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研究成果報告

Mental Health
精神健康

Microbiology
微生物學

Oral Health
口腔健康

Traditional Chinese Medicine
傳統中醫藥

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Traditional Chinese Medicine

Dissemination reports are concise informative reports of health-related research supported by funds administered by the Food and Health Bureau, namely the *Research Fund for the Control of Infectious Diseases* (RFCID), the *Health and Health Services Research Fund* (HHSRF), the *Health Care and Promotion Fund* and the *Health Services Research Fund*. In this edition, 11 dissemination reports of funded projects related to mental health, microbiology, oral health, and traditional Chinese medicine are presented. Two of the reports are highlighted, owing to their potentially significant findings, impact on health care delivery and practice, and/or contribution to health policy formulation in Hong Kong. In addition, a summary of the Health Research Symposium 2010 organised by the Food and Health Bureau is presented.

The Health Research Symposium 2010 was held on 11 September 2010 at the Hong Kong Academy of Medicine. It was the second such event organised by the Food and Health Bureau and aimed to (1) disseminate significant findings of research projects supported by the Bureau's funds to the local research community, (2) facilitate the exchange of ideas between invited overseas experts and local researchers on aspects of health-related research, and (3) acknowledge outstanding local researchers. The two themes of the Symposium were *Application of statistical and mathematical models to understand infectious disease dynamics* (RFCID theme) and *Cost-effectiveness in health services research* (HHSRF theme). To ensure coverage of a wide spectrum of research outcomes, these themes were explored and discussed from the perspectives of multiple stakeholders, including researchers, funders, policy makers, administrators, clinicians, and patients. The Symposium attracted more than 500 delegates and over 250 poster presentations. There were 22 presentations from distinguished overseas and local speakers. Awards for research excellence were given to eight local investigators.

Cystitis or urinary tract infection affects one third of women at some point in their lives. Its microbial aetiology is well established, with *Escherichia coli* being the predominant pathogen accounting for 80% of cases. Antibiotic treatment is mainly determined by the prevailing antimicrobial resistance of this organism. Ho et al[1] used molecular typing to evaluate antimicrobial resistance rates of *E coli* and other pathogens isolated from 352 local adult women with community-acquired cystitis. Patients were recruited from 54 centres including general practitioner offices, general outpatient clinics, and emergency departments. Regarding all *E coli* isolated, the rates of antimicrobial resistance were 52.8% for ampicillin, 29.5% for co-trimoxazole, and 12.9% for ciprofloxacin. Nitrofurantoin and fosfomycin remain active against >90% of the isolates. Age over 51 years and recent antibiotic treatment were significantly associated with fluoroquinolone resistance. The authors suggest that in future revision of management guidelines, the patient's age should be considered as a variable in the approach to empirical therapy.

The outbreak of severe acute respiratory syndrome in 2003 was the inspiration to develop a herbal formula Kwan Du Bu Fei Dang (KDBFD) which was expected to have antiviral and immunomodulatory properties. Eighty healthy adult Chinese volunteers were randomised to receive a single oral dose of KDBFD or placebo every day for 7 days. Immune markers were monitored on days 0, 7 and 21. Fung et al[2] observed that cell numbers of CD8+ suppressor plus cytotoxic T-lymphocytes and CD4+ helper T-lymphocytes were significantly increased after 7 days of treatment, although the effect was not sustained or significant compared with placebo. These studies may be useful in developing traditional Chinese medicines to enhance the immune response against respiratory and other pathogens.

We hope you will enjoy this selection of research dissemination reports. Electronic copies can be downloaded from the Research Fund Secretariat website (**http://www.fhb.gov.hk/grants**). Researchers interested in the funds administered by the Food and Health Bureau also may visit the website for detailed information about application procedures.

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2. Fung KP, Leung PC, Tsui KW, et al. Immunomodulatory activities of the herbal formula Kwan Du Bu Fei Dang in healthy subjects: a randomised, double-blind, placebo-controlled study. Hong Kong Med J 2011;17(Suppl 2):41-3.

Health Research Symposium 2010: improving health and recognising excellence

The Health Research Symposium 2010 was held on 11 September 2010 at the Hong Kong Academy of Medicine. The symposium was organised by the Food and Health Bureau (FHB) and aimed to (1) disseminate significant findings of research projects supported by its funds to the local research community, (2) facilitate the exchange of ideas between invited overseas experts and local researchers on aspects of health-related research, and (3) acknowledge outstanding local researchers.

The FHB supports health-related research via two funds, namely the *Health and Health Services Research Fund* (HHSRF) and the *Research Fund for the Control of Infectious Diseases* (RFCID). To date, the two funds have supported 334 investigator-initiated research projects.

The HHSRF supports research in public health, health services and Chinese medicine. To date, 81 of 117 HHSRF projects have been completed. The RFCID was established in 2003 after the severe acute respiratory syndrome (SARS) outbreak to fund research related to the control of infectious diseases. The RFCID supports commissioned as well as investigator-initiated research. From 2004 to 2009, four large-scale commissioned studies worth HK$93.2 million were conducted by The University of Hong Kong, the Chinese University of Hong Kong, a consortium led by the Hospital Authority, and the Centre for Health Protection. In total, 105 separate projects were supported in this first phase of commissioning, of which at least 93 have been completed. A second phase of commissioning worth HK$79 million has started to disburse grants to the two universities over 5 years from 2009 to 2014. In addition, 157 of 217 RFCID investigator-initiated projects have been completed. Thus, there is a large body of locally generated and relevant research that will benefit from further dissemination.

The two themes of the symposium were *Application of statistical and mathematical models to understand infectious disease dynamics* (RFCID theme) and *Cost-effectiveness in health services research* (HHSRF theme). To ensure coverage of the wide spectrum of research outcomes, these themes were explored and discussed from the perspectives of multiple stakeholders, including researchers, funders, policy makers, administrative users, and patients.

Morning plenary session

The symposium commenced with a welcome speech from Dr York Chow, the Secretary for Food and Health. He welcomed the more than 500 delegates and presented souvenirs to the keynote speakers. The morning keynote session was moderated by Dr PY Lam, Director of Health. The first keynote presentation was made by Prof Christl Donnelly (Department of Infectious Disease Epidemiology, Imperial College London, UK). Her presentation was titled *Using statistical and mathematical models for infectious diseases at the science – public policy interface*. Prof Donnelly noted that the analysis of a newly identified disease, or a new strain of a familiar one, is particularly challenging as the data are not always sufficient to provide early answers to key scientific and policy questions. Epidemiologists can help public policy makers control disease without too much disruption to society through appropriate risk communication. Risk communication is a key component of the scientist–policy maker interaction as well as an important interactive process which actively involves stakeholder groups from the outset.

The second keynote speaker was Prof Joseph Sung (Vice-Chancellor and President of the Chinese University of Hong Kong). Having played a key role in controlling the spread of SARS in Hong Kong in 2003, Prof Sung's presentation was titled *Research in infectious diseases: past, present and future*. Prof Sung observed that with globalisation and frequent international travel, the spread of infectious disease is much faster and much more difficult to control. As with SARS, the identification of the causative organisms and their natural reservoirs hold the key to the control of infectious disease and its clinical management. International collaboration coordinated by World Health Organization coupled with the use of information technology and molecular biology are essential components for effective outbreak control. Dr PY Lam moderated a question-and-answer session after the presentations.

After the morning keynote session, the delegates attended parallel sessions related to either HHSRF or RFCID topics.

Health and Health Services Research Fund parallel session

Prof Jean Woo (School of Public Health and Primary Care, The Chinese University of Hong Kong) gave a presentation titled *Health services research questions in elder care*. Prof Woo noted that a prime aim of conducting health services research is to use the findings to engage with the general public and policy makers, and also to facilitate debates about

priorities for health service delivery and planning. Important health service issues in the care of the elderly include prevention and management of geriatric syndromes (eg frailty, falls, cognitive and functional decline), service provision models (complex interventions), and patients' perspective of the service provided. Changing patient profiles and service settings generate research questions that contribute to continuous quality improvement, evidence-based practice, provide cost-effectiveness and cost-benefit data to guide service providers to formulate policies.

Prof Kenneth Lee (School of Medicine and Health Sciences, Monash University Sunway campus, Malaysia) gave a presentation titled *Application of health technology assessment to drug evaluation*. Health technology assessment is a form of policy research that examines short- and long-term consequences of the application of a health care technology, including drugs, biologics, devices, procedures, support systems, and health programmes. The goal of health technology assessment is to inform policy makers of policy alternatives. For any given technology, properties and impacts assessed may include technical properties, evidence of safety, efficacy, real-world effectiveness, cost, and cost-effectiveness as well as estimated social, legal, ethical, and political impacts.

Prof Sarah McGhee (School of Public Health, The University of Hong Kong) gave a presentation titled *Cost-effectiveness in diabetic retinopathy care*. Prof McGhee commented that more health service research is needed to help formulate policy and practice in Hong Kong. One example is diabetic retinopathy, a leading cause of blindness in those under 65 years of age. Screening for diabetic retinopathy is usually highly cost-effective, but such screening often involves a co-payment, because of the mixed medical economy incorporating private and public services. Preliminary data indicate that even a low co-payment may deter some from screening and consequently they may be at higher risk. Further work to determine whether existing financial safety nets could avoid inequity is needed.

Prof Tze-wai Wong (School of Public Health and Primary Care, The Chinese University of Hong Kong) gave a presentation titled *Morbidity and mortality attributed to air pollution: evidence and challenges*. The harmful effects of air pollution on health have long been recognised, and associations between air pollution and morbidity/mortality reported. Nonetheless, many questions on air pollution and health remain unanswered. Challenges to future studies include the development of more precise exposure assessment techniques, a better understanding of the joint effects of air pollutants and their interactions with climate, the mechanisms of action of some air pollutants on the cardiovascular and respiratory systems, the fate of inhaled ultrafine particulates (nanoparticles) and their effects on different organs and systems, and the development for more specific indicators and biomarkers of body responses to air pollution.

Dr Irene Wong (School of Public Health, The University of Hong Kong) gave a presentation titled *Cost-effectiveness of mammography screening*. Such screening has been accepted in most western populations. Nonetheless, this may not readily apply to Chinese women who have a much lower breast cancer incidence and different age profile. A state-transition decision model was developed to simulate breast cancer progression and to determine the cost-effectiveness of alternative mammography screening strategies among Hong Kong Chinese women aged 40 years or older. The results suggest that mass biennial screening may not be cost-effective for Hong Kong in terms of allocation of resources.

Prof Samuel Wong (School of Public Health and Primary Care, The Chinese University of Hong Kong) gave a presentation titled *Evaluation of general outpatient clinics using the primary care assessment tool*. In Hong Kong, the main goal of publicly funded general outpatient clinics is to provide primary medical services for the financially vulnerable. In a territory-wide telephone survey comparing primary care experiences of general outpatient clinic users and private general practitioner users, Prof Wong reported that the latter had better primary care experiences. This was largely due to the greater accessibility and better interpersonal relationships offered by private general practitioners.

Research Fund for the Control of Infectious Diseases parallel session

Dr Ben Cowling (School of Public Health, The University of Hong Kong) gave a presentation titled *Clinical effectiveness of seasonal influenza vaccination against pandemic and seasonal influenza*. Vaccination is effective in preventing infection and illness associated with seasonal influenza viruses when circulating strains match the vaccine strains. Dr Cowling described a double-blind randomised trial on 431 individuals belonging to 119 Hong Kong Chinese households. One child aged 6 to 15 from each household was randomised to receive one dose of inactivated trivalent seasonal influenza vaccine or saline placebo. The vaccine recipients had lower rates of serologically confirmed seasonal A/H1N1 infection and A/H3N2 infection but higher rates of serologically confirmed pandemic A/H1N1 infection. These data indicated that vaccination against seasonal influenza protected against strain-matched infection in children. Naturally acquired seasonal influenza infection appeared to confer cross-protection against pandemic influenza. Whether prior seasonal influenza vaccination predisposes to a higher risk of infection with the pandemic strain requires further investigation.

Dr Steven Riley (School of Public Health, The University of Hong Kong) gave a presentation titled *Serological studies and the transmission dynamics of influenza*. During and between pandemics, good knowledge of the transmission dynamics of influenza can help to improve public health decisions. Dr Riley described a paired serological survey of human swine influenza exposure in a largely representative cohort of households in Hong Kong. The survey also gathered data on severe cases from the whole population. The data indicated that the human swine influenza epidemic in Hong Kong infected more children than adults. The rate of infection in older adults was low but the infection was more severe. Dr Riley concluded that predicting the peak of an outbreak of a novel pathogen is difficult without accurate knowledge of the rate of infections in different transmission groups. Surveillance of currently circulating strains of influenza should focus on older individuals, so as to detect any antigenic evolution that renders the pandemic strain more infectious to older adults.

Prof Shui-shan Lee (School of Public Health and Primary Care, The Chinese University of Hong Kong) gave a presentation titled *Exploratory research in infectious disease epidemiology - the HIV example*. Epidemiology has assumed a central role in HIV research. Prof Lee identified three key characteristics of epidemiological studies of HIV. First, cohort studies have assumed an important position in describing epidemiology, as exemplified by the Multicentre AIDS Cohort Study. There are more than 200 HIV/AIDS cohorts globally, which continue to generate new knowledge that informs clinical and public health interventions. Second, public health surveillance has become an expanded concept in epidemiology, which covers not just clinical HIV disease but infection and behaviour. Third, methodological exploration has led to the widespread use of molecular approaches, spatial studies, and social network analysis, which have advanced our understanding of the transmission dynamics of the virus and its determinants at individual, social and population levels.

Dr Joseph Wu (School of Public Health, The University of Hong Kong) gave a presentation titled *Cost-effectiveness of HPV vaccination*. Cervical cancer causes significant morbidity and mortality among women worldwide. Human papillomavirus (HPV) infection of the cervix is the cause of cervical cancer. In addition to cervical screening for early detection, vaccines that prevent infection of the two most prevalent HPV types (16 and 18) have been developed and are commercially available in Hong Kong. Mathematical models were built to perform cost-effectiveness analyses to evaluate the public health impact of large-scale HPV vaccination. In addition, surveys were conducted to investigate knowledge and receptiveness of HPV vaccination among females. Of 2254 adolescent girls and 1023 women who had daughters under 18 years of age, 33% of adolescent girls and 45% of women would consider vaccinating themselves and their daughters. Age of vaccination was the main factor influencing tendency to vaccinate. Both groups expressed that the most suitable age for vaccination was 15 to 16 years, which was older than that recommended by vaccine manufacturers (9-12 years). Such vaccination costs US$150 000, 94 000 and 77 000 per life-year after vaccination has begun for 20, 40 and 60 years, respectively. This suggested that adding a long-term HPV vaccination programme to current screening practice may be cost effective in reducing the burden of cervical cancer in Hong Kong.

Prof David Hui (School of Public Health and Primary Care, The Chinese University of Hong Kong) gave a presentation titled *Exhaled air dispersion during application of common respiratory therapies*. Viral pneumonia such as SARS and influenza may be spread by airborne transmission. Prof Hui used laser smoke visualisation techniques to examine exhaled air dispersion during application of common respiratory therapies in a hospital setting. Substantial exposure to exhaled air occurs within 0.4 m and 0.8 m in patients receiving oxygen via a simple mask and treatment via a jet nebuliser, respectively. Nasal positive pressure ventilation provided via a range of equipment also poses a risk of infection as exhaled air may be dispersed in excess of 0.95 m in some circumstances. In addition, substantial exposure to exhaled air occurs within 1 m from the end of the bed in patients receiving oxygen via nasal cannula in large isolation rooms with efficient air exchange, whereas diffuse room contamination occurs in smaller isolation rooms with less efficient air exchange in specific hospital settings. Health care workers may need to take extra infection-control precautions when managing patients with pneumonia and respiratory failure in these small isolation room settings.

Prof Yu-guo Li (Department of Mechanical Engineering, The University of Hong Kong) gave a presentation titled *Modelling infectious diseases from an engineering perspective*. Engineering control measures such as air cleaning and ventilation play an important part in infection control and should be considered together with administrative measures and use of personal protective equipment. Prof Li described his studies on the fundamental properties and behaviour of airborne infectious droplets. Respiratory droplets are commonly between 35 and 100 microns in diameter (range, 0.1-1000 microns). Droplets larger than 60 microns are involved in large droplet transmission of diseases. Sneezing expels air at a velocity of 50 m/s, and potentially infectious droplets can be carried more than 6 m. In contrast, coughing and breathing can expel air at a velocity of 10 m/s and 1 m/s and carry droplets up to 2 m and 1 m, respectively. Large droplets are removed by deposition on to exposed surfaces, whereas fine droplet nuclei are removed by ventilation. Properly designed natural ventilation is an accepted measure for infection control. The size and ventilation properties of isolation

rooms are therefore important parameters to the control of respiratory infectious diseases.

Final plenary session

After the parallel sessions, the delegates reassembled for the final plenary session moderated by Prof Sian Griffiths (Professor and Director of the School of Public Health and Primary Care, the Chinese University of Hong Kong) and Prof TH Lam (Chair Professor and Head of the School of Public Health, The University of Hong Kong). The third keynote speaker was Prof Karen Kuntz (Division of Health Policy and Management, School of Public Health, University of Minnesota, USA), whose presentation was titled *The role of economic evaluation for the allocation of healthcare resources*. Prof Kuntz noted that in an environment of escalating health care costs, relying on evidence of quality, safety, and efficacy of an intervention may not be sufficient for determining coverage decisions. Economic evaluations provide a framework for maximising the level of health care that can be achieved within a population, thus providing a measure of 'value for money' associated with health care interventions. Economic evaluations, which include cost-effectiveness and cost-utility analyses, provide an explicit, quantitative, and systematic approach to synthesising information on the clinical benefits of an intervention or programme, the associated risks and harms, and the economic costs. Prof Kuntz gave an overview of the methods used to conduct economic evaluations and discussed the role and implications of the perspective of the analysis (eg government, societal) and how results may differ by perspective.

The final keynote speaker was Prof KY Yuen (Department of Microbiology, The University of Hong Kong), whose presentation was titled *Emerging microbial agents in humans and animals in Hong Kong and Southern China*. Prof Yuen noted that emerging infections originating in Mainland China may actually be first detected in Hong Kong, because of its better surveillance and laboratory infrastructure. This places Hong Kong in a leading position in the discovery of novel microbes associated with human or animal diseases. Prof Yuen described the wide range of novel or emerging pathogens that have been discovered in recent years in Hong Kong, including viruses (eg human, civet and bat SARS-coronaviruses) and bacteria (eg *Laribacter hongkongensis*, *Streptococcus sinensis*). Most of these microbes were originally found in clinical specimens such as blood or pus before some were traced back to an animal source. In some cases, clinical findings enhance health policy, as was seen in the SARS epidemic, where ecological and epidemiological investigations helped to prevent re-emergence of SARS by encouraging the banning of game food in live animal markets in southern China.

Award ceremony

After a question-and-answer session moderated by Prof Griffiths and Prof Lam, the symposium ended with an award ceremony to acknowledge outstanding investigators whose research findings have influenced health policy and practice in Hong Kong.

Best Poster (HHSRF) was awarded to Dr Simon SM Ng (Department of Surgery, The Chinese University of Hong Kong) for his work titled *Electroacupuncture for postoperative ileus after laparoscopic colorectal surgery: a randomized sham-controlled study.*

Best Poster (RFCID) was awarded to Prof Annie NY Cheung (Department of Pathology, The University of Hong Kong) for her work titled *Integrated human papilloma virus analysis as adjunct for triage of atypical cervical cytology.*

Most Promising Young Researcher Award was given to Prof Nelson Lee Lai-shun (Department of Medicine and Therapeutics, The Chinese University of Hong Kong) for his work titled *Influenza virus load in hospitalised patients*.

