developed in this study is extremely versatile. This assay is apparently more cost effective than conventional methods for the detection of MRSA.

Specific primers were designed based on the conserved and non-conserved regions of the target genes used in the monoplex and multiplex PCR assays. The sizes of the 5 PCR products, including IC, were designed to obtain a staircase-like pattern when separated by agarose gel electrophoresis. A total of 5 primer pairs were designed using VectorNTI Advance 9 software (Invitrogen Corporation, California, USA). Out of those 5 pairs, one pair was designed to amplify a specific gene of the *staphylococcus* genus (16S rRNA gene).

The ClustalW interface in VectorNTI software was used to align all the downloaded sequences. The conserved and non-conserved regions of these alignments were visualized using GeneDoc software. Based on the conserved region on the alignment, a specific primer pair was designed to amplify the 16S rRNA while the non-conserved regions were used to design primer pairs for *fem*A *S. aureus, mec*A, *and lukS* genes. The designed primers were BLAST analysed through GenBank for specificity (http://www.ncbi.nlm.nih.gov/BLAST/). Search results showed none of the designed primers had similarity with other non-specific gene; hence they were specific for their target genes.

After successfully developing a complete multiplex PCR for the identification of MRSA with virulent toxin we went on thermostabilized the multiplex PCR reagents by freeze-drying in the presence of an enzyme stabilizer. In accelerated stability evaluation test it was found that the thermostabilized multiplex PCR mix was stable at 10°C for more than one year. The diagnostic accuracy of the thermostabilized multiplex PCR at the genus level was determined using 231 nasal swabs and found to be 100% sensitive, specific, positive and negative predictive values for staphylococci isolates. The *fem*A gene in the multiplex PCR assay was able to rule out non *S. aureus* staphylococci. The present study is believed to be the first to develop a combined molecular test for the rapid identification and discrimination of the *Staphylococcus* genus from others, with simultaneous discrimination of methicillin-resistant from susceptible staphylococcal strains, *S. aureus* from CoNS, and concomitant detection of PVL genes.

7. Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.
[Sila gunakan kertas berasingan]

Sila lihat lampiran

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	Bahasa Inggeris
Bakteria	*Bacteria*
Kultur	*Culture*
PCR	PCR
MRSA	MRSA

8. Output dan Faedah Projek
Output and Benefits of Project
(a) * **Penerbitan Jurnal**
Publication of Journals

Published 2 papers in indexed journal:

1. *A pentaplex PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus and panton-valentine leucocidin.* Hassanain Al-Taib, Chan Yean Yean, Alyaa Al-Khateeb, Habsah H., Kirnpal-Kaur B.S., Karim Al-Jashamy, Ravichandran M. ***BMC Microbiology*. 2009. 9:113**. DOI:10.1186/1471-2180-9-113 **(Impact Factor: 2.89)**

2. *Comparative evaluation of five culture media with triplex PCR assay for the detection of methicillin-resistant Staphylococcus aureus (MRSA).* Hassanain I Al-Talib, Chan Yean Yean, Alyaa R Al-khateeb, Habsah Hassan, Kirnpal-Kaur Banga Singh, Karim A Al-Jashamy and Manickam Ravichandran. ***Current Microbiology*. 2010. 61: 1-6.** DOI 10.1007/s00284-009-9567-8. **(Impact Factor: 1.33)**

Conference/Seminar Presentations:

1. Comparative Evaluation of Eight Methods for Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA). H Al-Talib, Y.Y. Chan, A. Al-khateeb, H. Habsah,K. Al-Jashamy, M. Ravichandran. 13th International Conference of Infectious Diseases, Kuala Lumpur, Malaysia, June 19-22, 2008. **(Poster presentation).**
2. Prevalence of Methicillin-Resistant *Staphylococcus aureus* Infection Among HUSM Patients. Hassanain Al-Talib, Habsah H., Karim Al-Jashamy. 2nd National conference on Infectious Diseases. 14th-15th February 2007, Grand Riverview Hotel Kota Bharu-Kelantan **(Poster presentation).**
3. In vitro study of the antimicrobial potential effects of Malaysian honey on some pathogens. Hassanain Al-Talib, Chan Yean Yean, Alyaa Al-khateeb, Kirnpal-Kaur Banga Singh, Karim Al-Jashamy, Ravichandran M. 10th Symposium of the Malaysian Society of Applied Biology. November 6-8 2008, Merdeka Palace Hotel, Kuching, Sarawak, Malaysia **(oral presentation)**
4. *A pentaplex PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus and panton-valentine leucocidin.* Hassanain Al-Taib, Chan Yean Yean, Alyaa Al-Khateeb, Karim Al-Jashamy, Kirnpal-Kaur B.S., Habsah H., Ravichandran M. 7th International Symposium on Antimicrobial Agents and Resistance. Bangkok Convention Centre, Bangkok, Thailand. 18-20 March 2009. **(Poster presentation).**