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INFORMATION

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RESEARCH PAPERS

Using High Performance Liquid Chromatography to Quantitate Artemether and Artesunate Anti-Malarial Tablets in the National Capital District, Papua New Guinea

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ABSTRACT:

Malaria is a major public health problem in Papua New Guinea (PNG). The Artemisinin-based combination therapy is widely used as the first-line treatment for malaria in PNG. This study was to assess the quantity of the Artemether and Artesunate ingredients in the antimalarial drugs used for the treatment of malaria in the National Capital District (NCD) PNG.

Artemether and Artesunate tablets were purchased from various pharmacies in NCD. Artemether and Artesunate solutions were prepared according to the Standard United States Pharmacopoeial protocol for assay of active ingredients by high performance liquid chromatography (HPLC). The results indicated that the percent Artemether content in the three brands (ART 01, ART 02 and ART 03) of Artemether purchased in the NCD were 93.2%, 87.6% and 89.3% respectively. Four brands (ATS 01/02, ATS 03/04, ATS 05, and ATS 06) of Artesunate were purchased in the NCD. The % Artesunate content in the four brands were 109.0%, 110.0%, 101.2% and 96.2%% respectively. The three Artemether brands (100%) and two (ATS 01/02 and ATS 03/04) of the Artesunate brands (50%) did not satisfy the USP specifications for the amount of active ingredients in the drugs.

Our data indicate that poor quality Artemether and Artesunate antimalarial drugs are sold in the National Capital District in PNG. This indicates the urgent need to advocate for more efficient drug monitoring and effective enforcement of regulations that prevents importation of substandard drugs into the NCD.

Key words: Substandard, Antimalarial drugs, Artemether, Artesunate, Papua New Guinea
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INTRODUCTION:

Malaria is a major public health problem in Papua New Guinea (PNG). It is the commonest cause of admission to health facilities in most areas of PNG and continues to increase in all regions of the country in its severity [1, 2]. Effective antimalarial therapy is one of the major strategies recommended for the control and eradication of malaria.

In PNG, the Artemisinin-based combination therapy is widely used as the first-line treatment for malaria, after widespread resistance to other existing drugs by the malarial parasites [3 – 6]. This is in line with World Health Organisation (WHO) recommendations in the fight against malaria [7].

In recent years, there is considerable interest in determining the burden of diseases, such as malaria, in tropical countries where most of the world's infant and child deaths occur [8 - 9]. There has also been a marked increase in the number of research and clinical trials conducted to determine the most efficacious and appropriate local treatments, their cost-effectiveness, and factors determining the gap between efficacy and effectiveness, including the molecular genetics of drug failure in the management of malaria in resource limited countries [10 - 12]. However, there has been relatively little apparent interest in the quality of drugs used to treat malaria, despite the logical implication that poor quality drugs reduce the effectiveness of therapy and encourage drug resistance [11– 13].

Despite evidence suggesting that substandard counterfeit or degraded medicines are major problems of global importance, there are very few reliable data describing their epidemiology, or their effects on health and drug resistance [11, 13]. Such research and monitoring projects appear relatively difficult to find funding for and to publish despite their obvious and immediate relevance.

Drug quality is an essential translational link between epidemiology, clinical trial research, and improved public health [8, 12]. Translating evidence on drug treatment outcomes into treatment policy is futile if the drugs actually used are inferior in terms of efficacy or toxicity compared with the drugs originally evaluated [12, 13].

Anecdotal reports indicate widespread distribution of non-genuine Artemisinin drugs in the National Capital District (NCD) and

other provinces in PNG. The reports further indicate that there appears to be cases of treatment failures in some patients using the Artemisinin-derived anti-malarial drugs. The general concept that leads to spurious reporting of drug resistance and toxicity cannot be justified by these reports. This is because good quality anti-malarial drugs are often misused in treating malaria because of under-dosing and poor adherence, which could lead to treatment failures and development of drug resistance [7].

The use of counterfeit or substandard mono-therapies may further endanger malaria chemotherapy in the NCD. Thus, the express need to provide appropriate research data needed to answer the following questions. Are there poor quality Artemisinin drugs used in NCD? What is the prevalence of poor quality Artemisinin drugs in NCD? Appropriate answers to these and other questions are necessary for improving malaria treatment and in the development and implementation of actions designed to improve the quality of treatment. The lack of appropriate research data needed to respond to some of these questions was the justification for this project.

The aim of this study was to assess the quality of the Artemisinin drugs used for the treatment of malaria in NCD. The major objective was to determine the quantity of active ingredients in the Artemether and Artesunate antimalarial drugs used for treatment of malaria in NCD. Another objective was to produce some scientific data and make recommendations that can be translated into policies needed to increase access to good quality Artemisinin drugs, which may ultimately increase the rates of effective malaria treatment in NCD.