Excellent Research Award in Health and Health Services Research was given to Dr Irene Wong Oi-ling (School of Public Health, The University of Hong Kong) for her work titled *A cost-effectiveness analysis of mammography screening in Hong Kong Chinese using state-transition Markov modeling*.

Excellent Research Award in Public Health was given to Dr Benjamin Cowling (School of Public Health, The University of Hong Kong) for his work titled *Comparison of statistical algorithms for early detection of the start of the annual influenza peak season in Hong Kong using sentinel surveillance data*.

Excellent Research Award in Clinical Studies was given to Prof Henry Lik-yuen Chan (Department of Medicine and Therapeutics, The Chinese University of Hong Kong) for his work titled *Role of hepatitis B virus covalently closed circular DNA in determination of treatment outcome*.

Excellent Research in Basic and Laboratory Science was awarded to Prof Guan Yi (Department of Microbiology, The

University of Hong Kong) for his work titled *Genetic characterisation of H5N1 influenza viruses isolated from different regions of southern China.*

Excellent Research in Basic and Laboratory Science was awarded to Prof Yuen Kwok-Yung (Department of Microbiology, The University of Hong Kong) for his work titled *Wild animal surveillance for coronavirus HKU1, a novel coronavirus associated with pneumonia in patients in Hong Kong, and potential variants of other coronaviruses that infect humans.*

Closing remarks

Prof Gabriel Leung, Under Secretary for Food and Health, made the closing remarks. He thanked all the delegates for attending and outlined areas for consideration of future research. These included the four ongoing 'epidemics' of emerging infectious diseases, chronic disease, environmental insults, and social inequality. He also noted the existence of methodological gaps including the lack of robust decision analytic models and health economic studies and the need for more community-based studies.

The Food and Health Bureau is committed to supporting local research to provide evidence-based information for health policy formulation and to enhance public health through continuous improvement in health care practices.

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Postnatal depression among Hong Kong Chinese fathers

Key Messages

1. The prevalence of postnatal depression in Hong Kong Chinese fathers was 4.9%.
2. Postnatal depression in fathers was closely linked with postnatal depression in mothers.
3. Risk factors differed between fathers and mothers. Fathers who had life events, stress, worries over unemployment, poor marital relationships, low social support, and *peiyue* care were more likely to have postnatal depression. Mothers who had antenatal depression, past depression, past neurasthenia, and unhelpful *peiyue* care were more likely to have postnatal depression.
4. The association between paternal and maternal postnatal depression was unlikely the result of commonly perceived risk factors. Instead, the partner's mood per se was an important factor in the aetiology of postnatal depression.

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Introduction

Worldwide, postnatal depression affects 10 to 15% of recently delivered women. As paternal involvement in childcare becomes more prevalent, paternal postnatal depression is not uncommon and has been estimated to affect 5 to 24% of fathers in western societies. Paternal postnatal depression is closely linked to maternal postnatal depression. Hence, the affective experiences of the couple should be studied as a whole.

Factors that place fathers at risk of depression include unemployment, poor marital relationship, stress, life events, lack of social support, and a psychiatric history. Some of these factors are also related to maternal postnatal depression.

Sociocultural factors help shape the affective well being of Chinese mothers. The practice of *peiyue* (literally 'accompanying the month') mandates family support of the mother in the first postnatal month, which helps to reduce postnatal depression in mothers.[1] This practice could also be relevant to the adjustment of fathers in the postnatal period. However, men may have gender-specific risk factors that are absent or less influential in women. For example, work-related stress and the relationship with their partner may be more important for the adjustment of fathers than for mothers. Thus, it is vital to identify the psychosocial factors that predispose fathers to postnatal depression, and examine whether these factors are shared with maternal depression.

The present study attempted to determine the prevalence and incidence of postnatal depression among Hong Kong Chinese fathers, and identify psychosocial risk factors of paternal postnatal depression, and examine whether these risk factors are also present in maternal depression.

Method

Study design

This prospective cohort study was conducted from September 2005 to April 2007 at the Prince of Wales Hospital. All Chinese couples consecutively admitted to the postnatal ward were invited to participate. The inclusion criteria were Chinese ethnicity and permanent residency in Hong Kong.

Eligible participants were interviewed by trained research nurses immediately following delivery. Information on seven categories of risk factors was obtained. Participants completed the Chinese versions of the Edinburgh Postnatal Depression Scale (EPDS) and the Beck Depression Inventory (BDI).

At postpartum week 8, the participants repeated the EPDS and BDI. Information on *peiyue* care was solicited from the maternal participants. All participants who scored above a BDI cut-off score of 10.5 or the EPDS cut-off score of 9.5 (high scorers) were invited for a psychiatric diagnostic interview—the Chinese non-patient version of the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. Forty percent of participants who scored below the BDI and EPDS cut-off (low scorers) were randomly selected for the interview. The prevalence and incidence of paternal postnatal depression were calculated by means of a two-phase design using reverse weighting.

Sample size

A total of 2351 couples were approached; the overall response rate was 44%. At postpartum week 8, 551 couples returned for the follow-up assessment.

Study instruments

The EPDS is a 10-item scale for assessing postnatal depression. Its reliability and validity has been demonstrated. The Chinese version of the EPDS has been validated among Chinese women and its psychometric performance is comparable to the original scale.

The BDI is a 21-item self-rating scale for measuring the severity of depression. The Chinese version of the BDI has been validated among Chinese women to identify postnatal depression and has been shown to have satisfactory psychometric properties.

The Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition is a semi-structured interview that allows the interviewer to use additional questions to enquire about indigenous idioms of distress so that it can be used in a culturally-informed way. It is widely used as the gold standard of psychiatric diagnosis and the Chinese version has been validated.

Results

The sociodemographics of the participants are summarised in the Table. Fathers who completed the follow-up study did not differ from those who dropped out in terms of baseline EPDS and BDI scores. However, those who completed the study were more likely to be married and less likely to have a history of psychiatric illness.

Prevalence and incidence of paternal postnatal depression

The prevalence of paternal postnatal depression was 4.9% (2.4% major and 2.5% minor). The incidence was 3.5% (1.6% major and 1.9% minor) per year.

Relations between paternal and maternal depression

Paternal and maternal postnatal depression was significantly associated (P=0.04, McNemar test). When a father was diagnosed with depression, his partner had a 31% risk of suffering from depression.

Risk factors of postnatal depression

Multiple regression analysis indicated that paternal postnatal depression was predicted by maternal postnatal depression (β=0.30, P=0.00), life events (β=0.13, P=0.00),

Table. Sociodemographics of paternal participants

Characteristics	Fathers assessed at post-partum week 8 (n=551)		Fathers not assessed at postpartum week 8 (n=57)		Statistics	
	No.	%	No.	%	χ^2	P value
Place of birth					1.39	0.50
Hong Kong	476	86.4	51	89.5		
Mainland China	67	12.2	5	8.8		
Others	8	1.5	0	0		
Marital status					24.49	0.00
Married	533	96.7	47	82.5		
Cohabiting	17	3.1	9	15.8		
Separated or divorced	0	0	0	0		
Single	1	0.2	1	1.8		
Education level					1.56	0.82
No formal education	1	0.2	0	0		
Kindergarten	1	0.2	0	0		
Primary	18	3.3	2	3.5		
Secondary	309	56.1	27	47.4		
University	222	40.3	27	47.4		
Occupation					4.42	0.35
Unskilled	17	3.1	1	1.8		
Semi-skilled	18	3.3	0	0		
Skilled	323	58.6	29	50.8		
Semi-professional	145	26.3	19	33.3		
Professional	48	8.7		12.3		
Homemaker	0	0	0	0		
Social class					4.51	0.34
I	48	8.7		12.3		
II	144	26.1	19	33.3		
III	324	58.8	29	50.9		
IV	187	3.37	07	0		
V	17	3.1	1	1.8		
Medical history					1.91	0.38
Nil	460	83.5	42	73.7		
Inactive	19	3.4	0	0		
Active	69	12.5	5	8.8		
Psychiatric history					7.41	0.03
Nil	538	97.6	44	77.2		
Inactive	3	0.5	2	3.5		
Active		1.3	1	1.8		
	Mean	SD	Mean	SD	z	P value
Age (years)	33.4	5.9	32.4	5.5	-0.50	0.62
No. of children	1.3	0.5	1.3	0.5	-0.46	0.65
Monthly household income (HK$)	37 493	85 823	45 075	101 300	-0.55	0.58
Edinburgh Postnatal Depression Scale score	4.9	4.3	5.4	5.2	-0.36	0.72
Beck Depression Inventory score	3.8	5.0	5.2	7.1	-0.71	0.48

perceived stress (β=0.16, P=0.00), potential threat of self unemployment (β=0.14, P=0.00), poor martial relationship (β= –0.14, P=0.00), lack of social support during pregnancy (β= –0.11, P=0.00), and the presence of *peiyue* care (β=0.10, P=0.00).

On the other hand, maternal postnatal depression was independently predicted by paternal postnatal depression (β=0.31, P=0.00), past depression (β=0.21, P=0.00), antenatal depression (β=0.12, P=0.00), past neurasthenia (β=0.11, P=0.01), and unhelpful *peiyue* care (β= –0.16, P=0.00).

Discussion

The prevalence of Hong Kong Chinese fathers suffering from postnatal depression was 4.9%, which is similar to 1.8% reported in Singapore and 2.9% in Australia.[2,3] This suggests that a substantial proportion of fathers experienced emotional problems during the transition to parenthood. The role of fathers has changed and they are expected to share greater responsibilities in childcare. Thus, postnatal depression should be conceptualised as a mental health problem affecting both fathers and mothers. It is essential for health care providers to be aware of the mental health needs of fathers during the transition to fatherhood.

Postnatal depression in one partner correlated with postnatal depression in the other. This association has been consistently demonstrated, but is not likely to be related to common risk factors.

Paternal postnatal depression was predicted by life events and stress (work-related stress in particular). Perhaps fathers are primary providers for material support for the family. The arrival of a new family member may increase economic pressure on the father, thus compromising his psychological well being. Social support and marital support also play an important role in the affective experiences of fathers during the early postnatal period. Perhaps men often rely on their spouse for emotional support as their social networks are more restricted. When the marital relationship is unsatisfactory and the amount of spousal support deteriorates, fathers may be more negative toward their paternal role and exhibit more adjustment problems.

For mothers, the most reliable predictor of postnatal depression is a history of depression. Our findings support this possibility, as maternal postnatal depression was associated with past depression, antenatal depression, and past neurasthenia.

The association between paternal and maternal depression was not likely to be the result of common risk factors. Instead, a partner's mood per se was a risk factor in the aetiology of postnatal depression. Mood emerged as the most important predictor when other risk factors were considered. This highlights the importance of studying the couple as a whole for a comprehensive understanding of the affective experiences of new parents. Although routine screening in all fathers may not be plausible in clinical setting, our findings suggest that when the mothers are depressed, screening for paternal depression can be useful in identifying fathers at risk. In our hospital, it is a routine practice for mothers to complete the EPDS at postpartum weeks 6 to 8. Those who score above the cut-off score are referred to the postnatal depression care unit, so long as they consent. Male partners of these high scorers could also be evaluated for depressive symptoms using the EPDS. Fathers can also be involved in the management of maternal depression. When the father was involved in treatment, not only was the recovery of maternal postnatal depression expedited, but the father also benefited by lowering his own risk of becoming depressed.[4]

Health care providers should be cautioned about the gender-specific nature of risk factors when identifying new parents who are likely to develop postnatal depression. Each partner may have particular concerns, types of distress, and vulnerabilities that are specific to their gender role during the transition to parenthood. It is likely that their affective experiences are shaped by different types of factors. Hence, the intervention and management of postnatal depression should be designed in a way that addresses the specific needs of new parents.

The practice of *peiyue* care may render new parents vulnerable to depressive symptoms. The mere presence of *peiyue* placed fathers at a heightened risk of depression in the early postnatal period, as mother-in-laws were the most frequently reported providers of *peiyue* care in our sample. Conflicts between mother-in-law and daughter-in-law remain a common issue in modern Chinese families. The father is often caught in between the role of a filial son and a loving husband. Thus, the transgenerational in-law conflicts and its accompanying distress may further add onto the burden of new fathers. Mothers who perceived the *peiyue* care as unhelpful had a greater tendency to report depressive symptoms. Perhaps societal values have changed and discrepancies may exist in the attitudes towards childcare and customary practice between the two generations. Conflicts, instead of support, may arise and parents' affective well being is hampered. It is important to rethink whether cultural practices, which are legacies from the past, continue to benefit contemporary parents.

The present study highlights the importance of incorporating the fathers' affective experiences to derive a more comprehensive representation of postnatal mental health problems. Follow-up studies over a longer time period are needed to examine the course of paternal postnatal depression, and to develop intervention programmes to help fathers in need.

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Supported employment versus traditional vocational rehabilitation for individuals with severe mental illness: a three- ear study

Key Messages

1. We developed an innovative Integrated Supported Employment (ISE) service protocol, which amplifies the effect of the Individual Placement and Support (IPS) model by the addition of work-related social skills training.
2. Participants in the ISE outperformed those in the IPS and traditional vocational rehabilitation (TVR) with respect to employment rate, job tenure, and some psychological outcomes, when assessed 39 months after admission to such services.
3. Self-efficacy was improved in ISE and IPS (but not TVR) participants after receiving service for 18 months.
4. The long-term employment rate of participants was higher in the ISE than the IPS programme.
5. The ISE is potentially applicable to other Chinese communities including mainland China.

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Introduction

Work has long been regarded as a potential contributor and a tool for the treatment of mental illness. Returning to work was regarded as a significant factor for recovery by health care professionals in Hong Kong.[1] However, only 20.3% (174 000 people) of those with mental illness in Hong Kong were actively employed or actively participated in economic activities.

Traditional vocational rehabilitation (TVR) adopts a step-wise approach, which offers pre-vocational training at the beginning. Participants are paid less than the minimum wage, and rates for competitive employment are usually less than 20%. Owing to the limitations of TVR, a supported employment service is recommended.

We developed an Integrated Supported Employment (ISE) programme, which amplifies the vocational outcomes of the Individual Placement and Support (IPS) model, by the addition of work-related social skills training (WSST). Other than the WSST module,[2] on-going support is also provided. After 15 months of service, ISE participants had significantly higher employment rates (78.8% vs 53.6%) and longer job tenure (23.84 vs 12.34 weeks) than those receiving IPS.[3]

Studies regarding the influence of such programmes on self-efficacy, psychiatric symptom control, quality of life, and psychosocial well being are limited, as are studies on long-term vocational outcomes of supported employment in a non-US culture. We therefore examined long-term vocational and psychological outcomes of ISE in Hong Kong for up to 3 years. To faciliate the understanding of both short- and long-term outcomes, data collected in the previously funded project HSRF-S121014 were included where necessary.

This study aimed to compare the long-term influence of ISE and IPS in terms of vocational (job satisfaction, job tenure, job stress coping, and job mobility) and psychological (symptom control, relapse rate, self-efficacy, and subjective personal well being) outcomes. The hypothesis was that the group receiving ISE would have better vocational and psychological outcomes than the groups receiving IPS or TVR.

Methods

This randomised controlled trial was conducted from September 2005 to August 2007. The subjects were randomly assigned to three different vocational services: TVR, IPS, and ISE.

Traditional vocational rehabilitation involves comprehensive vocational assessment and pre-vocational training carried out in vocational rehabilitation centres.

Individual Placement and Support represents a synthesis and standardisation of eight principles of support employment[4]: (1) a single-minded focus on

competitive employment; (2) eligibility for services being based solely on client choice, with no exclusions related to work readiness, substance use problems, lack of motivation, treatment non-compliance, etc; (3) a rapid job search upon programme admission, using the 'place then train' approach; (4) attention to client preferences in the job search, rather than dependence on job availability; (5) close integration between employment services and the mental health treatment team; (6) ongoing, individualised support and job training after the clients obtain employment; (7) systematic benefits counselling; and (8) consultation with the employer or job supervisor including advocacy accommodations.

Integrated Supported Employment combined IPS and 10 sessions of WSST. Training of social skills was provided to ISE participants before they obtained employment. A problem-solving approach was used to help participants handle interpersonal conflicts throughout the follow-up period.

Between 2003 and 2006, 189 participants were randomly assigned to TVR (n=66), IPS (n=65), and ISE (n=58) [Table 1 and Fig]. The subjects were recruited from three community mental health service units and two day hospitals, based on the following selection criteria: (1) severe mental illness (schizophrenia, schizo-affective disorder, bipolar disorder, recurrent major depression, or borderline personality disorder), (2) at least 2 years of major role dysfunction, (3) medium-to-high functioning capacity and free from serious role dysfunction in the past 3 months, (4) unemployed, (5) willing and cognitively competent to give informed consent, (6) no memory impairment, learning disorder and neurological or medical illness that would preclude working or participating in research interviews, (7) completion of primary education, and (8) a desire to work. The diagnosis of the participants was based on medical records kept by certified psychiatrists in Hong Kong according to DSM-IV criteria. Informed consent was obtained from each participant.

The outcome measures included the vocational (employment rate, job tenure, salary, and number of job terminations) and psychological aspects. The following psychometrically valid instruments were used: (1) medical history, work history, and demographic data, (2) Employment Outcome Checklist, (3) the 21-item Chinese Job Stress Coping Scale, (4) the Chinese Job Termination Checklist, (5) Personal Well Being Index, (6) the Chinese General Self-efficacy Scale, and (7) the Chinese Job Satisfaction Scale.

From the commencement of the 3-month initial service provision, both ISE and IPS programmes lasted 39 months, whereas the TVR programme lasted 15 months. Assessments were conducted at baseline and month 7, 11, 15, 21, 27, 33, and 39 by an independent, trained, and blinded registered occupational therapist (Fig).

Three registered occupational therapists as employment specialists implemented the ISE and IPS protocols. Social workers (who referred cases to our study) were also involved. Prior training for employment specialists was provided by Prof Gary Bond, Dr Robert Drake, Ms Debbie Becker, and the author. The 15-item supported employment fidelity scale was adopted to ensure the quality of our service. The PI met with the employment specialists at month 1, 4, 9, 15, 21, 27, and 33 and used the scale to check adherence of the two protocols to the principles of supported employment. The scores of IPS participants ranged from 66 to 68 out of 75 (88-91%) and those of ISE participants ranged from 65 to 68 out of 75 (87-91%). Both protocols demonstrated good fidelity to supported employment implementation. For TVR participants, services were provided by staff members of service centres that provided sheltered vocational training in the community. Employment specialists met with the participants mainly in their workplace or restaurants close to their workplace. If face-to-face meeting could not be

Table 1. Participant characteristics of Integrated Supported mployment (ISE), Individual Placement and Support (IPS), and traditional vocational rehabilitation (TVR) groups

Characteristics	No. (%) of participants			χ^2	*df*	P value
	ISE (n=58)	IPS (n=65)	TVR (n=66)			
Mean±SD age (years)	34.12±8.68	34.08±9.01	36.50±7.57	-	-	0.195; F-value, 1.647 (2, 186)
Gender				1.570	2	0.456
Male	26 (44.8)	36 (55.4)	31 (47.0)			
Female	32 (55.2)	29 (44.6)	35 (53.0)			
Education				15.272	6	0.018
Below primary	0 (0)	0 (0)	1 (1.5)			
Primary	4 (6.9)	3 (4.6)	12 (18.2)			
Secondary	47 (81.0)	49 (75.4)	50 (75.8)			
Post-secondary	7 (12.1)	13 (20.0)	3 (4.5)			
Diagnosis				1.207	2	0.547
Schizophrenia	45 (77.6)	47 (72.3)	53 (80.3)			
Others	13 (22.4)	18 (27.7)	13 (19.7)			
Employment history				1.576	2	0.455
Yes	53 (91.4)	57 (87.8)	62 (93.9)			
No	5 (8.6)	8 (12.3)	4 (6.1)			

arranged, discussion took place via phone calls.

Intention-to-treat analyses of the employment rates were conducted on the entire sample (n=189). As we aimed to compare the long-term outcomes of IPS and ISE, only these two groups were followed up at month 21, 27, 33, and 39. The demographic variables of the three groups were compared using the Chi-square statistic or one-way ANOVA. Independent sample *t*-tests were used to compare the number of contacts for the ISE and IPS groups by the employment specialists. The Chi-square statistic was used to compare the overall job nature, and the programme attrition rates and the cumulative employment rates of the three groups. Post-hoc testing of employment rates was implemented by exact logistic regression. Success in competitive employment was defined as having continuously worked in the job for ≥2 months for at least 20 hours per week. Repeated measures ANOVA with post hoc analysis was used to determine whether there were significant differences between groups in terms of job tenure, job satisfaction, and psychological outcomes. The job tenure referred to the longest duration (in weeks) a participant worked for the same job during the study.

In terms of salary and number of job terminations, comparisons were only made for ISE and IPS groups, because only a few TVR participants were employed. Missing data were prominent when the participants were unemployed or their job was not terminated during the follow-up assessments. Thus, an independent sample *t*-test and Chi-square statistic were used to evaluate whether IPS or ISE participants experienced more workplace interpersonal difficulties resulting in job terminations.

Results

From the beginning to month 15 and from month 16 to 39, the number of telephone (t= -1.34, P=0.19) and face-to-face (t= -0.89, P=0.38) contacts in ISE and IPS groups not significantly different.

The attrition rates of the three groups did not differ significantly; 44 (75.9%) ISE and 41 (63.1%) IPS participants completed the final follow-up at month 39, whereas 54 (81.8%) TVR participants completed the final follow-up at month 15.

During the corresponding follow-up periods, 48 (82.8%) ISE and 40 (61.5%) IPS participants successfully obtained competitive employment, but only four (6.1%) TVR participants did so. Employment rates of the three groups were significantly different at month 7, 11 and 15.

There were 27 (57.4%) ISE, 21 (55.3%) IPS, and three (100%) TVR participants worked full-time, whereas 20 (42.6%) ISE and 17 (44.7%) IPS participants worked part-

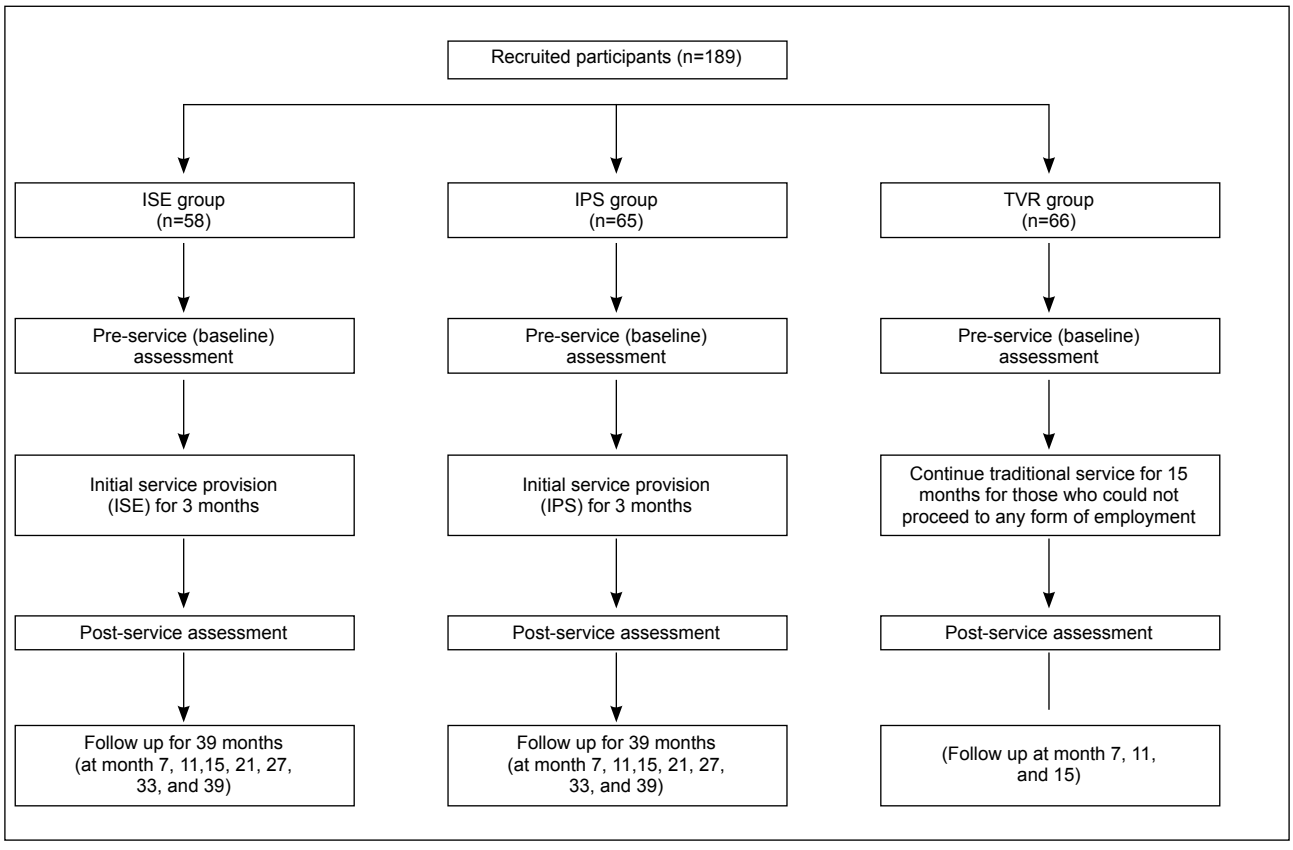

Fig. Data collection protocol of Integrated Supported Employment (ISE), Individual Placement and Support (IPS), and traditional vocational rehabilitation (TVR) groups

time. Data of four participants were missing. Job nature of the three groups was not significantly different (χ^2=2.294, *df*=1, P=0.318, Table 2).

At the 7-, 11-, and 15-month follow-ups, ISE participants stayed longer in a job than IPS (P<0.001 to P=0.008) and TVR (P<0.001) participants. Job tenure of the three groups was significant different *(F*=9.526, *df*=4,316, P<0.001).

Data in the baseline and month 21 to 39 of the ISE and IPS groups were compared. Overall significance (*F*=3.394, *df*=4,100, P=0.012) on time x group interactions between the two groups was found. The results of post-hoc comparison indicated that job tenure of the ISE group was significantly longer than that of the IPS group at 21-month follow-up (P<0.011). Although no significant difference was noted between the two groups for the remaining follow-ups, the same trend was consistently noted at the 27-, 33- and 39-month follow-ups.

The two groups did not significantly differ in terms of salary at all follow-ups.

The mean number of unwanted job terminations for IPS participants was significantly higher than for ISE participants at the 7-month (P=0.031), 11-month (P=0.004) and 15-month (P=0.001) follow-ups. Although there was no significant difference at the 21-, 27-, 33-, and 39-month follow-ups, the number of job terminations in the IPS group was greater. Only 8.7% of ISE participants had faced interpersonal problems in quitting their jobs, compared to 24.2% in the IPS group.

Regarding the mean scores of the Chinese Job Satisfaction Scale, there was no significant difference between the ISE and IPS groups throughout the period nor between the three groups at 7-, 11-, and 15-month follow-ups. Nonetheless, there was a trend for the mean score of ISE participants to be higher than that of IPS participants.

The ISE (P<0.001) and IPS (P=0.004) participants obtained higher mean scores for the Chinese General Self-efficacy Scale than the TVR participants at the 15-month follow-up. Nonetheless, the mean scores of Job Stress Coping Scale, the Personal Well Being Index, and the Chinese General Self-efficacy Scale between the ISE and IPS groups were not significantly different.

Discussion

Numerous empirical reports from United State and other western countries have demonstrated the enhanced vocational outcomes of IPS among participants with severe mental illness. Based on a review on nine randomised controlled trials of IPS programmes,[4] on average 56% of participants obtain competitive employment. The employment rate of our IPS participants (61.5%) and that reported in another Hong Kong study is similar. This indicates the superiority of IPS over conventional vocational programmes. The employment rate of our ISE participants at 39-month follow-up was 82.8%, which was significantly higher than that for IPS participants (61.5%). This superiority was probably due to the efforts of the employment specialist who upgraded job interview skills of the participants. It may also be due to the WSST component; employers perceived more social skills in these job applicants when they made hiring decisions.[5] The job interview skills of the participants would have played a crucial role in influencing the decision to hire. Our hypothesis that ISE programme would result in higher employment rate with a long-lasting effect was supported.

The mean job tenure of our ISE participants at 39-month follow-up was 47 weeks, which was 10 weeks longer than for IPS participants. Our hypothesis that ISE outperforms IPS in terms of ability to enhance job maintenance was therefore supported. The social competence of ISE participants was enhanced by the WSST module, which was then maintained throughout the follow-up period. Meanwhile, the employment specialists worked together with the participants to set and upgrade individualised behavioural goals, so as to help them solve interpersonal conflicts. When participants handled interpersonal difficulties successfully, encouragement would be given. Although IPS participants received similar services, they lacked the skills training element (WSST module), which was an important difference between IPS and ISE protocols.

Many problems leading to job termination pertained to interpersonal difficulties; ISE participants were less perplexed by interpersonal conflicts and were better able to resolve them than the IPS participants, despite not significantly. This suggests that social competence at the workplace may not be the only factor affecting job terminations. Other impairments such as neurocognitive and social cognition also play a role.

Vocational outcomes (including job titles, job nature,

Table 2. Types of jobs obtained by Integrated Supported mployment (ISE), Individual Placement and Support (IPS), and traditional vocational rehabilitation (TVR) participants

Job title	No. of participants		
	ISE	IPS	TVR
Office assistant	14	12	0
Cleaning worker	6	6	0
Construction worker	1	1	0
Delivery worker	4	2	0
Healthcare worker	1	1	0
Security guard	5	2	1
Sales or shop assistant		4	2
Tutor	17	37	0
Waiter	67	27	0
Questionnaire	1	0	0
Leaflet distributor	1	3	0
Library attendant	0	1	0
Car repair worker	0	1	0

salary, and job satisfaction) of the three groups were not significantly different, because our IPS and ISE participants shared similar socio-economic characteristics (low educational level, lack of professional qualifications), which are significant predictors of these aspects. In addition, demographic variables between the stayers and pre-mature leavers in these three groups were not significantly different.

Scores for general self-efficacy were significantly higher in both ISE and IPS participants than in TVR participants at the 15-month follow-up. Nonetheless, the difference in ISE and IPS groups was not significant, because of the small sample size. Vocational outcomes were positively associated with self-efficacy, as working facilitates self-efficacy of individuals.

Limitations

The samples were small and heterogeneous and may not be generalised. The methodology would have been more rigorous had we evaluated fidelity to the WSST by therapists and acquisition of skills by participants, and the use of those skills in the workplace. More local validation of data obtained from some of these instruments was needed. Improvement in the social skills of the ISE participants should have been demonstrated by a validated social skills assessment. The symptom control and relapse rate of the participants were not reported due to administrative and practical restraints. The reasons for attrition were not officially collected. The possible effects of allegiance should be noted, as our employment specialists were not blinded to the treatment assignment of the subjects.

Conclusions

The long-term effectiveness of the ISE programme in enhancing employment rates and job tenure among individuals with severe mental illness has been demonstrated. Further studies with larger sample sizes and better control of confounding variables are needed. The ISE programme is a newly developed service protocol and needs to be promoted in various community-based mental health settings. Clinicians should be encouraged to adopt this model as the approach for vocational rehabilitation for people with severe mental illness.

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Rapid molecular methods for epidemiological investigation of food-borne outbreaks

Key Messages

1. Pulsed-field gel electrophoresis (PFGE) was used to type more than 1000 strains of salmonellae belonging to the major serotypes isolated in Hong Kong. Some strains of *S enteritidis* probably belonged to a few clusters, whereas almost all strains of the other serotypes tested were different and due to sporadic spread. ERIC and BOX typing may be used as quick alternatives to PFGE in discriminating strains.
2. A total of 128 strains of *Shigella flexneri* and *S sonnei* were typed by PFGE, ERIC and BOX typing. All except 22 strains of *S sonnei* belonged to a heterogeneous population.

Introduction

Salmonellae and shigellae are important causes of diarrhoeal disease in Hong Kong, the former being the most common bacterial pathogens isolated from stool.[1-4] They have the potential to cause outbreaks, as they are readily transmissible via contaminated food or drinks as well as the faecal-oral route. Patients who have recovered may carry the organism for extended periods during which they act as a source of infection, especially if they are food handlers. This can lead to grave consequences in terms of public health and health care burden.

To prevent such outbreaks, prompt identification of sources of infection, in addition to strict adherence to infection control practices is required. Microbiological investigation of diarrhoeal cases is routinely performed by culture, identification and typing of the causative organisms. Various methods involving characterising bacterial DNA or protein have been developed, but no single technique is considered definitive. Thus, at least two methods should be used and results collated. Bacterial evolution leads to minor divergence among strains of the same outbreak, rather than novel genetic changes. Thus, typing results of strains isolated within a short period should not be affected by bacterial evolution.

We aimed to develop discriminatory, simple, quick and economical methods for genotyping of *Salmonella* and *Shigella* species for routine infection control. We also aimed to optimise and evaluate these methods by investigating both genuses isolated during 1990-2004.

Methods

This study was conducted from September 2005 to August 2007. All surviving *Salmonella* serotypes with >50 strains isolated during 1990-2004 from hospitals in the New Territories East Cluster, Hong Kong SAR were tested. Some serotypes that were isolated in smaller numbers were randomly selected for testing. All surviving *Shigella* stains of *S flexneri* and *S sonnei* isolated during 1996-2004 were also tested. Strains of *S typhimurium*, *S enteritidis*, *S derby*, *S typhi*, *S flexneri* and *S sonnei* that were tested in previous studies were not tested. The tested strains were subjected to pulsed-field gel electrophoresis (PFGE),[5] and then randomised for variable number tandem repeats (VNTR) typing[6] (using EXCEL gels to separate amplification products), multilocus sequence typing (MLST),[7] rDNA spacer region typing,[8] ERIC-PCR typing,[9] BOX typing,[9] IS200 typing (for salmonellae only),[10] and amplified fragment length polymorphism (AFLP)[11] analysis.

Results

Salmonella sp

Out of the 2234 strains of salmonellae isolated during 1990-2004, 1155 surviving strains belonging to 24 serotypes were tested, giving a survival rate of 52%. All strains were subjected to PFGE, a method regarded as the gold standard to which results of other typing methods are compared. The 1155 strains (representing 55% of the total number of strains isolated) were pulsotyped using *Xba*I-digested

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DNA. More than 50% of the strains of each serotype were tested except for *S anatum*, *S derby*, *S newport* and *S stanley*. A total of 49 strains of *S flexneri* and 97 of *S sonnei* were tested.

S enteritidis, *S typhimurium* and *S derby* were the three most common *Salmonella* serotypes isolated. We only studied strains of *S enteritidis* and *S typhimurium* isolated during 1997-2004, strains of *S derby* isolated during 1995-2004, and strains of *S typhi* isolated during 1998-2004. Those isolated outside these periods had been tested in previous studies. For the remaining 21 serotypes, strains isolated during 1990-2004 were tested.

The isolates were from 565 male and 599 female patients (male:female=1:1.06); 41% of the patients were aged ≤1 year, 13% were aged 2-9 years, 25% were aged 10-39 years, and 21% were aged 40-99 years. During the 5 months from June to October, ≥10% of isolates were obtained each month, constituting 57% of isolates. Isolates obtained during the other 7 months ranged from 3% (February) to 9% (May).

Each strain with a unique PFGE banding pattern was assigned an individual pattern. The patterns were analysed, using the BioNumerics software (Applied Maths, Saint-Martens-Latem, Belgium), by cluster analysis using the Dice coefficient for band matching with 1.0% position tolerance and an unweighted pair group method with an averaging algorithm. Patterns with ≥90% similarity were placed in the same group. *S typhimurium*, *S enteritidis*, *S derby* and *S london* were then tested by VNTR typing. The bands differed by approximately 6 bp in size and could only be discerned by separating on EXCEL gels. However, no difference in banding patterns was observed in all the tested strains. For MLST typing, the same banding patterns were obtained using different primers[7] for all *S typhimurium*, *S enteritidis*, *S derby* and *S london* strains tested. For rDNA spacer region typing, the same strains of *S typhimurium*, *S enteritidis*, *S derby* and *S london* were typed. Similarly, all banding patterns were the same. ERIC and BOX typing were applied in the typing of 8-90 each of the 12 serotypes tested. Patterns obtained using both methods were combined and analysed. Almost all banding patterns were distinctly different and were comparable to those of PFGE. However, patterns with ≥90% similarity should not be grouped since the differences were too small. Grouping these patterns together would give a false impression of relatedness of the strains. Both IS200 typing and AFLP analysis failed to give discriminatory banding patterns on the tested strains.

Shigella sp

Of the 241 strains of *S flexneri* and *S sonnei* obtained during 1996-2004, 88 strains survived and were tested, giving a survival rate of 37%. Both *Shigella* sp were isolated throughout the year with no preponderance during any particular season. All except two of the 40 *S flexneri* strains tested belonged to an individual PFGE pattern. Eight of these patterns could be grouped into four groups each with two patterns that were ≥90% similar, whereas there were nine patterns that could be grouped into two groups each with four and five similar patterns in one group. Similarly, all except two strains of *S sonnei* belonged to a distinct PFGE pattern. While 42 strains could be grouped into 11 groups, there was one group that comprised 22 strains. ERIC and BOX typing also revealed that strains of both *Shigella* sp were heterogeneous.

Discussion

Only about 50% of *Salmonella* and *Shigella* isolates survived for testing. The survival rate was low, as isolated strains were stored on agar slants in bijoux bottles and could dry up if the screw caps were not tightly replaced or the bottles misplaced.

S enteritidis, *S typhimurium* and *S derby* remained the most common *Salmonella* serotypes isolated, with approximately 300-1000 single patient strains isolated during 1990-2004. These were followed by *S london*, *S anatum* and *S blockley* with >100 strains isolated during the same period. The other serotypes had <100 strains isolated.

Using PFGE, we were able to reveal the diverse heterogeneity of strains of the more common *Salmonella* serotypes as well as *S flexneri* and *S sonnei*. Almost all the strains of each *Salmonella* serotypes and the two *Shigella* sp were quite distinct, as inferred from their different PFGE patterns. Thus, we most likely experienced sporadic salmonella and shigella infections in the community. Probably, there were a few clusters of *S enteritidis*, *S flexneri* and *S sonnei* circulating, eg, one cluster comprising 15 *S enteritidis* strains that belonged to 10 individual patterns that were ≥90% related, and one cluster comprising 22 *S sonnei* strains that belonged to 20 patterns with ≥90% similarity.

It was disappointing to find that typing methods such as VNTR typing, MLST typing, rDNA spacer region typing, IS200 typing, and AFLP were not useful in distinguishing our strains, as almost all showed the same banding patterns. ERIC and BOX typing were probably satisfactory for discriminating individual strains, but their use in determining strain similarities was questionable. ERIC and BOX typing might be a rapid alternative to PFGE in typing salmonellae or shigellae, as they could provide results within the same day and were more economical and much less technically demanding than PFGE.