MATERIALS AND METHODS:

Artemether and Artesunate tablets were purchased and obtained from various pharmacies in the NCD, including the Port Moresby General Hospital (PMGH) pharmacy. Pure Artemether powder, donated by "Kunmign Pharmaceutical Corporation, China" was used as reference standard. Reagents used include Acetonitrile (EM Science/Merck KGaA Germany), Potassium Di-hydrogen Phosphate (Wako pure Chemicals, Ltd., China) and Orthophosphoric Acid. All reagents used were of Analytical grade. Solutions of Artemether and Artesunate standards and samples were prepared as indicated in the USP [14].

Preparation of standard solutions [14]: Artemether: Pure Artemether powder (100.0mg) was used to prepare a 10.0mg/ml

solution in Acetonitrile:Water (60:40). Artesunate: 50.0mg of pure Artesunate powder was dissolved in a total volume of 12.5ml Acetonitrile to give a solution containing 4.0mg/ml. Preparation of samples for HPLC analyses [14]:

For assay of Artemether: two 50.0mg tablets were crushed, ground to fine powder and then dissolved in Acetonitrile:Water (60:40) to obtain a 10.0mg/ml solution [14]. The solution was agitated for about 30 minutes to achieve complete dissolution. It was then filtered into a clean collecting tube and stored away from sunlight [14]. The same procedure was used to prepare duplicate solutions of other Artemether brands.

For assay of Artesunate: One 50.0mg tablet was crushed and ground to fine powder, which was then dissolved in Acetonitrile to obtain a 4.0mg/ml solution [14]. The solution was agitated for 30 minutes to achieve complete dissolution. The Artesunate solution obtained was filtered into a clean collecting tube and stored away from sunlight [14]. Similar procedure was used to prepare duplicate solutions of other Artesunate brands.

The Varian 920 HPLC fitted with a 4.6mm x 25.0cm column containing 5.0um L-1 C-18 packing was used to analyse both the Artemether and Artesunate solutions [14, 16]. Column temperature was 30^{o}C. The injection volume of each drug sample was 20.0ul. The mobile phases used were, Acetonitrile:Water (60:40) for Artemether; Acetonitrile:Phosphate Buffer pH 3.0 (12:13) for Artesunate [14]. The flow-rate of the mobile phase was set at 1.0ml/min in each analysis. The operating HPLC system used was the Galaxie Chromatography data software Version 1.9, configured for analyses of Artemether and Artesunate active ingredients against external standards. The wavelengths used were 210nm for Artemether and 216nm for Artesunate [14].

RESULTS:

The labelled brand strength for both Artemether and Artesunate tablets were 50.0mg. All brands procured were not expired at the time of purchase and expiry date for all had more than six months before their labelled expiry dates at the time of the study. Three different brands of Artemether were procured and labelled as ART 01, ART 02 and ART 03.

Table 1 shows the summary statistics of the Artemether content obtained by the HPLC analyses of the three brands of Artemether. The results presented are the means for 7, 11 and 9 separate analyses of ART 01, ART

02 and ART 03 brands respectively. The mean (± standard deviation) for the ART 01, ART 02 and ART 03 was 46.6 ± 1.1mg, 43.8 ± 3.3mg and 44.6 ± 2.4mg respectively.

For further analysis of the data the active Artemether ingredient per brand (Brand strength) indicated by the manufacturers was compared with the mean Artemether content obtained in the HPLC analysis of each brand. The results were also expressed as percent of Artemether content in each brand.

The data obtained is presented in Table 2, which also shows the % USP (United States Pharmacopeia) specification indicating the acceptable range for Artemether content in genuine tablets. The results indicated that the percent Artemether content in ART 01, ART 02 and ART 03 were 93.2%, 87.6% and 89.3% respectively.

A total of six batches comprising four different brands of Artesunate preparations were procured for analysis of Artesunate content. The samples were given identification labels ATS 01 to 06, respectively. ATS 01 was the same brand as ATS 02; ATS 03 was the same brand as ATS 04. A number of tablets ranging from two to six were analysed for their Artesunate content from the four different brands.

Table 3 shows the summary statistics of the Artesunate content obtained by the HPLC analyses of the four brands of Artesunate. The results presented are for the means of 4, 6, 2 and 4 separate analyses of ATS 01/02, ATS 03/04, ATS 05, and ATS 06 brands respectively.

The mean for the ATS 01/02, ATS 03/04, ATS 05, and ATS 06 was 54.5 ± 10.5mg, 55.0 ± 23.4mg, 50.6 ± 0.5mg and 48.1 ± 1.6mg respectively. The data were further analysed to compare the active Artesunate ingredient per brand (Brand strength) indicated by the manufacturers with the mean Artesunate content obtained in the HPLC analysis of each brand.

The results were also expressed as percent of Artesunate content in each brand. The results obtained are presented in Table 4, which also shows the % USP specification indicating the acceptable range for Artesunate content in genuine tablets.

The % Artesunate content in ATS 01/02, ATS 03/04, ATS 05, and ATS 06 were 109.0%, 110.0%, 101.2% and 96.2%% respectively.