However, no two typing methods could provide the same results. Although PFGE was regarded as the gold standard, it could only detect differences within the fragments containing the restriction sites of the digesting enzymes, whereas ERIC and BOX typing could only detect differences in regions between the primers. Thus, at least two methods should be used and the results combined in

order to obtain more reliable interpretation.

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Antimicrobial resistance among uropathogens causing cystitis in women

Key Messages

1. Among *Escherichia coli* from adult women with acute cystitis, the rates of antimicrobial resistance were 52.8% for ampicillin, 29.5% for co-trimoxazole, and 12.9% for ciprofloxacin. Nitrofurantoin and fosfomycin remain active against >90% of the isolates.
2. The respective age-stratified rates for co-trimoxazole and ciprofloxacin resistance were 26.4% and 9.6% for women aged 18-50 years, and 35.5% and 19.4% for women aged ≥51 years. Being aged ≥51 years (Odds ratio [OR]=2.3, 95% confidence interval [CI]=1.1-4.8, P=0.02) and receipt of recent antibiotic treatment (OR=2.5, 95% CI=1.1-5.8, P=0.03) were significantly associated with fluoroquinolone resistance.
3. Ampicillin and co-trimoxazole should not be used as first-line agents for empirical treatment of acute cystitis. As fluoroquinolone resistance was less than 10% among isolates obtained from younger women, these agents are still useful for empirical treatment. Nonetheless, the high rates of fluoroquinolone resistance among women who were older or who had received antibiotic treatment recently indicates the need to consider alternatives such as nitrofurantoin, fosfomycin and amoxicillin-clavulanate as initial therapy.
4. Molecular analysis of the multidrug resistant strains showed that they were genetically diverse, with no evidence of epidemic strains. Nonetheless, resistant strains possess virulence traits distinct from susceptible isolates, suggesting that they may evolve from other sources and not by acquisition of resistance mutations in susceptible isolates from humans.

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Introduction

Cystitis or urinary tract infection (UTI) affects one-third of women at some stage during their lifetime. In one quarter to one third of such patients, the infection is recurrent. The microbial aetiology of UTI is well established. *Escherichia coli* is the predominant pathogen (80%); the antibiotic treatment is mainly determined by the prevailing antimicrobial resistance of this organism. First-line agents recommended include ampicillin, co-trimoxazole and fluoroquinolones, but resistance rates to these first-line agents among community isolates of *E coli* in Hong Kong were reported to be 66%, 57%, and 23%, respectively.[1] Whether alternative agents are needed for some or all women is unknown, because all previous local studies failed to take into account patient demographics, types of infection and the clinical settings. The present study was therefore conducted to address this issue.

This study evaluated antimicrobial resistance rates of *E coli* and other pathogens isolated from patients with community-acquired cystitis. The epidemiological relation of the antibiotic-resistant isolates was evaluated using molecular typing. The correlation between bacterial virulence factors and antimicrobial resistance was also assessed.

Methods

This prospective cross-sectional study was conducted from January 2006 to June 2008. It comprised 54 centres including general practitioner offices, general outpatient clinics and emergency departments. Adult women (aged ≥18 years) diagnosed with uncomplicated cystitis were enrolled. Patients with loin pain, fever, renal stones and indwelling urinary catheters were excluded. A standardised questionnaire was used to collect patient demographics, history, and underlying medical details. A mid-stream urine sample was obtained for culture and processed by standard methods. Bacterial identification and antimicrobial susceptibility testing was performed using established methods.[2]

Beta-lactamases related to the CTX-M families were sought by polymerase chain reaction and sequencing, using primers previously described.[2] Selected isolates were studied by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA (Amersham Pharmacia Biotech, Little Chalfont, UK), and patterns were analysed with Gelcompar II software (Applied Maths). Isolates were tested for 30 virulence-associated traits using established multiplex polymerase chain reactions.[3]

The Chi-squared test, Fisher's exact test, or Student's *t*-test were used for statistical analysis. A two-tailed P value of <0.05 was considered significant.

Results

Patient demographics and antimicrobial susceptibilities

A total of 592 patients with uncomplicated cystitis were recruited. Among these, 359 were enrolled from general practitioner offices, 101 from general outpatient clinics, and 132 from emergency departments (Table 1). In 237 (40%) of the patients, the urine cultures did not grow any bacteria, and in three (0.5%) others

the cultures were contaminated. Thus, the study population consisted of 352 (59.5%) patients, whose urine samples grew a single uropathogen on culture. The mean age of these patients was 44.9 years (standard deviation, 16.3 years); most (96.8%) of whom were Chinese. Underlying comorbidities were present in 38 patients, the commonest being diabetes mellitus (n=20), hypertension (n=6) and heart disease (n=4).

The rates of antibiotic-resistant *E coli* were 52.8% for ampicillin, 45.4% for nalidixic acid, 29.5% for co-trimoxazole, and 12.9 for ciprofloxacin (Table 2). Almost all isolates (>90%) were susceptible to nitrofurantoin and fosfomycin. Fourteen (5.2%) of the isolates were extended-spectrum beta-lactamases (ESBL) producers. The point prevalence of ESBL isolates in these samples was 2.8% (5/178) for women aged 18-50 years, and 9.7% (9/93) for women aged ≥51 years. The ESBL producer rate was 20.8% among triple drug resistant strains (defined as co-resistance to ampicillin, ciprofloxacin, and co-trimoxazole) versus 3.6% among non–triple drug resistant strains (P<0.001). The resistance rates to ampicillin (60.2% vs 48.9%), nalidixic acid (54.8% vs 40.4%), ciprofloxacin (19.4% vs 9.6%), and co-trimoxazole (35.5% vs 26.4%) among women aged ≥51 years were higher than those in younger women (aged 18-50 years), although only the difference for nalidixic acid

Table 1 Epidemiological characteristics of 352 patients ith cystitis

Epidemiological characteristics	No. (%) of patients with cystitis		
	All (n=352)	18-50 years old (n=235)	≥51 years old (n=117)
Underlying comorbidities	38 (10.8)	11 (4.7)	27 (23.1)
Prior cystitis	94 (26.7)	53 (22.6)	41 (35)
Recent antibiotic use	46 (13.1)	28 (11.9)	18 (15.4)
Presentation setting			
General practitioner office	61 (17.3)	177 (70.2)	75 (29.8)
General outpatient clinic	39 (11.1)	18 (46.2)	21 (53.8)
Emergency department	252 (71.6)	40 (65.6)	21 (34.4)
Uropathogen			
Escherichia coli	271 (77)	178 (75.7)	93 (79.5)
Other *Enterobacteriaceae*	50 (14.2)	35 (14.9)	15 (12.8)
Staphylococci	18 (5.1)	15 (6.4)	3 (2.6)
Others	13 (3.7)	7 (3)	6 (5.1)

Antibiotic	% of patients					
	E coli (n=271)			Other *Enterobacteriaceae* (n=50)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Ampicillin	40.6	6.6	52.8	26.5	0	73.5
Amoxicillin-clavulanate	84.9	13.3	1.8	79.6	8.2	12.2
Nalidixic acid	52.8	1.8	45.4	75.5	16.3	8.2
Ciprofloxacin	87.1	0	12.9	95.9	2	2
Co-trimoxazole	69.4	1.1	29.5	83.7	0	16.3
Nitrofurantoin	92.3	1.1	6.6	30.6	14.3	55.1
Fosfomycin	98.2	0	1.8	91.8	2	6.1

Table 3 Distribution of bacterial characteristics among two *Escherichia coli* subsets

Bacterial characteristics*	No. (%) of isolates*		P value
	Susceptible (n=42)	Multidrug resistant (n=34)	
Adhesin VF genes			
focG (F1C fimbriae)	**7 (21)**	1 (3)	0.03
papA (P-fimbriae structural subunit)	**26 (77)**	18 (53)	0.02
papC (P-fimbriae assembly subunit)	**27 (79)**	19 (56)	0.02
papEF (P-fimbriae tip pilin)	**24 (71)**	13 (38)	0.01
papG (P-fimbriae adhesin molecule)	**27 (79)**	17 (50)	0.01
papG allele III (P-adhesin variant III)	**22 (65)**	9 (27)	<0.01
sfa/foc DE (S and F1C fimbriae)	**24 (71)**	13 (38)	0.01
sfaS (S fimbriae)	**15 (44)**	8 (24)	0.05
Toxin VF genes			
cnf1 (cytotoxic necrotising factor)	**26 (77)**	12 (35)	<0.01
hlyA (haemolysin)	**26 (77)**	7 (21)	<0.01
Capsule polysaccharide VF genes			
K1 (K1 kpsMT II variant)	4 (12)	**11 (32)**	0.05
Siderophore and miscellaneous VF genesa			
cvaC (Colicin V)	1 (3)	**5 (15)**	0.04
iutA (aerobactin receptor)	3 (9)	**18 (53)**	<0.01
traT (serum resistance)	17 (50)	**27 (79)**	0.02
Phylogenetic group			
E coli reference group A	3 (9)	3 (9)	NS
E coli reference group B	0 (0)	2 (6)	NS
E coli reference group B2	29 (85)	26 (76)	NS
E coli reference group D	2 (6)	3 (9)	NS

* Virulence factors are shown only if P≤0.05. Boldface indicates those factors occurred in higher frequencies. Multidrug resistant is defined as co-resistance to three or more drugs: ampicillin, ceftriaxone, ciprofloxacin, co-trimoxazole, fosfomycin, gentamicin, nitrofurantoin and tetracycline. Susceptible subset (control) isolates were sensitive to all eight drugs.

and ciprofloxacin reached statistical significance.

Risk factor for antibiotic-resistant Escherichia coli

Antibiotic use in the past 6 weeks significantly increased the risk of infection by co-trimoxazole-resistant *E coli* (odds ratio [OR]=2.8, P=0.003) and ciprofloxacin-resistant *E coli* (OR=2.3, P=0.03). Resistance rates to co-trimoxazole and ciprofloxacin among patients with a history of recent antibiotic use were 50% and 23.7%, respectively. Older age (≥51 years) was also associated with infection by ciprofloxacin-resistant *E coli*.

Molecular analysis

A subset of 68 isolates including 34 multidrug resistant (MDR) isolates and 34 susceptible controls were analysed further by PFGE and virulence factor analysis. Based on PFGE, the MDR isolates had highly diverse banding patterns, and there was little overlap with the susceptible controls. All 14 ESBL-positive isolates were also analysed by PFGE and noted to be genetically distinct and not clonally related. Virulence profiling showed that adhesin and toxin genes were significantly more frequent among the susceptible subset than the MDR subset. In contrast, the K1 (capsule), *iutA* (siderophore), *cvaC* (colicin V) and *traT* (serum resistances) genes were more commonly found in the MDR strains (Table 3). Resistance to ciprofloxacin but not ampicillin and co-trimoxazole was associated with lower aggregate VF scores.

In the conjugation experiments, the ESBL phenotype could be transferred from five of the 14 isolates. The frequency of transfer was 10^{-3} to 10^{-2} per donor cells. Polymerase chain reaction and sequencing showed that the presence of CTX-M-14 in 11 isolates, CTX-M-24 in two isolates, and CTX-M-9 in one isolate.

Discussion

Since the early and mid-1990s in Hong Kong, resistance rates to ampicillin and co-trimoxazole reached 50-60% and 10-20%, respectively. In 1991, the rate of fluroquinolone resistance among community *E coli* was 6%. Our data showed that ampicillin resistance remains at the same level but fluoroquinolone and co-trimoxazole resistance has increased substantially. Nonetheless, the fluoroquinolone (12.9%) and co-trimoxazole (29.5%) resistance rates were substantially lower than those reported in the Department of Health sentinel surveillance (21-26% and 37-46%, respectively) during 2005-2007. The spuriously higher rates of resistance in the Department of Health figures were likely a result of bias from excessive inclusion of elderly patients and those with complicated infections.

Our data documents the spread of ESBL producers among patients with UTIs. The detection of ESBL isolates from young women is a concern because such community-associated ESBL isolates were previously reported mainly from older women. In agreement with our recent work,[2] CTX-M-14 is the predominant enzyme type among the ESBL-producers. As the isolates were not clonally related, the CTX-M determinant could possibly have spread through dissemination of epidemic plasmids. Additional investigation into the plasmid epidemiology is warranted. As food animals have been suggested to be important reservoirs for the CTX-M enzymes, future work should focus on the relation between the CTX-M encoding plasmids in human and animal isolates.[4]

In accordance with current guidelines,[5] the empirical use of co-trimoxazole as first-line therapy for women with community-acquired UTIs should be avoided. For younger women with cystitis, fluoroquinolones remain valuable as first-line agents. As ciprofloxacin-resistant isolates usually prevail in post-menopausal women, drugs other than fluoroquinolones may need to be re-considered for this patient population, especially those with a history of recent antibiotic treatment. Nitrofurantoin and fosfomycin remain active against most *E coli* isolates. In using nitrofurantoin, the duration of treatment should be extended to 5 to 7 days as there is insufficient data to support the efficacy of 3-day therapy. In selection of alternative agents, *in vitro* activity, clinical efficacy and side effects are important considerations. In future revision of management guidelines, we suggest inclusion of age as a variable in the approach to empirical therapy.

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Photodynamic nactivation of multi-drug resistant pathogens n Hong Kong

Key Messages

1. Photodynamic therapy could be an alternative treatment for highly prevalent local antibiotic-resistant pathogens.
2. Photodynamic inactivation using toluidine blue O was observed on both reference strains and clinical samples, including methicillin-resistant *Staphylococcus aureus*, extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*, *Candida glabrata* and *Candida krusei*.

Introduction

The widespread systemic use of antibiotics is a cause of multi-drug resistance and super-infection. In addition to multi-drug resistant (MDR) bacteria, fungal species such as *Candida glabrata* and *Candida krusei* have become increasingly prevalent due to resistance to traditional antifungal agents in immunocompromised patients.[1] Innovative methods to reduce MDR bacteria and fungi are required. Photodynamic inactivation (PDI) may be a useful approach in treating MDR bacteria and fungi.[2] It employs a non-toxic dye—photosensitiser (PS)—that selectively targets its destination cells while sparing the host cells. Upon illumination by specific wavelengths of visible light and in the presence of oxygen, reactive species are generated that destroy the pathogens. The extent of killing depends on the type of photosensitiser used, light dose, drug dose and the bacterial growth phase. There is also a difference in susceptability to PDI between Gram-positive and Gram-negative bacteria due to differences in physiology and cytoplasmic membrane protein structure.[2,3]

The efficiency of PDI depends on the match between PS and light illumination. The selectivity of PS to various bacteria is one of the key concerns of PDI. In general, neutral or anionic PS molecules bind efficiently to and photo-inactivate Gram-positive bacteria. In contrast, they may bind to the outer membrane only of Gram-negative bacteria, which are not inactivated. However, PSs with an overall cationic charge efficiently kill both Gram-positive and Gram-negative species. This has been shown for cationic porphyrins, phthalocyanines and phenothiazines.[4] Cationic phenothiazines such as methylene blue (MB) and toluidine blue O (TBO) have also been studied to photo-inactivate both Gram-positive and Gram-negative bacteria *in vitro* and in *ex vivo* samples.[2,5]

This study aims to elucidate the *in vitro* PDI efficacy of three PSs: MB, TBO, and delta-aminolevulinic acid (ALA) against highly prevalent antibiotic-resistant pathogens, namely methicillin-resistant *Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL) producing strains of *Escherichia coli* and *Klebsiella pneumoniae*, MDR *Pseudomonas aeroginosa*, *C krusei*, and *C glabrata*. One clinical isolate of each strain plus wild-type reference strains were comparatively studied.

The objectives of this study were: (1) to compare the *in vitro* PDI efficacy of the three PSs at different concentrations and light dose combination to these clinical MDR isolates, (2) to determine the minimum bactericidal concentration (MBC) of the three PSs on different pathogens, (3) to quantitate and compare the uptake kinetics of ALA-induced porphyrin for the proposed pathogens by flow cytometry (drug uptake is the major dependent factor for effective killing), and (4) to evaluate the PDI effect for the virulence factor, lipopolysaccharide (LPS), at pre- and post-PDI.

Methods

This study was conducted from February 2006 to December 2006. A total of eight bacterial and four fungal isolates were investigated. They included ATCC wild-type strains, clinical wild-type isolates, and clinical MDR isolates. Wild type strains included *S aureus* (ATCC 25923), *E coli* (ATCC 23922), *K pneumonia* (clinical wild type isolate), *P aeruginosa* (ATCC 27853), *C glabrata*

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(ATCC 90030) and *C krusei* (ATCC 6258). Clinical MDR isolates included MRSA, ESBL-producing Gram-negative pathogens included *E coli* and *K pneumonia*, *P aeruginosa*, *C glabrata* and *C krusei*. All clinical isolates were archived samples collected from the Department of Microbiology, United Christian Hospital, Hong Kong SAR, China.

Photosensitisers

Of the three PSs, MB is cationic, TBO is a phenothiazinium salt, which is also moderately effective cationic, and ALA is a pro-drug, which could increase the intra-cellular level of PpIX. Stock solutions of each PS were prepared by dissolving them in sterile distilled water, and then stored at 4ºC in the dark.

Study design

Regarding PDI efficacy of each PS on wild-type and MDR pathogens by MBC, bacterial strains (*S aureus*, *E coli*, *K pneumonia* and *P aeruginosa*) were grown in suspensions of cell density of $1x10^8$ cells/mL at 37ºC in nutrient broth, and *C glabrata* and *C krusei* isolates were grown in suspensions of a cell density of $1x10^6$ cells/mL at 37ºC in Sabouraud dextrose broth. Aliquots of bacterial suspensions were sensitised with a range of PS concentrations for 30 minutes. The PS-loaded cells were centrifuge-washed with sterile phosphate-buffered saline and resuspended in broth. Aliquots of 150 μL treated cells were placed in a 96-well microtitre plate and irradiated with light range (5-30 Jcm^{-2}) emitted from a 400-W quartz-halogen lamp equipped with a heat isolation filter and a long-pass filter (600 nm). Then 100 μL broth was withdrawn and serially diluted in phosphate-buffered saline; 10 μL from each dilution mixture was spread on nutrient agar plates in triplicate. The plates were incubated at 37ºC overnight. The number of colonies was enumerated to determine the survival fractions. Six independent experiments were conducted and the results were presented as mean and standard error of the mean (SEM) values. Light-alone controls (with no-PS) and dark controls (PS-treated cell suspension without light) were included.

Regarding bacterial uptake of ALA, all pathogens were sensitised with different drug concentrations and incubated for 4 hours for drug uptake. The fluorescence intensity of PpIX was determined by flow cytometry.

Regarding the PDI effect for the virulence factor of LPS using the limulus amoebocyte lysate (LAL) assay, the amount of LPS after MB, TBO and ALA mediated PDI was determined by the LAL assay (Associates of Cape Cod Inc, USA). In the presence of LPS, enzymes in the LAL assay can be activated and result in clot formation. Different concentrations of MB, TBO or ALA were separately mixed with LPS to attain a final LPS concentration of 0.25 or 0.03 EU/mL (LPS detection limits of LAL assay kits). After light inactivation, individual PS-LPS mixtures were incubated with the LAL. During the incubation, enzyme activation in the LAL was catalysed by the LPS resulting in clot formation. If more LPS is bound or destroyed by PDI, less free LPS remains to trigger the enzyme activation, resulting in no clot formation and vice versa. Positive controls were included with LPS alone at the concentration of 0.25 and 0.03 EU/mL. LAL reagent water from the commercial LAL kit served as a negative control. The experiments were conducted in triplicate.

Data analysis

Results were presented as mean and SEM values of six independent experiments. Statistical analyses were conducted by one-way ANOVA; a P value of <0.05 was considered statistically significant.

Results

Regarding the PDI efficacy of MB (Fig. a), with 3 μM MB at 30 Jcm^{-2}, 6.5 log killing was obtained for the *S aureus* (ATCC 25923) and 7 log killing was obtained for MRSA. With 8 μM MB at 30 Jcm^{-2}, 6 log killing was obtained for both clinical wild-type *E coli* and ESBL-producing *E coli*. With 10 μM MB at 30 Jcm^{-2}, 5.9 log killing was obtained for clinical wild-type *K pneumonia* and 4.8 log killing was obtained for ESBL-producing *K pneumonia*. However, MB-PDI demonstrated only 2.8 log killing for *P aeruginosa* MDR isolate at 200 μM MB at 30 Jcm^{-2}. Applying 1 μM MB at 30 Jcm^{-2}, no PDI effects were demonstrated for both MDR *Candida* species.

Regarding PDI efficacy of TBO (Fig. b), with 0.5 to 10 μM TBO and light doses (10, 20 and 30 Jcm^{-2}) applied to four groups of bacteria and two groups of fungi, a significant reduction ($P<0.01$) of viability count was noted. With 1 μM TBO at 30 Jcm^{-2}, 5.9 log killing was obtained for the *S aureus* (ATCC 25923) and 4.3 log killing for MRSA. With 2 μM TBO at 30 Jcm^{-2}, 6 log killing were obtained for both *E coli* (clinical wild-type) and ESBL-producing Gram-negative *E coli*. With 5 μM TBO at 30 Jcm^{-2}, 4.8 log killing was obtained for *K pneumonia* (clinical wild-type isolate) and 6 log killing for ESBL-producing *K pneumonia*. With 10 μM TBO at 30 Jcm^{-2}, 2.5 log killing was obtained for *P aeruginosa* (ATCC 27853) and 3.9 log killing for *P aeruginosa* clinical MDR isolate. With 100 μM TBO at 30 Jcm^{-2}, the *C glabrata* MDR isolates responded better with 4.6 log killing, compared to just 4 log killing for *C glabrata* (ATCC 90030). In contrast, only with 40 μM TBO at 15 Jcm^{-2}, near 5 log killing was obtained for both clinical MDR isolates and *C krusei* (ATCC 6258). These findings demonstrated that TBO mediated PDI was effective for both wild type and clinical MDR bacterial and fungal isolates. The poorest response was observed for *P aeruginosa* strains.

Regarding PDI efficacy of ALA (Fig. c), by applying up to 6 μM ALA at 30 Jcm^{-2}, all groups of pathogens (*S aureus*, MRSA, *E coli*, ESBL *E coli*, *K pneumonia*, MDR *K pneumonia*, *P aeruginosa* and MDR *P aeruginosa*) showed <1 log killing. This may be due to poor ALA uptake by

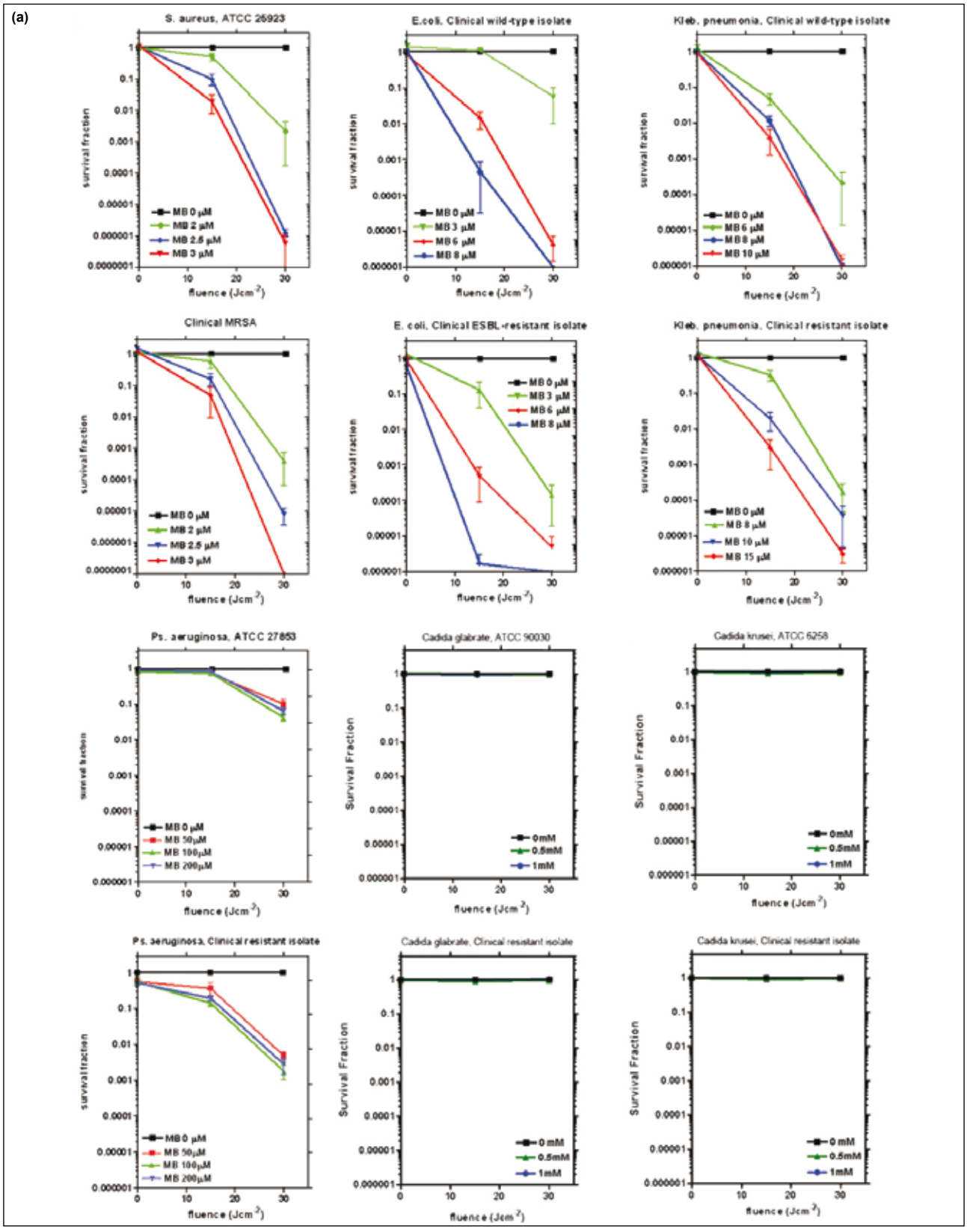
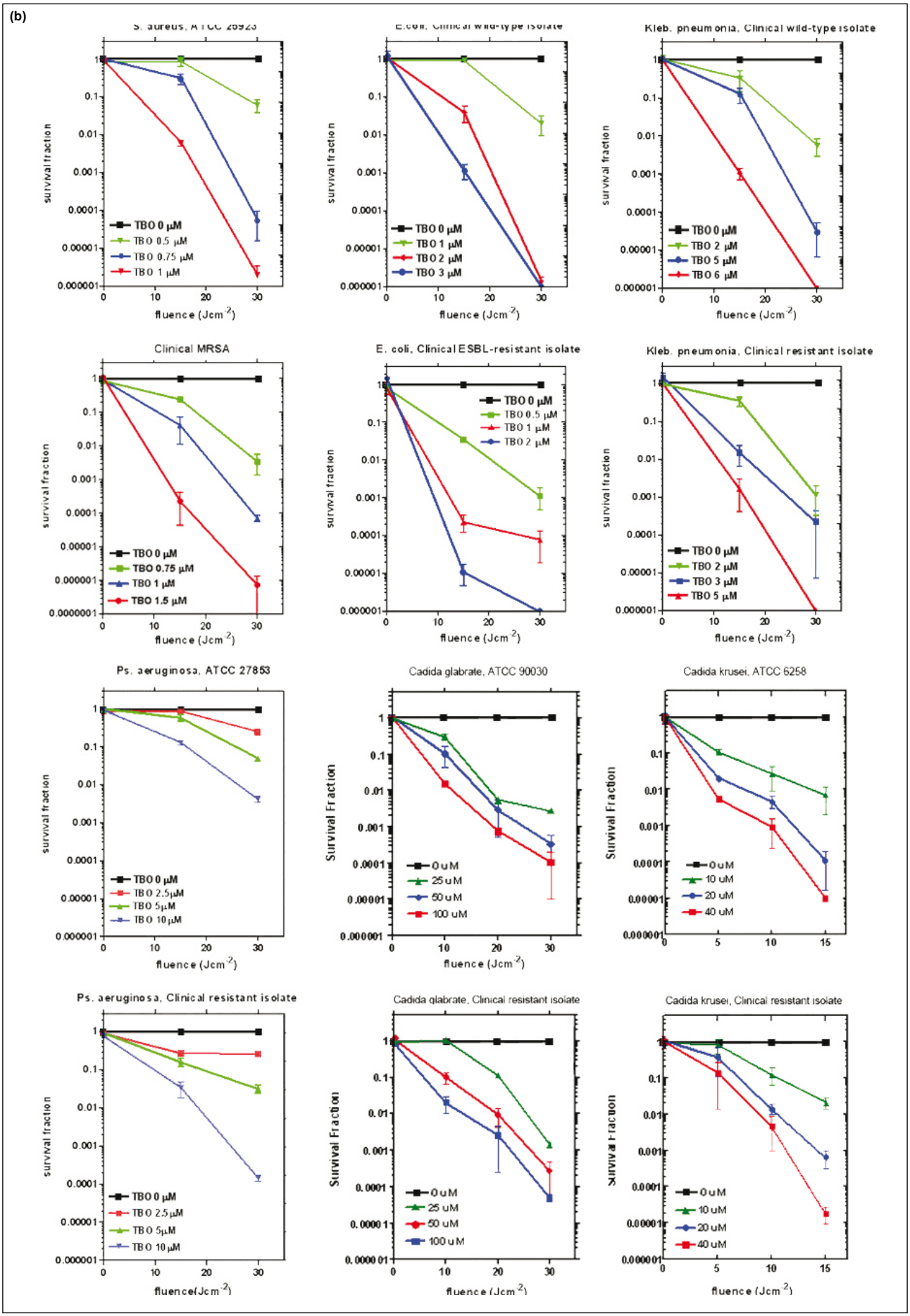
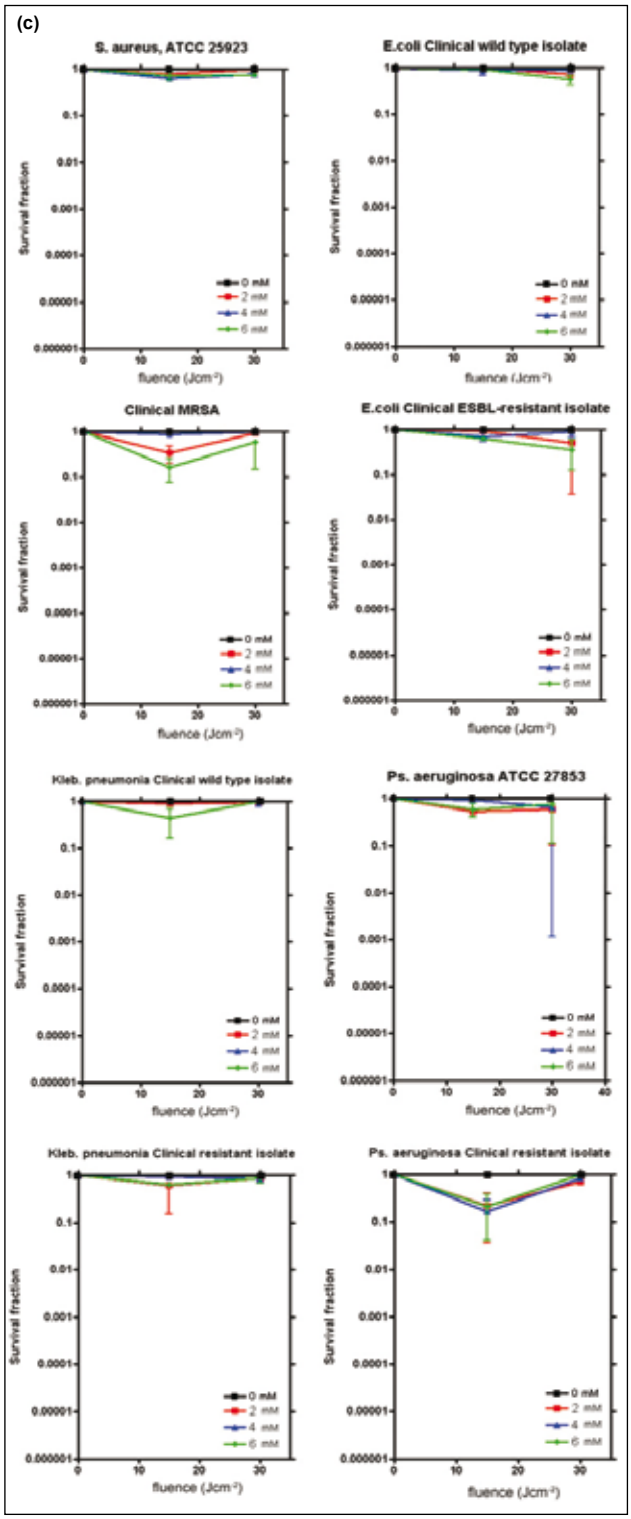


Fig. The photodynamic inactivation efficacy of (a) methylene blue, (b) toluidine blue O, and (c) delta-aminolevulinic acid against different pathogens using minimum bactericidal concentration

(1) *Staphylococcus aureus* (ATCC 25923) and methicillin-resistant *S aureusk* (2) *Escherichia coli* (clinical wild-type isolate) and extended-spectrum β-lactamase (ESBL)-producing Gram-negative *E colik*(3) *Klebsiella pneumonia* (clinical wild-type isolate) and ESBL-producing Gram-negative *K pneumoniak* (4) *Pseudomonas aeruginosa* (ATCC 27853) and its clinical multidrug resistant (MDR) isolate, (5) *Candida glabrata* (ATCC 90030) and its clinical MDR isolate, and (6) *Candida krusei* (ATCC 6258) and its clinical MDR isolate. Results are presented as means and standard error of the means of six independent experiments (P<0.01).


(b)
S. aureus, ATCC 29923
survival fraction
fluence (Jcm-2)
TBO 0 μM
TBO 0.5 μM
TBO 0.75 μM
TBO 1 μM
E.coli, Clinical wild-type isolate
TBO 0 μM
TBO 1 μM
TBO 2 μM
TBO 3 μM
Kleb. pneumonia, Clinical wild-type isolate
TBO 0 μM
TBO 2 μM
TBO 5 μM
TBO 6 μM
Clinical MRSA
TBO 0 μM
TBO 0.75 μM
TBO 1 μM
TBO 1.5 μM
E. coli, Clinical ESBL-resistant isolate
TBO 0 μM
TBO 0.5 μM
TBO 1 μM
TBO 2 μM
Kleb. pneumonia, Clinical resistant isolate
TBO 0 μM
TBO 2 μM
TBO 3 μM
TBO 5 μM
Ps. aeruginosa, ATCC 27853
TBO 0 μM
TBO 2.5μM
TBO 5μM
TBO 10μM
Cadida glabrate, ATCC 90030
Survival Fraction
0 uM
25 uM
50 uM
100 uM
Cadida krusei, ATCC 6258
0 uM
10 uM
20 uM
40 uM
Ps. aeruginosa, Clinical resistant isolate
TBO 0 μM
TBO 2.5μM
TBO 5μM
TBO 10μM
Cadida glabrate, Clinical resistant isolate
0 uM
25 uM
50 uM
100 uM
Cadida krusei, Clinical resistant isolate
0 uM
10 uM
20 uM
40 uM



the strains measured in the eight tested strains using flow cytometry. There was no significant PpIX accumulation in either the reference strain or the clinical isolates for all the tested pathogens (data not shown).

All three tested PSs (at 200 μM MB, 10 μM TBO and 3 μM ALA) were able to reduce the LPS level after PDI using LAL assay at the sensitivity limits of 0.25 and 0.03 EU/mL.

Discussion

This *in vitro* study showed the photo-antimicrobial efficacy of MB, TBO and ALA against eight bacterial suspensions (reference strains and clinical wild-type and MDR strains) and four fungal strains using different PDI doses. The killing effect of PDI depends on the generation of reactive oxygen species. Further experiments are required to confirm the PDI effect and more wild type and MDR strains should be included. Moreover, application of the PS on cell surfaces should be investigated as this will provide valuable information in the clinical setting. With appropriate selection and improvement of PS, PDI may become a useful therapeutic adjunct to address the antibiotic resistance and MDR problem.

Acknowledgements

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Methionine aminopeptidase as a ovel target for antibiotic therapy against *Staphylococcus aureus:* a proteomic approachn

Key Messages

1. Methionine aminopeptidase (MetAP) is an essential enzyme in *Staphylococcus aureus* and a potential target for novel antibiotics.
2. Two-dimensional electrophoresis gel identified more than 100 differences in protein expression between wild type and MetAP-deficient strains of *S aureus*.
3. Using mass spectroscopic techniques, 63 differentially expressed proteins were identified, of which some were related to purine biosynthesis and methionine metabolism.

Introduction

Staphylococcus aureus is a common aetiologic agent of pneumonia in the hospital setting, often as a consequence of influenza, with a mortality of 15 to 20%.[1] Surgical site infections constitute approximately 15% of the infections reported to the National Nosocomial Infections Surveillance System.[2] These infections increase the economic burden of the hospital system, partly owing to the prolonged length of hospital stay. Thus, research in the pathogenesis of *S aureus* and identification of novel targets for antibacterial therapy against this pathogen are necessary.

Co- and post-translational modifications are essential steps for the maturation of proteins for proper folding, regulation, function, targeting and eventually degradation.[2] Translation of proteins is initiated with methionine in eukaryotes and N-formyl methionine in prokaryotes, mitochondria, and chloroplasts. The N-formyl group is removed from proteins in prokaryotes and eukaryotic organelles by a deformylase, leaving a methionine residue at the amino terminus. However, most mature proteins do not retain the initiator residue. Methionine aminopeptidase (MetAP) is the enzyme responsible for the catalytic cleavage of the N-terminal methionine from proteins, when the penultimate residue has a small and uncharged side-chain. MetAPs is essential in Gram-negative bacteria (such as *Escherichia coli* and *Salmonella typhimurium*) and in *Saccharomyces cerevisiae*. Its physiological importance guided researchers to design antimicrobial inhibitors that specifically target this enzyme. However, the precise roles of MetAP in cellular functions are still elusive. By applying proteomic techniques, the wild type and MetAP-deficient strains of *S aureus* showed similar protein profiles. Of more than 600 proteins detected on the two-dimensional electrophoresis (2-DE) gel, more than 100 showed differences in the expression level. We quantified and compared 63 differentially expressed proteins. Using mass spectrometric techniques, the identities of these selected proteins were confirmed and some were identified to be those related to purine biosynthesis and methionine metabolism.

Methods

This study was conducted from February 2006 to January 2008. Whole cell lysates of *S aureus* were prepared using a modified protocol.[3] Protein (600 μg) was applied onto an IPG Strip (18 cm, pH 4-7) by overnight passive rehydration. Isoelectric focusing was performed using Ettan IPGphor II (GE Healthcare, USA). The strip was then equilibrated for 15 minutes in 10 mL SDS equilibration buffer with 100 mg dithiothreitol (DTT), followed by an equilibration step for 15 minutes in 10 mL SDS equilibration buffer with 250 mg iodoacetamide. The second dimension electrophoresis was carried out on 1.5 mm thick 12.5% self cast polyacrylamide gel in a Ettan DALT*six* electrophoresis apparatus (GE Healthcare) at 17 W/gel with a maximum voltage of 300 V at 10ºC until the dye front reached the bottom of the gel. Three separated batches of cell lysates were analysed by three independent 2-DE experiments. Gels were stained using Coomassie brilliant blue G250, as described previously.[4]

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Stained gels were digitalised using an ImageScanner (GE Healthcare). Intensity calibration was carried out by using the Kodak Step Tablet number 2 (Kodak, USA) before image acquisition. Comparative analysis of the gel images was carried out using the ImageMaster 2D Platinum version 5.0 software (GE Healthcare) according to the manufacturer's instructions.

Protein spots were picked manually and de-stained in 1:1 solution of 100 mM ammonium bicarbonate and acetonitile. After de-staining, enzymatic digestions were carried out as previously described.[5]

Results

Using 2-DE, we were able to compare the proteomes of RKC261 (MetAP-sufficient *S arueus*) and RKC264 (MetAP-deficient *S aureus*). As the MetAP-deficient *S aureus* was barely growing in extreme pH conditions and elevated temperatures, proteomes were compared using brain-heart infusion broths. Among more than 600 protein spots detectable in the 2-DE, we were able to quantify and compare the expression levels of differentially expressed proteins between RKC261 and RKC264 (data not shown). We further classified the identified proteins into related biological processes (data not shown). In the MetAP-deficient state, 80% of the differentially expressed proteins detected by 2-DE were down-regulated. Apart from recognising more general biological processes such as protein synthesis, energy generation, etc, proteins involved in methionine biosynthesis/metabolism were identified. In addition, a number of proteins involved in purine biosynthesis/metabolism were identified.

Discussion

In this study, the 2-DE gel results indicated a down-regulation of particular proteins, which are required and implicated in the *de novo* synthesis of inositol-monophosphate IMP,[6] the precursor of ATP and GTP, for example, phosphoribosylaminoimidazole-succinocarboxamide synthase (purC) and phosphoribosylamine-glycine ligase (purD). The enzymes involved in the salvage and interconversion of purine nucleosides and nucleotides, for example, adenylosuccinate synthase (purA), and purine nucleoside phosphorylase (deoD) were also noted to be down-regulated. These purC and purD genes belong to the putative purine biosynthesis operon purEKCSQLFMNHD in *S aureus*, but purA and deoD also have their independent promoters. Yet they are all involved in the biosynthesis of IMP and are linked in this manner. At the same time the gene for inositol-monophosphate dehydrogenase (guaB) was found to be up-regulated, implying that the cell uses more IMP in order to synthesise GMP.

In addition, MetAP deficiency in *S aureus* may lead to impaired methionine recycling and consequently down-regulation in the production of S-adenosyl-L-methionine. Deficiency in S-adenosyl-L-methionine will lead to a decrease in the production of polyamines. This may lead to a slower production of methylthioadenosine (MTA), which is believed to lead to the formation of the purine adenine. Methionine has been involved in purine salvage and purine recycling pathways and therefore a sufficient decrease in the production of this amino acid may lead to a decrease in the formation of purines downstream.[7]

Another very important protein that has been detected by the 2-DE gel analysis was the branched-chain amino acid aminotransferase. This enzyme is important in the conversion of ketomethiobutyrate (KMTB) to methionine.[8] According to the 2-DE gel results, its production is severely down-regulated in the MetAP-deficient state. This enzyme is also found to be linked with MTA recycling to methionine. As methionine production is costly in terms of energy requirements, the existence of this unique pathway, which recycles methionine from MTA, serves to conserve energy. The final step in methionine regeneration is the transamination of KMTB by the branched-chain amino acid aminotransferase. Our study suggests that the impact of MetAP deficiency in *S aureus* (and possibly in other organisms too), is profound and multidimensional. The expression profile of a wide range of proteins, spanning nucleotide biosynthesis, energy production, protein synthesis, glycolysis, electron transport, vitamins biosynthesis and metabolism, transcription regulation, amino acid production, and fatty acid metabolism, is affected by the deficiency of MetAP. Our study has provided evidence that MetAP may be linked to the essential methionine recycling and purine biosynthesis pathways, suggesting that it could be a useful target for antibiotic development.

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Targeting glutamate synthase for tuberculosis drug development

Key Messages

1. The *Mycobacterium tuberculosis* glutamate synthase consisting of alpha and beta subunits can be purified in a heterologous *Escherichia coli* expression system.
2. The beta subunit can be expressed in the soluble protein fraction under low temperature conditions.
3. The alpha subunit enters inclusion bodies in *E coli* but can be refolded and subsequently purified.

Introduction

Tuberculosis is a contagious disease mainly caused by the infection of high guanine plus cytosine content Gram-positive mycobacteria, mainly *Mycobacterium tuberculosis*. According to the World Health Organization (WHO) 2007 Tuberculosis Facts, the annual incidences of tuberculosis are stable or falling in all six WHO regions and have peaked globally.[1,2] Nonetheless, the total number of cases in developing countries is still increasing. Tuberculosis remains a leading cause of death worldwide and is a potentially lethal infectious disease in Hong Kong.

The complete genome sequence of *M tuberculosis* enables better understanding of this bacterium and the potential targets that may be useful in therapeutic interventions. By using postgenomic methods, identification of essential genes opened a window of opportunity to pursue novel therapeutic strategies to develop antituberculosis agents. Recent research identified a group of essential genes based on a postgenomic *in silico* approach, and suggested that the glutamate synthase is one of the essential genes in the *M tuberculosis* life cycle.[3] As both large and small subunits of glutamate synthase, *gltB* and *gltD* respectively, are absent from the human genome, targeting the glutamate synthase may be an excellent means of treating tuberculosis. This study may provide the foundation for new therapeutic strategies against tuberculosis, by cloning and purifying both subunits of the *M tuberculosis* glutamate synthase.

Methods

This study was conducted from January to June 2007. The *E coli* strain TOP10 was used for gene cloning and deoxyribonucleic acid (DNA) sequencing, whereas BL21 (DE3) was used for protein expression. pGEM-T Easy Vector (Promega, USA) was used for cloning polymerase chain reaction (PCR) products, whereas pET-28a(+) vector (Novagen, USA) was used for protein expressions.

Oligonucleotide primers were purchased from Tech Dragon Limited in Hong Kong, and DNA sequencing was also performed by this company. Amplification of PCR was performed with Pwo SuperYield DNA Polymerase (Roche) using genomic DNA of *M tuberculosis* H37Rv provided by colleagues. The *gltB* and *gltD* open reading frames were amplified by PCR. The PCR products were cloned into pGEM-T Easy Vector (Promega) and then transformed into *E coli* strain TOP10 for propagating plasmids and then cloned into the NheI/HindIII site of pET-28a(+) Vector (Novagen) to yield plasmid pET28a-gltB, pET28a-gltD and pET28a-gltBD for protein expressions in *E coli* strain BL21 (DE3).

Plasmids pET28a-gltB and pET28a-gltD that encoded the α and β subunits of glutamate synthase were transformed into *E coli* BL21 (DE3). The cells were maintained in LB broth supplemented with 50 μg/mL of kanamycin. For protein expression, 1 M of IPTG was added to the culture to a final concentration of 0.5 mM IPTG to induce protein expression when the cells grow to the log phase (OD_{600}≈0.6). The cells were further incubated at room temperature or 37ºC for protein expression. For protein expression with addition of chemical chaperones, different concentrations of dimethyl sulfoxide and 4-phenylbutyric acid were added to the culture before IPTG induction.

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After expression of proteins, cells were pelleted by centrifugation and extraction buffer (50 mM Tris, pH 7.5, 0.3 M NaCl, 20 mM imidazole, and 10 mM β-mercaptoethanol, 1% Triton-X 100, 1X protease inhibitor cocktail [Roche]) was added to the pellet in a 1:100 ratio. The cell suspension was incubated on ice for 30 minutes and then sonicated by a tip probe sonicator. The soluble protein fraction and insoluble protein fraction were separated by centrifugation. For refolding of the α subunit, the insoluble fraction was dissolved in solubilising buffer (6 M urea, 50 mM Tris, pH 7.5, 20 mM imidazole, 0.3 M NaCl, 10 mM β-mercaptoethanol, 1X protease inhibitor cocktail [Roche]) in a 1:50 ratio. The solublised proteins was then rapidly diluted in 1 L of extraction buffer for protein refolding. This protein solution was centrifuged at 24000x g to remove the insoluble fraction, and the supernatant was collected for protein purification.

Both of the subunits were purified by using HisTrap FF column (Amersham Biosciences). The column was equilibrated in a 3 bed volume of 20 mM imidazole in elution buffer (50 mM Tris, pH 7.5, 0.3 M NaCl, 10 mM β-mercaptoethanol, 1% Triton-X 100, 1X protease inhibitor cocktail [Roche]), and then the protein solution was loaded on the column. For the α subunit, a column was washed with 50 mM imidazole in elution buffer, and the α subunit was eluted using 300 mM imidazole in elution buffer. For the β subunit, a column was washed by 75 mM imidazole in elution buffer, and the β subunit was eluted using 100 mM imidazole in elution buffer. Regarding screening for the protein expressions with added chemical chaperones, Ni-NTA Magnetic Agarose Beads (Qiagen) were used for purification of the α subunit. The purification step was carried out according to the manufacturer's instructions. Briefly, the soluble fraction of the cell extracts were loaded on the pre-washed beads and the beads were washed by extraction buffer. Subsequently, proteins were eluted using 500 mM imidazole in elution buffer. The purification of α and β subunits of glutamate synthase were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Glutamate synthase activity was detected by the assay described by Miller and Stadtman in 1972 with some modifications.[1] Glutamate synthase activity was determined by measuring the rate of oxidation of NADPH at 340 nm. Standard assay mixtures (250 μL) contained 0.16 mM NADPH, 1 mM α-ketoglutaric acid, 2 mM L-glutamine, 1 mM EDTA, 50 mM potassium phosphate buffer, pH 7.5, and sufficient enzyme to produce an absorbance change at 340 nm at 30ºC.

The absorbance at 280 nm and 440 nm were detected by spectrophotometer at room temperature. Also, for the detection of the flavin bound to the subunits, the emission spectrum of the purified subunits were recorded between 450 and 600 nm with excitation at 440 nm in a spectrofluorometer.[2]

Results

The glutamate synthase consisted of two subunits, *GltB* and *GltD*, which were cloned and purified independently. The genes were cloned and ligated into a heterologous protein expression vector in *E coli* using a hexahistidine tag for facile purification. The α subunit misfolded upon expression in *E coli,* but refolding and purification of the alpha subunit of the protein was successfully performed (Fig 1a). The β subunit expressed in the soluble form under low temperature expression conditions and was expressed and purified successfully, using immobilised metal affinity chromatography (Fig 1b).

Discussion

Previous research had identified a group of essential genes using a postgenomic *in silico* approach, and suggested that the glutamate synthase was an essential gene for the *M tuberculosis* life cycle.[3] By comparing the deduced amino acid sequences of *M tuberculosis gltB* and *gltD* with other

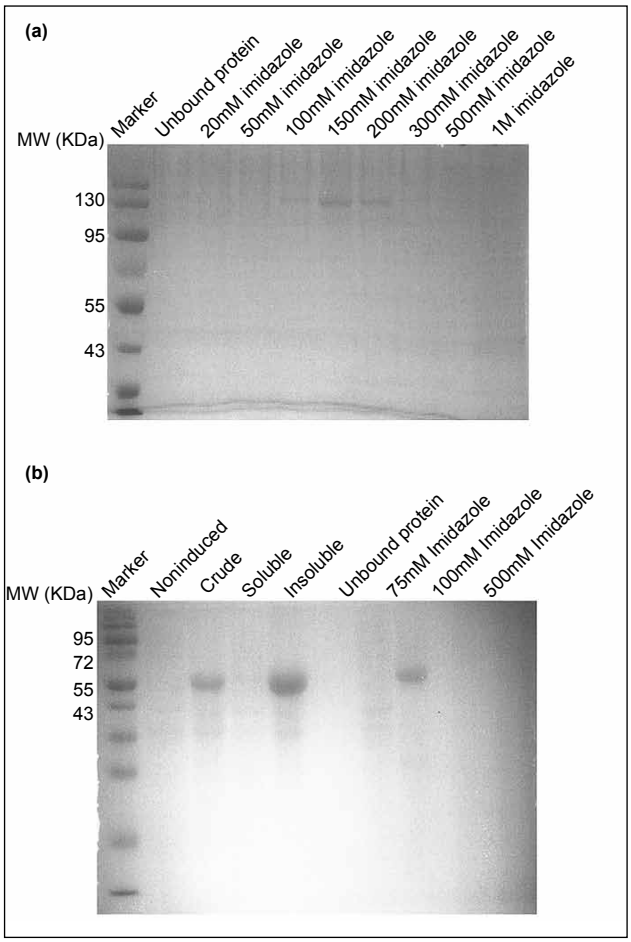

Figure 1. Purification of the α and β subunits of glutamate synthase using nickel affinity chromatography column: (a) purifying refolds the α subunit, whereby the α subunit is eluted using 100 mM to 200 mM imidazole in elution buffer; (b) purifying the soluble β subunit, where the β subunit is eluted using 100 mM imidazole in elution buffer.

mycobacteria, it was inferred that these proteins were highly conserved. This suggested that the cloning and expression of glutamate synthase genes might provide a foundation for the development of inhibitors against *Mycobacterium* growth.

We aimed to express the relevant glutamate synthase in a soluble and functional form. However, only the β subunit could be expressed in a soluble form. Even with assistance of chemical chaperones, the α subunit was expressed as an insoluble fraction. We therefore refolded the protein before purification.

Previous studies suggested that glutamate synthase was a multicomponent iron-sulphur flavoprotein, composed of two dissimilar subunits (α and β). The α subunit was believed to contain one flavin mononucleotide and β subunit one flavin adenine dinucleotide (FAD). As the glutamate synthase activity depends on the cooperation of both flavin co-factors, which the β subunit serves to transfer reducing equivalents from NADPH to the FMN co-factor through the FAD co-factor and the iron-sulfur cluster. Therefore, loss of co-factor during refolding may lead to loss of glutamate synthase activity. We also noted that in future we should scan the atomic absorption spectrum of both subunits to assess whether the iron-sulfur clusters persist in the purified glutamate synthase.

In this project, we successfully cloned the subunits, *gltB* and *gltD*, of glutamate synthase and the proteins encoded by these two open reading frames were expressed in *E coli* BL21 (DE3). The α subunit was expressed in an insoluble form and the β subunit as a soluble fraction. We were able to purify the α subunit after refolding. Further studies are required to fully characterise the purified and reconstituted enzyme before targeting this protein as part of a therapeutic strategy against tuberculosis.

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Inequalities in oral health and oral health care delivery among adults in Hong Kong: an analysis of extant data

Key Messages

1. Socio-demographic variations in oral health knowledge, attitudes and behaviour exist in Hong Kong; older adults living in institutions have poorest oral health knowledge, attitudes, and behaviour.
2. Socio-demographic variations in oral health exist with respect to clinical oral health status and perceived burden of oral health on quality of life. Older adults living in institutions have poorest dental caries status.
3. Socio-demographic variations in the use of oral health care services exist. Older people, particularly those living in institutions, have less accessible oral care services.
4. This study highlights inequalities in oral health and oral health care delivery among adults in Hong Kong. Appropriate provision of oral health care services for older people is needed.

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Introduction

In the past, local health service research has focused on mapping the prevalence and severity of various diseases over time. A key indication of the success of a health care system is the overall reduction in the prevalence of diseases that are life threatening or detrimental to quality of life. Although oral health care has improved markedly in recent decades, gross variations remain with respect to oral disease patterns among populations, which are primarily related to socio-demographic factors.[1] It is now widely viewed that a true measurement of the effectiveness of any health care system is how small are the variations in health status within the population. Information on social inequalities in oral health care in Hong Kong is scant. We analysed an extant data set of oral health in Hong Kong (using the Hong Kong Oral Health Survey conducted in 2001)[2] and assessed socio-demographic variations in oral health knowledge, attitudes, behaviour, and clinical status, as well as in the impact of oral health on quality of life, and in the use of oral health care services accounting for predisposing and enabling factors.

Methods

This project was conducted from November 2005 to October 2006. Socio-demographic variations in oral health knowledge scores (derived from knowledge of the causes of dental caries and periodontal disease and how to prevent them), oral health attitude scores (derived from attitudes towards dental check-up, dental treatment and tooth loss), and oral health behaviour scores were assessed using a 2-way analysis of variance (ANOVA). Oral health status comprised clinical oral health status (dental caries status according to the number of untreated decayed, missing, filled teeth [DMFT]), periodontal status (Community Periodontal Index), and perceived burden of oral health on quality of life (Oral Health Impact Profile scores) and was assessed using a 2-way ANOVA taking account of age-gender interactions. Finally, a regression model was created where dental attendance pattern (subjects categorised as 'regular' or 'irregular' dental service attenders) was the dependent variable, whereas oral health knowledge score, oral health attitude score, oral health behaviour score, and oral health status (of different age groups and genders) were independent variables.

Results

Variations in oral health knowledge, oral health attitudes, and oral health behaviour existed with respect to age ($P<0.001$, Table 1). Younger adults (aged 35-44 years) had better oral health knowledge, attitudes, and behaviour. Those aged ≥60 years living in institutions had the poorest oral health knowledge, attitudes, and behaviour. Gender variations in oral health knowledge, attitudes, and behaviour were not apparent ($P>0.05$).

Variations in clinical oral health status existed with respect to age and gender (Table 2). Age was associated with the dental caries status (the number of DMFT) [$P<0.001$]. Those aged ≥60 years living in institutions had the poorest

oral health. Gender variations existed among older people living in institutions with respect to the number of DMFT (P<0.001). Age had a borderline significant association with respect to Community Periodontal Index ratings (P=0.05). Variations in the impact of oral health on quality of life (OHIP14 scores) were apparent with respect to age (P<0.001). Older people had higher OHIP14 scores than younger adults aged 35 to 44 years (Table 2). Variations in the impact of oral health on quality of life with respect to residency (community versus institution) among older adults were not apparent (P>0.05). Gender variations in the impact of oral health on quality of life were not apparent (P>0.05).

Having accounted for oral health knowledge, attitudes, behaviour, and status, as well as socio-demographic factors

Table 1. Socio-demographic variations in oral health knowledge, attitudes, and behaviour

Socio-demographic (Mean±SD)	Adult (aged 35 to 44 years)			Non-institutionalised older people			Institutionalised older people		
	Male (n=157)	Female (n=218)	Total (n=375)	Male (n=157)	Female (n=159)	Total (n=316)	Male (n=116)	Female (n=247)	Total (n=363)
Oral health knowledge score*	3.54±1.69	3.64±1.64	3.60±1.66	1.97±1.52	1.86±1.50	1.91±1.51	1.27±1.74	1.11±1.78	1.16±1.77
Oral health attitude score†	1.92±0.95	1.93±0.92	1.93±0.93	1.36±1.02	1.32±1.02	1.34±1.02	1.02±0.90	0.88±0.90	0.93±0.90
Oral health behaviour score‡	1.32±0.97	1.42±0.94	1.38±0.93	1.24±1.01	1.19±1.04	1.21±1.03	0.88±0.76	0.76±0.72	0.89±0.83

* 2-way ANOVA: age group, P<0.001; gender, P=0.946/0.995; age*gender, P=0.394

† 2-way ANOVA: age group, P<0.001; gender, P=0.793/0.810; age*gender, P=0.762

‡ 2-way ANOVA: age group, P<0.001; gender, P=0.523/0.726; age*gender, P=0.643

Table 2. Socio-demographic variations in oral health status

Socio-demographic	Adult (aged 35 to 44 years)			Non-institutionalised older people (NOP)			Institutionalised older people		
	Male (n=157)	Female (n=218)	Total (n=375)	Male (n=157)	Female (n=159)	Total (n=316)	Male (n=116)	Female (n=247)	Total (n=363)
Mean±SD dental caries status (No. of untreated decayed, missing, filled teeth)	7.3±4.7	7.7±4.9	7.5±4.8	16.9±8.9	17.7±9.4	17.3±9.2	22.2±7.4	25.8±8.5	24.7±8.3
t-test within gender (P value)	0.390			0.451			<0.001		
t-test within age group: adult & NOP (P value)	<0.001								
Periodontal status (%)									
No periodontal disease	0.0	1.4	0.7	0.0	0.0	0	0.0	0.6	0.4
Bleeding only	4.5	3.2	3.4	1.6	2.3	1.8	0.0	0.0	0.0
Calculus	47.4	52.1	49.9	40.6	46.2	43.1	42.7	52.8	49.2
Shallow pockets	39.1	37.3	38.9	42.2	43.8	43.6	42.7	33.1	36.7
Deep pockets	9.0	6.0	7.1	15.6	7.7	11.5	14.6	13.5	13.7
Chi-square test within gender (P value)	0.390			0.244			0.334		
Chi-square test within age group: adult & NOP (P value)	0.052								
Mean±SD oral health–related quality of life score (OHIP-14 score)	4.4±5.6	3.8±4.7	4.1±5.1	7.0±8.0	6.7±8.8	6.9±8.4	6.2±9.3	4.6±8.9	5.1±9.0
t-test within gender (P value)	-0.229			0.781			0.114		
t-test within age group: adult & NOP (P value)	<0.001								

Table 3. Summary of logistic regression analyses*

Irregular dental service attender	Regression coefficient	Standard error	Odds ratio	95% CI	P value
Age (1=older adults living in institutions, 0=all other adults)	0.45	0.17	1.56	1.12-2.19	0.009
Gender (1=female, 0=male)	-0.34	0.17	0.71	0.52-0.98	0.04
Oral health attitude score	0.36	0.09	1.44	1.21-1.70	<0.001
Oral health behaviour score	0.40	0.19	1.49	1.03-2.14	0.03
Periodontal status (1=periodontal pockets, 0=no periodontal pockets)	-0.37	0.16	0.69	0.50-0.95	0.02

* Dental caries status (No. of untreated decayed, missing, filled teeth), oral health–related quality of life (C-OHIP14) and oral health knowledge score are not associated with dental attendance patterns (P>0.05)

of age and gender, older people residing in institutions were 1.56 times more likely to be irregular dental service attenders than other adults (P=0.009; 95% confidence interval [CI], 1.12-2.19; Table 3). Women were 29% less likely to be irregular dental service attenders than men accounting for other factors in the model (P=0.04; 95% CI, 0.52-0.98). An increase in oral health attitude score of 1 was associated with a 1.44 times more likelihood of being irregular dental service attenders (P<0.001; 95% CI, 1.21-1.70) accounting for other factors in the model. An increase in oral health behaviour score of 1 was associated with a 1.49 times greater likelihood of being irregular dental service attenders (P<0.03; 95% CI, 1.03-2.14) accounting for other factors in the model. Subjects with periodontal pockets were 31% less likely to be irregular dental service attenders than those without periodontal pockets accounting for other factors in the model (P=0.02; 95% CI, 0.50-0.95).

Discussion

Variations in oral health knowledge, attitudes, and behaviour exist with respect to age. Since the 1980s, the Department of Health in Hong Kong has provided school dental services and oral health promotion activities. Most local young adults participated in the school dental service during childhood and benefited from it.[3] Among older people, particularly those living in institutions, there is a need to improve oral health knowledge and attitudes. Furthermore, positive oral health behaviour (snacking control and tooth brushing) among older people, particularly those living in institutions, should be encouraged.

Socio-demographic variations in clinical oral health status exist among Hong Kong adults. Older people have poorer overall dental caries status, particularly those living in institutions. It is unclear whether institutionalised older people, who have more ill health than the rest of the population, are also more likely to have less self-care ability, and thus have more oral diseases.[4] This is a gross inequality for which urgent attention is required.

Variations in the use of oral health services exist. Accounting for other factors in the final regression model, older people living in institutions were >50% more likely to be irregular dental service attenders than other adults. This highlights the need for the provision of oral health care in Hong Kong. It is important to determine whether usage of oral health care services is an issue related to accessibility.

Socio-demographic variations in oral health and oral health care delivery exist in Hong Kong. Older people living in institutions are deprived the most. Appropriate provision of oral health care services for older people, particularly those living in institutions, is needed.

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Therapeutic effect and safety of a traditional Chinese medicine for atopic dermatitis in children: a randomised, double-blind, placebo-controlled study

Key Message

The traditional Chinese medicine concoction using five herbs was palatable and well tolerated and was efficacious in reducing topical corticosteroid usage in children with moderate-to-severe atopic dermatitis.

Introduction

Atopic dermatitis (AD) is a common chronic relapsing skin disease affecting about 15% of children aged less than 15 years. As there is no definitive cure for the condition, the use of traditional Chinese herbal medicine (TCHM) is a potential adjunctive therapy. In an open-label study, a concoction of five herbal extracts twice daily has been found to be beneficial.[1] The five herbs included *Flos lonicerae* (*Jinyinhua*), *Herba menthae* (*Bohe*), *Cortex moutan* (*Danpi*), *Rhizoma atractylodis* (*Cangzhu*) and *Cortex phellodendri* (*Huangbai*). The formulation was based on a widely used traditional concoction, with no corticosteroid or related compound.[2] This study aimed to determine the therapeutic efficacy, tolerability, and safety of this concoction in children with AD.

Methods

This was a randomised, placebo-controlled, double-blind study conducted from November 2004 to November 2005. The sample size calculation was based on our pilot data.[1] In each arm (TCHM and placebo) of the study, it was estimated that 40 subjects would be required to achieve an 80% power (b=0.20) and an α error of 0.05 (2-tailed) for detecting a 25% difference (13-point changes) in mean total SCORAD between the two groups. The SCORAD is a validated scoring system that assesses objective parameters (area [A] and intensity [B] signs) and subjective symptoms (pruritus and sleep loss [C]). As the dropout rate was estimated to be 5%, 84 children were needed to be recruited.

The diagnosis of AD was based on criteria defined by Hanifin and Rajka.[3] Patients aged 5 to 21 years with moderate-to-severe AD (defined as a SCORAD of >15)[4] and attended the paediatric dermatology outpatient clinic of our university hospital were invited to participate. Patients were excluded if they had any other inflammatory dermatitis (eg psoriasis, seborrhoeic dermatitis, ichthyosis) or experienced overt asthmatic symptoms (eg cough, wheeze, shortness of breath or exercise-induced bronchospasm) in the preceding 4 weeks. Patients were not recruited if they had had systemic corticosteroids, immunomodulating drugs or other TCHMs in the preceding 4 weeks. The Clinical Research Ethics Committee of the Chinese University of Hong Kong approved the study. Written informed consent was obtained from each patient.

Patients were randomised to receive either TCHM or placebo. Both were matched, manufactured, packaged, and labelled by the Chinese Medicine Industry Development Centre, of the Hong Kong Institute of Vocational Education, which fulfilled Good Manufacturing Practice standards. The formula consisted of *Flos lonicerae* (*Jinyinhua*) 2 g, *Herba menthae* (*Bohe*) 1 g, *Cortex moutan* (*Danpi*) 2 g, *Rhizoma atractylodis* (*Cangzhu*) 2 g, and *Cortex phellodendri* (*Huangbai*) 2 g. The dosage was based on the standard prescription for this concoction for children (aged ≥7 years) and teenagers.[1] It was formulated into standard-weight capsules according to established procedures under the supervision of the Clinical Trials Section. The medications were distributed monthly, and three capsules were to be taken twice daily for 12 weeks.[2] As all patients had moderate-to-severe disease, it

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was not advisable to discontinue other routine medications during the trial, which included emollients, bath oils, soap substitutes, topical corticosteroids, and oral or systemic antihistamines. Patients were asked to record the frequency of both the trial and routine medications used. The amount of topical corticosteroids used was also recorded.

Participants were followed up at regular intervals at baseline (before treatment), and 4, 8, 12, and 16 weeks later. The severity of AD was assessed using the SCORAD.[4,5] Symptoms of coexisting allergic rhinitis (sneezing, watery rhinorrhoea, nasal congestion, itching nose, itching eyes, and eye watering) were quantified by the Allergic Rhinitis Score. All severe adverse events were investigated to determine whether they were directly related to the drugs or underlying condition. Hospitalisation was considered a severe adverse event. Blood samples for complete blood counts, eosinophil counts; total IgE levels as well as liver and renal function were assessed before treatment, and at the end of the 12-week treatment course. Unused trial and routine medications were quantified. Statistical analysis of the clinical and laboratory data was performed independently by a statistician not involved in the clinical trial.

Results

A total of 85 patients (42 taking TCHM and 43 on placebo) participated in the trial. The characteristics of the two groups were similar; there was no significant difference in pre-treatment SCORAD, Allergic Rhinitis Scores, IgE values, and eosinophil counts. Patients were treated with a combination of emollients, moderately potent topical corticosteroids (93% were on 0.1% mometasone furoate and 7% received no regular corticosteroids) or oral antihistamines (86% were taking chlorpheniramine and 14% received no regular antihistamine). Of 85 patients, 71 had co-existing allergic rhinitis.

In the TCHM and placebo groups, 93% and 92% of the prescribed capsules were taken, respectively. The respective mean SCORADs decreased from 58.3 to 49.7 and from 56.9 to 46.9 (Fig), but there was no significant difference between the groups at the end of treatment, nor in their component SCORAD scores. In contrast, there was a >30% improvement in the Children's Dermatology Life Quality Index in the TCHM group at the end of treatment (P=0.008) but no improvement for the placebo-treated patients (Fig).

Corticosteroid usage in the TCHM group was significantly reduced by a mean of 4 days per month compared to baseline usage, whereas in the placebo group it was reduced by one day. Of 79 patients using 1% mometasone furoate as the topical corticosteroid, the amount used was also significantly reduced in the TCHM group, but antihistamine usage was not significantly different between the groups. Addition of a more potent topical corticosteroid and antihistamine occurred during follow-up in two and seven patients on TCHM, and 3 and 5 patients on placebo, respectively. Anti-staphylococcal antibiotics, including cloxacillin and erythromycin were prescribed for 16 and 17 patients in the TCHM and placebo groups, respectively.

In the respective TCHM and placebo groups, 35 and 36 patients had symptoms of allergic rhinitis, but the total allergic rhinitis scores and most corresponding symptoms were not significantly different. Only sneezing score improved significantly in the TCHM group from 1.5±0.9 to 1.1±1.1.

The TCHM was well tolerated. There was no significant difference in the frequency of adverse events. Analysis of biochemical data also revealed no significant change in IgE levels, or haematological (complete blood counts, eosinophil counts) and biochemical (electrolytes, renal and liver function) parameters monitored. All patients had normal renal and liver functions following TCHM treatment. No patient complained that the capsule was unpalatable.

Discussion

In both the TCHM and placebo groups, the SCORADs, the extent of disease, the intensity of lesions and subjective symptoms decreased during the study period, but no significant difference was observed between the two groups. However, compared to baseline, the duration and quantity of corticosteroid (mometasone furoate) usage in the TCHM group was significantly reduced but not so in the placebo group. This suggests that TCHM may possess corticosteroid-sparing effects and that the improvement in the placebo group was at the expense of a greater use of topical corticosteroids. Using thin-layer chromatography,

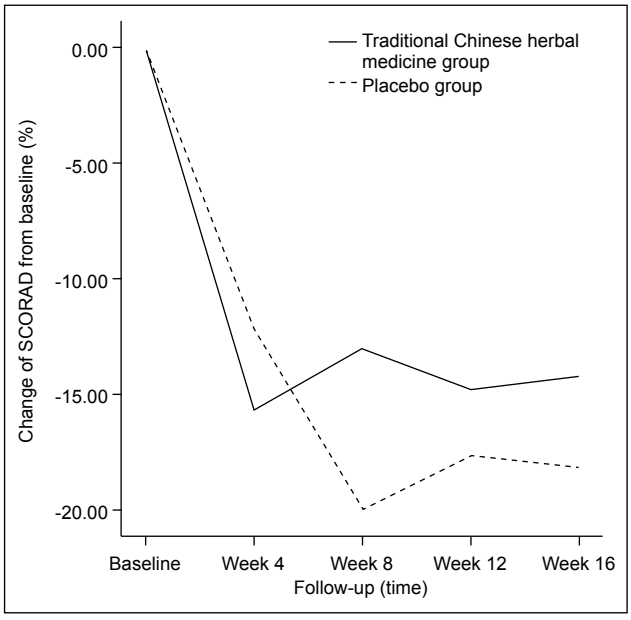

Fig. Percentage improvement of SCORAD from baseline

infrared spectrophotometry and liquid chromatography–mass spectrometry, we have previously reported that there were no corticosteroid or related precursors in this TCHM formulation.[2]

Adverse effects from this TCHM were uncommon, mild and self-limiting. There was no significant difference in haematological and biochemical parameters at baseline or following use of trial medications, and no derangement of liver and renal function during the 3 months of assessment. Unlike many bitter-tasting TCHM soups, children found the capsule easy to swallow and palatable.

This TCHM concoction was efficacious in reducing topical corticosteroid usage in children with moderate-to-severe AD. The formulation was palatable and well tolerated, and there was no derangement in haematological or biochemical parameters after treatment. This formulation can probably be used as an adjunctive treatment for children with refractory AD.

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Immunomodulatory activities of the herbal formula Kwan Du Bu Fei Dang in healthy subjects: a randomised, double-blind, placebo-controlled study

Key Messages

1. The herbal formula Kwan Du Bu Fei Dang transiently modulated the human immune system.
2. *Coriolus versicolor* had an immunostimulatory effect in mouse splenic lymphocytes.
3. *Ganoderma lucidum* inhibited SARS-CoV RNA-dependent RNA polymerase.
4. *Houttuynia cordata* possessed both immunostimulatory and anti-viral activities.
5. Kwan Du Bu Fei Dang, *Houttuynia cordata* and *Coriolus versicolor* were essentially non-toxic to laboratory animals after oral administration of 16 g/kg.

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Introduction

Severe acute respiratory syndrome (SARS) was a life-threatening form of pneumonia caused by SARS-coronavirus (SARS-CoV). Symptoms included cough, high fever and headache that could progress to interstitial infiltrates in the lung. From late 2002 to mid-2003, SARS affected more than 8000 persons worldwide, mostly in China. Due to the absence of definitive therapeutic western medicines, agents active against this disease are still needed.

The herbal formula Kwan Du Bu Fei Dang (抗毒補肺湯) [KDBFD] was an innovative product packaged in the form of sachets. It was based on two classical, popularly used formulae for treating influenza-like diseases known as Wan Bin (溫病). The formula was a combination of Sang Ju Yin (桑菊飲) and Yu Ping Feng San (玉屏風散) plus two other herbs with well-known antiviral properties. The herbs in the formula were Folium Mori (3.75 g), Flos Chrysanthemi (1.5 g), Semen Armeniacae Amarum (3.0 g), Fructus Forsythiae (2.5 g), Herba Menthae (1.25 g), Radix Platycodonis (3.0 g), Radix Glycyrrhizae (1.25 g), Rhizoma Phragmitis (3.0 g), Radix Scutellariae (6.0 g), Folium Isatidis (8.0 g), Radix Astragali (7.5 g) and Radix Saposhnikovia (5.0 g). These raw herbs were boiled to form a decoction and then freeze-dried into granules and packaged (4.0 g per sachet), ready for reconstitution as a tea-like drink. The granules were prepared according to standard Good Manufacturing Practice.

In addition to KDBFD, four traditional Chinese medicines (TCMs) were included in the present study: *Houttuynia cordata* (HC), which has been used to relieve lung-related symptoms and its anti-inflammatory and anti-viral effects are supported by scientific data; *Sinomenium acutum* (SA), which has been shown to possess anti-inflammatory effects and has been used to treat rheumatoid arthritis in China for over 2000 years; *Coriolus versicolor* (CV) and *Ganoderma lucidum* (GL), which are well known for their immunomodulatory actions.

Methods

This study was conducted from May 2005 to May 2007 and comprised two clinical trials. The first was a self-controlled study and the second was a randomised, double-blinded, placebo-controlled study. Only the results of the second study are reported. The results of the first study as well as the immunomodulatory and anti-SARS activities of HC have been published elsewhere.[1,2]

Volunteers were screened to ensure they were healthy, based on results of their complete blood count, levels of lactate dehydrogenase and creatine kinase, renal function and liver function. A total of 80 healthy subjects were enrolled. Each eligible subject was randomised to receive placebo or KDBFD treatment, according to a computer-generated code list. Neither the investigators nor subjects knew the treatments assigned. The placebo was prepared to appear, smell, and taste indistinguishable from the KDBFD, and was devoid of pharmacological activity and toxicity. Subjects were required to take one sachet of KDBFD (4 g) or placebo once per day for 7 days. Blood samples were collected from them on

days 0, 7 and 21. Changes in immune markers including the *ex vivo* production of cytokines, and the percentage and absolute numbers of $CD4^+$ and $CD8^+$ T-lymphocytes, the CD4/CD8 ratio, $CD56^+$ NK cells, and $CD19^+$ B-lymphocytes were evaluated.

The immunomodulatory activities and anti-viral effects of KDBFD, HC, SA, GL and CV were investigated. Proliferation of mouse splenic lymphocytes was used as the first-line screening assay to evaluate whether the KDBFD or TCMs possessed immunostimulatory activity. Samples with immunostimulatory activity were further studied for their effects on cytokine production and T cell populations. The effects of the KDBFD or TCMs on two SARS-CoV enzymes, RNA-dependent RNA polymerase and 3C-like protease, were evaluated.

The acute oral toxicity test was carried out according to the Procedures and Methods for Toxicological Assessment on Food Safety - Acute toxicity test (GB15193.3-94) issued by Ministry of Health, People's Republic of China.

Results

The numbers of T-lymphocytes, $CD8^+$ suppressor plus cytotoxic T-lymphocytes, $CD4^+$ helper T-lymphocytes, and $CD56^+$ NK cells in KDBFD treatment group were significantly elevated at day 7, compared to day 0 (all $P<0.05$). However, such significant elevations of cell numbers were generally not observed at day 21 compared to day 7 or 0 (all $P>0.05$). The proportion of NK and NK cell numbers was significantly elevated at day 21, compared to day 0 and 7, and day 0, respectively (all $P<0.05$). In the placebo group, only the cell number of $CD8^+$ suppressor plus cytotoxic T-lymphocytes showed a significant decrease from day 0 to day 7 ($P<0.05$). Liver function and renal function parameters in the two groups were not significantly different throughout the study period.

Regarding the *ex vivo* cytokine production of IL-1β, IL-6, IL-8, IL-12, TNF-α and IL-10, there were no significant differences in the stimulation (%) of cytokines released on day 7 or 21 compared to day 0 in the KDBFD and placebo groups (all $P>0.05$). Inter-group comparison analysis of *ex vivo* cytokine production and lymphocyte subsets in the two groups showed that the increase in TNF-α production (day 21 and day 7 vs day 0) were significantly higher in the placebo than KDBFD group, but the increase in NK cells (day 21 vs day 7) were significantly higher in the KDBFD group (all $P<0.05$). However, most of the measured immunological parameters were not significantly different (all $P>0.05$).

Immunological assays

Splenic lymphocytes of Balb/c mice were incubated with the KDBFD or TCM extracts at concentrations of 0-400 μg/mL in the presence of polymyxin B sulfate for 48 and 72 hours. Extract of CV was found to significantly stimulate the proliferation of mouse splenic lymphocytes in a dose-dependent manner, whereas KDBFD and SA were immunosuppressive at high concentrations. In contrast, GL was neither stimulatory nor suppressive. Using flow cytometry, it was found that CV extract increased the proportion of $CD4^+$ and $CD8^+$ T cells. These data indicated that CV stimulated T cell proliferation *in vitro*. After 48 and 72 hours of treatment, the CV extract increased the levels of IL-2 and IL-10 significantly, even at relatively low concentration, ie 100 μg/mL. Moreover, it stimulated the secretion of IL-4 and IFN-γ to a certain extent.

Anti-viral assays

Extracts of KDBFD/TCM inhibited SARS-CoV RNA-dependent RNA polymerase in a dose-dependent manner and their effectiveness in descending order was: GL>CV>SA>HC>KDBFD. Their IC_{50} values were 41.9, 108.4, 198.6, 251.1 and 471.3 μg/mL, respectively. In the SARS-CoV 3C-like protease assay, only the HC extract possessed dose-dependent inhibitory activity on this viral enzyme, whilst other TCM extracts/KDBFD showed insignificant effects at doses up to 1000 μg/mL.

Acute oral toxicity test

There was no significant difference in body weights between KDBFD/HC/CV and the control groups. All live animals appeared normal throughout the 7-day observation period. From our results, KDBFD/HC/CV were essentially non-toxic to laboratory animals following oral administration at 16 g/kg. In contrast, SA and GL caused 70-100% mortality within the same period.

Discussion

In this study, cell numbers of $CD8^+$ suppressor plus cytotoxic T-lymphocytes and $CD4^+$ helper T-lymphocytes were significantly increased after taking KDBFD for 7 days. No such increase was observed at day 21 after KDBFD was stopped as well as in the placebo group. Most immunological parameters in the KDBFD and placebo groups did not differ significantly (all $P>0.05$). This may have been due to insufficient sample sizes in each group.

The herbal preparation had transient beneficial effect on some immune functions in healthy subjects, but not significant when compared to the placebo group. No adverse event was observed during the study period; liver function and renal function remained normal.

During SARS-CoV infection, lymphocytes are the first line of defence. Attrition of these cells may result in a compromised immune response and eventually the development of disease. In SARS patients, lymphopaenia was usually observed during the initial phase of infection and virus-induced apoptosis was considered to be the major cause of lymphopaenia.[3] The results in mouse splenic lymphocytes demonstrated that HC and CV extracts had an

immunostimulatory effect. They were found to stimulate the proliferation of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T cells, which may in turn help to prevent the development of lymphopaenia and the pathogenesis of SARS.

SARS-CoV RNA-dependent RNA polymerase (RdRp) and SARS-CoV 3C-like protease ($3CL^{pro}$) are two enzymes important in the viral replication processes. RdRp is responsible for both positive and negative strand RNA synthesis. It is the essential enzyme in a replicase complex that contains additional viral and cellular proteins. In contrast, $3CL^{pro}$ is responsible for releasing the key replicative enzymes such as RdRp and helicase from the polyprotein precursors.[4] The functional importance of RdRp and $3CL^{pro}$ in the life cycle of SARS-CoV make them the key targets for the development of drugs directed against the virus.[5] From our results, GL and HC were the most potent TCM agents in inhibiting SARS-CoV RdRp and $3CL^{pro}$, respectively. They may be able to slow down viral growth and minimise its destructiveness.

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Efficacy and active components of herbal extracts on the treatment of tinea pedis

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Key Messages

1. Ethanolic extracts of Fructus Psoraleae (補骨脂), Rhizoma Curcumae Longae (姜黃), and Folium Eucalypti Globuli (藍桉葉) possess *in vitro* antifungal activities (against *Trichophyton mentagrophytes* and *Trichophyton rubrum)*.

2. An herbal formula, comprising these ethanolic extracts in the ratio of 1:1:1, could effectively alleviate tinea pedis caused by *T mentagrophytes* in guinea pigs (P<0.01).

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Introduction

Tinea pedis (also known as athlete's foot) is a superficial fungal infection of the feet. In a prospective epidemiological study conducted in Hong Kong in 1999, its prevalence was 20.4% in 1014 subjects.[1] Tinea pedis is more commonly found in males, the middle-aged or elderly, and in those with diabetic or other underlying medical (particularly immune system) problems that favour fungal growth. The most common causal organisms are the dermatophytes: *Trichophyton mentagrophytes, Trichophyton rubrum* and *Epidermophyton floccosum*.

Treatment for tinea pedis involves the use of topical antifungals (such as ketoconazole, terbinafine, econazole, and cicloprox creams) and oral agents (such as griseofulvin, itraconazole, fluconazole, and terbinafine). Inadequate spectrum of activity, drug resistance, toxicities, and drug-drug interactions limit successful results, for which reason alternative medicines may be desirable. In a review on the antifungal activities of over 1000 species of traditional Chinese medicines (TCMs) recorded in Pharmacopoeia of the People's Republic of China (2005 edition), 83 have been demonstrated to possess antifungal activity of which 11 are efficacious against *Trichophyton* strains. These include Bulbus Allii (大蒜), Fructus Galangae (紅豆蔻), Semen Cassiae (決明子), Herba Cichorii (菊苣), Rhizoma Curcumae Longae (姜黃) [RCL], Semen Juglandis (核桃仁), Herba Portulacae (馬齒莧), Cortex Pseudolaricis (土荊皮), Fructus Psoraleae (補骨脂) [FP], Fructus Chebulae (訶子), and Folium Eucalypti Globuli (藍桉葉) [FEG].

Methods

This study was conducted from December 2006 to December 2008. Aqueous and ethanolic extracts of the 11 TCMs were yielded by extracting in water or 95% ethanol under reflux. The antifungal efficacies of these extracts were compared using an *in vitro* antifungal test. Three most potent extracts were selected and subjected to activity-guided fractionation. A guinea pig model was used to evaluate the tinea pedis–treating effects of the active extracts and fractions. The *in vitro* assay was performed in duplicates and the *in vivo* assay was on at least four samples.

Results

In vitro antifungal susceptibility test

The aqueous extracts of Fructus Galangae, Herba Portulacae, Semen Juglandis, Semen Cassiae, and FP promoted fungal growth, whereas those of Fructus Chebulae and FEG were effective in inhibiting dermatophyte (*T mentagrophytes* and *T rubrum*) growth at 3.91 and 7.81 μg/mL concentrations, respectively (Table 1). Among ethanolic extracts, those of FP, RCL, and FEG possessed the most potent antifungal activity. Their minimum inhibitory concentration (MIC) values of 0 (for 100% inhibition) of both dermatophytes were low. After comparing the antifungal effects of all 22 extracts, ethanolic extracts of FP, RCL, and FEG were the most effective and selected for further fractionation.

Each potent extract was fractionated using solvent partition into five

fractions (n-hexane, dichloromethane, ethyl acetate, n-butanol, and water residue). Based on the MIC values determined in the *in vitro* antifungal assay, the n-hexane fraction (FP-EtOH-P1) and the dichloromethane fraction (FP-EtOH-P2) from FP, and the n-hexane fraction (FEG-EtOH-P1) from FEG exhibited the most potent inhibitory effect against dermatophytes. They were then subjected to column chromatography to give 10, 9, and 8 subfractions, respectively. Among these 27 samples, subfractions FP-EtOH-P1-C2, FP-EtOH-P2-C1, and FEG-EtOH-P1-C6 were the most active against dermatophytes (Table 2). Subsequent activity-guided fractionation led to two active compounds: bakuchiol from FP and macrocarpal C from FEG. Their antifungal activities have been reported.[2]

Table 1. Minimum inhibitory concentration (MIC) values of 11 traditional Chinese medicines extracted by water or 95% ethanol

Traditional Chinese medicines	Minimum inhibitory concentration (MIC)* values (μg/mL)											
	Aqueous extracts						Ethanolic extracts					
	Trichophyton mentagrophytes (ATCC 9129)			*T rubrum* (ATCC 28191)			*T mentagrophytes* (ATCC 9129)			*T rubrum* (ATCC 28191)		
	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2
Fructus Galangae (紅豆蔻)	PG†	PG	PG	PG	PG	PG	62.5	-	7.81	15.6	-	7.81
Herba Portulacae (馬齒莧)	PG	PG	PG	PG	PG	PG	125	62.5	31.25	125	62.5	-
Fructus Chebulae (訶子)	3.91	0.49	-	31.25	15.6	7.81	3.91	0.98	0.49	62.5	31.25	-
Semen Juglandis (核桃仁)	48	6	0.19	PG	PG	PG	-	-	500	-	250	125
Cortex Pseudolaricis (土荊皮)	7.81	1.95	0.49	31.25	15.6	7.81	3.91	0.98	0.49	62.5	-	-
Semen Cassiae (決明子)	PG	PG	PG	PG	PG	PG	125	31.25	7.81	250	125	62.5
Fructus Psoraleae (補骨脂)	PG	PG	PG	PG	PG	PG	7.81	3.91	-	15.6	7.81	3.91
Rhizoma Curcumae Longae (姜黃)	-	500	250	-	500	125	7.81	-	3.91	15.6	7.81	3.91
Bulbus Allii (大蒜)	>500	-	-	>500	-	-	500	250	-	500	250	-
Herba Cichorii (菊苣)	>500	-	-	>500	-	-		500	-	500	250	-
Folium Eucalypti Globuli (藍桉葉)	7.81	3.91	1.95	7.81	3.91	1.95	3.91	1.95	-	15.6	-	-

* MIC 0 denotes 100% inhibition, MIC 1 75% inhibition, and MIC 2 50% inhibition

† PG denotes promote growth

Table 2. Minimum inhibitory concentration (MIC) values of sub-fractions of the three selected fractions

Sub-fraction	Minimum inhibitory concentration (MIC)* values (μg/mL)					
	Trichophyton mentagrophytes (ATCC 9129)			*T rubrum* (ATCC 28191)		
	MIC 0	MIC 1	MIC 2	MIC 0	MIC 1	MIC 2
Fructus Psoraleae fraction (FP-EtOH-P1)						
C1	-	500.00	-	-	500.00	125.00
C2	7.81	3.91	1.95	31.25	3.91	1.95
C3	125.00	-	62.50	31.25	15.60	7.81
C4	125.00	-	62.50	31.25	15.60	7.81
C5	125.00	-	31.25	62.50	15.60	7.81
C6	125.00	62.50	-	62.50	31.25	15.60
C7	125.00	31.25	-	62.50	31.25	15.60
C8	500.00	125.00	62.50	31.25	15.60	7.81
C9	-	125.00	62.50	31.25	15.60	7.81
C10	-	250.00	125.00	125.00	62.50	15.60
Fructus Psoraleae fraction (FP-EtOH-P2)						
C1	7.81	0.98	0.49	15.60	3.91	1.95
C2	250.00	125.00	-	125.00	62.50	31.25
C3	31.25	15.60	7.81	62.50	31.25	15.60
C4	125.00	62.50	31.25	125.00	-	62.50
C5	62.50	-	31.25	125.00	62.50	31.25
C6	500.00	250.00	125.00	500.00	250.00	125.00
C7	31.25	-	15.60	125.00	31.25	15.60
C8	15.60	-	7.81	250.00	62.50	31.25
C9	-	-	500.00	-	250.00	31.25
Folium Eucalypti Globuli fraction (FEG-EtOH-P1)						
C1	15.60	3.91	1.95	62.50	31.25	7.81
C2	15.60	7.81	3.91	62.50	31.25	15.60
C3	15.60	3.91	1.95	62.50	15.60	7.81
C4	7.81	3.91	1.95	31.25	15.60	3.91
C5	1.95	0.98	0.49	15.60	-	3.91
C6	1.95	0.98	0.49	3.91	1.95	0.98
C7	7.81	3.91	0.98	31.25	15.60	7.81
C8	-	3.91	1.95	-	62.50	15.60

* MIC 0 denotes 100% inhibition, MIC 1 75% inhibition, and MIC 2 50% inhibition

Besides individual antifungal activity, synergistic effects among herbs (combination of three of the most potent TCM extracts) and among fractions (combination of three of the most potent fractions) were studied using the *in vitro* antifungal susceptibility test. The MIC 0 values of herbal combination on *T mentagrophytes* and *T rubrum* were 250 and 500 μg/mL, respectively, and were much higher than the values of the individual ethanolic extracts. For FP and RCL, their MIC 0 values for *T mentagrophytes* and *T rubrum* were 7.81 and 15.6 μg/mL, respectively, whereas for FEG, they were 3.91 and 15.6 μg/mL, respectively. Therefore, no synergism was evident for the herbal combination. On the contrary, the fractional combination was more effective than its component fractions FP-EtOH-P2 and FEG-EtOH-P1. Nevertheless, when compared to its third component fraction FP-EtOH-P1, enhancement in antifungal activity was not demonstrated in the fractional combination (their MIC 0 values on *T mentagrophytes* and *T rubrum* were equal).

Guinea pig model of tinea pedis–treating effects

Tinea pedis was induced in the right hind feet of guinea pigs by inoculating a fungal suspension of *T mentagrophytes* for 7 days. On day 7 post-infection, the infected feet showed scale formation on the toes and soles. In the negative controls, aqueous cream was applied topically to the infected feet for 12 days. At the end of experiment, the animals were killed and skin from their feet was excised for fungal culture. Among 10 skin blocks from each foot, on average there were eight to nine blocks retrieved for fungal growth on agar slants. On the other hand, the application of positive control terbinafin (Lamisil) cream completely cured the tinea pedis, as reflected by the low fungal burden score. With this successfully established and validated animal model, the tinea pedis–treating effect of ethanolic extracts of FP, RCL, and FEG (the three most potent TCM extracts in the *in vitro* antifungal assay) as well as their active fractions were investigated.

Topical application of ethanolic extracts of FP, RCL, and FEG as 5% cream to the infected guinea pig feet was able to alleviate tinea pedis. Although the results were not significant, the extracts reduced the mean±standard deviation fungal burden to 4.9±4.3, 6.0±3.3, and 7.0±2.8, respectively, compared to 8.5±2.5 in the controls. Although they were not very effective when used alone, synergism was demonstrated when used as a herbal combination. The herbal combination of these three ethanolic extracts (in the ratio of 1:1:1) significantly decreased the fungal burden to 2.5±1.8, compared to 8.0±2.9 in the controls ($P<0.01$, Table 3).

The most potent fractions inhibiting growth of dermatophytes *in vitro* were FP-EtOH-P1, FP-EtOH-P2, and FEG-EtOH-P1. Therefore, their tinea pedis–treating activity was studied in the guinea pig model. These fractions individually or in combination alleviated tinea pedis to some extent; the resulting fungal burdens (5.4-6.1) were lower than that of control group (~8.0), but not significantly.

Discussion

Eleven herbs were shortlisted for antifungal screening, because various aqueous and organic solvent extracts and pure compounds derived from them had been reported to show activity against *Trichophyton* strains. By using a standardised broth dilution method, the antifungal activities of aqueous and ethanol extracts of these 11 TCMs could be compared. The aqueous extracts of Fructus Galangae, Herba Portulacae, Semen Juglandis, Semen Cassiae and FP promoted fungal growth (Table 1). We speculated that the high sugar contents of these extracts provided nutrients for fungal growth. As determined by the anthrone-sulfuric acid test, the total sugar contents in these five extracts were high, and ranged from 19.37% w/w to 68.41% w/w (data not shown). The ethanolic extracts were more effective in inhibiting the dermatophyte. As in all clinical trials using herbs, attention should be paid to the variation in efficacy resulting from different extractions using different solvents.

Among the 11 TCMs, FP, RCL, and FEG extracted by ethanol were the most effective for inhibiting the growth of dermatophytes *in vitro* (Table 1). Fructus Psoraleae are dried ripe fruits of *Psoralea corylifolia* L. and are traditionally used to invigorate kidney function, alleviate asthma and relieve diarrhoea. The antidermatophytic activity of *Psoralea corylifolia* seed extracts have been evaluated using the disc diffusion method.[3] At 250 μg/mL, its ethanolic extract exhibited activity with an inhibition halo diameter of 24 mm and 23 mm against *T mentagrophytes* and *T rubrum*, respectively. Despite different assay methods, our results were in line with those previously documented, and confirmed the antifungal activity of FP.

Rhizoma Curcumae Longae are the dried rhizomes of

Table 3. Therapeutic efficacy of the herbal combination of Fructus Psoraleae, Rhizoma Curcumae Longae and Folium Eucalypti Globuli ethanolic extracts (1:1:1) by topical application for 12 days on tinea pedis of guinea pigs infected with *Trichophyton mentagrophytes*

Treatment	No. of animals	No. of culture-positive skin blocks/total no. of skin blocks	Fungal burden
Aqueous cream (control group)		56/70	8.0±2.9
Terbinafine cream	4	0/40	0*
Herbal combination	8	20/80	2.5±1.8*

* $P<0.01$, versus control group

Curcuma longa L. and are traditionally used to eliminate blood stasis, promote the flow of qi, stimulate menstrual discharge, and relieve pain. The ethanolic extract of RCL could exert 65% inhibition on *T longifusus*. We reported the inhibitory effect of RCL on *T mentagrophytes* and *T rubrum*. Turmeric oil and curcumin are two active components of RCL. Curcumin has no antifungal activity, whereas turmeric oil could inhibit dermatophytes, but at a relatively high MIC (>200 μg/mL).[4] Our results also showed that fractions of RCL were less effective than the crude ethanolic extract, indicating that the latter might be a more effective treatment against dermatophytes.

Folium Eucalypti Globuli are the fresh leaves of *Eucalyptus globules* Labill and are used to treat influenza, headache, cough, eczema and dermatomycosis. Its methanol-dichloromethane (1:1) extract was demonstrated to inhibit *T mentagrophytes*, with a MIC of 31 μg/mL.[5] Its ethanolic extract also had potent activity against this dermatophyte.

In TCM practice, treatment of tinea pedis involves the use of TCM decoctions of four to eight herbs. We investigated whether a formula composing of FP, RCL, and FEG could exert synergism and, thus, better antidermatophytic activity. Using an *in vitro* antifungal assay, this herbal formula showed only weak inhibitory effects. The combination might be antagonistic, instead of synergistic. Nonetheless, this formula could significantly reduce the fungal burden in the guinea pig model of tinea pedis. We speculate that involvement of host's immune response might play a role in the effectiveness of this herbal formula.

The herbal formula (FP, RCL, and FEG ethanolic extracts in the ratio of 1:1:1) used as a topical agent is efficacious for alleviating tinea pedis (Table 3). However, when compared with existing western medications, such as terbinafine (Lamisil) cream, it was much less effective, probably because the herbal extracts contain macromolecules that the presumed antifungal activity cannot penetrate effectively into the epidermis and thus their dermatophyte-killing actions remain superficial. To increase the efficacy of the herbal formula, the therapeutic concentration/dosage form/ratio of the three extracts needs refinement, or nano-technology could be applied to enhance transdermal absorption. The use of simple chemicals could also enhance the transcutaneous absorption and efficacy. At this point, the herbal formula was not powerful enough to replace terbinafine for treating tinea pedis. Nonetheless, it may be considered as an alternative/supplementary medicine for terbinafine-resistant cases.

Besides efficacy, safety issues should also be considered when developing the herbal formula. Liver injury associated with the oral intake of FP has been reported. Skin toxicity of these three herbs is rarely reported. Therefore, hypersensitivity and allergic responses following topical application of the herbal formula need evaluation. Our laboratory has done sensitivity tests for topical agents used for wound healing. The same platform may serve this purpose.

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AUTHOR INDEX

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