Table 1: Summary statistics of Artemether content in the three different brands of Artemether

	BRAND ID		
Parameters	ART 01	ART 02	ART 03
N	7	11	9
Mean (mg)	46.6	43.8	44.6
Median (mg)	46.5	42.7	43.9
Std dev	1.1	3.3	2.4
Range (mg)	45.2 – 48.5	41.3 – 51.5	42.7 – 50.6

Table 2: Comparison of brand strength of Artemether, mean Artemether content and USP Specifications

Brand ID	Brand strength	Mean Artemether content	Artemether content (%)	Deviation (%)	% USP Specifications	Status
ART 01	50.0mg	46.6mg	93.2	6.8	98.0 - 102.0	FAIL
ART 02	50.0mg	43.8mg	87.6	12.4	98.0 – 102.0	FAIL
ART 03	50.0mg	44.6mg	89.2	10.8	98.0 – 102.0	FAIL

Table 3: Summary statistics of Artesunate content in the from four different brands of Artesunate

	BRAND ID			
Parameters	ATS 01 & 02	ATS 03 & 04	ATS 05	ATS 06
N	4	6	2	4
Mean (mg)	54.5	55	50.6	48.1
Median (mg)	50.0	50.0	50.6	48.2
Std dev	10.5	23.4	0.5	1.6
Range (mg)	47.8 –70.0	38.4 – 101.3	50.6 – 50.6	46.4 – 49.5

Table 4: Comparison of brand strength of Artesunate, mean Artesunate content and USP specification (%)

Brand ID	Brand Strength	Mean Artesunate content	Artesunate content (%)	Deviation (%)	% USP Specification	Status
ATS 01/02	50mg	54.5mg	109.0	9.0	93-107	FAIL
ATS 03/04	50mg	55.0mg	110.0	10.0	93-107	FAIL
ATS 05	50mg	50.6mg	101.2	1.2	93-107	PASS
ATS 06	50mg	48.1mg	96.2	3.8	93-107	PASS

DISCUSSION:

According to the USP specification the acceptable content of Artemether in a genuine tablet ranges from 98.0 % to 102.0% of the brand strength indicated by the manufacturer [14]. The results presented in Tables 1 & 2 showed that the three brands of Artemether preparations tested had Artemether content less than the 50.0mg indicated by the manufacturers. The percent Artemether content of the ART 01, ART 02 and ART 03 were 6.8%, 12.4% and 10.8% respectively below the brand strength.

The absolute Artemether content obtained from HPLC analyses for each brand did not meet the USP specifications; all the brands were below the 98.0% minimum USP specification for genuine Artemether preparations. The three brands of Artemether can therefore be categorized as failed with respect to the USP specifications for Artemether.

The USP specification for acceptable content of Artesunate in a genuine tablet ranges from 93.0% to 107.0% of the brand strength indicated by the manufacturer [14].

The results for Artesunate tablets (Tables 3 & 4) showed two brands (ATS 01/02 & ATS 03/04) contained more than 50.0mg brand strength, one brand (ATS 05) contained about 50.0mg and the fourth brand (ATS 06) was below 50.0mg. The two brands that contained more than 50.0mg had absolute Artesunate content of 109.0% and 110.0% respectively. These values were above 107.0% upper limit of the brand strength indicated in the USP specification for

Artesunate. The absolute Artesunate content in the other two brands (ATS 05 and ATS 06) was within the range indicated in the USP specification. Thus brands ATS 01/02 and ATS 03/04 can be characterized as failed with respect to the USP specifications for Artesunate.

Data obtained in our present study indicated that substandard preparations of Artemisinin-derivatives are present within the NCD, and are sold to the general public for the treatment of malaria.

The use of these substandard preparations for the treatment of malaria could account for the treatment failures reported with the use Artemisinin-derived preparations in recent times.

In the present study, the 100 percent failure rate for Artemether preparations and 50 percent for Artesunate preparations is alarming and a timely reminder of the issue of poor quality artimisin-derivatives. The use of substandard artemisin-derivatives can further worsen drug resistance leading to increased morbidity and mortality among patients with malaria in the NCD. Other serious consequences include loss of consumers' confidence in healthcare providers.

Since Artemisinin-derivatives are our last real hope of keeping malaria under control, intercepting and removing poor quality preparations is of paramount importance. WHO [7] have reported that some of the factors facilitating the occurrence of poor quality drugs include poor legislation, weak penal sanctions, and weak or absent national drug regulatory authorities, weak enforcement of drug laws, corruption and conflict of interest. Most of these factors exist in PNG, hence the urgency to address this important issue while it is still in embryonic stages.

Furthermore, knowledge about the quality of antimalarial drugs provided by different suppliers in PNG is limited, because there is little or no published information on the prevalence of poor quality antimalarial drugs in PNG, especially data that distinguish counterfeit from substandard drugs. According to recent published data, estimates of the global prevalence of counterfeit and substandard drugs in resource-limited countries range from 1% to 50% and there is evidence of several cases of counterfeit anti-malarial drugs from 38 countries [8, 11–13].

PNG was not cited amongst the 38 countries because of non-availability of data. The widespread prevalence of counterfeit anti-malarial drugs in the Asian Pacific region should be of great public health concern because PNG is located in this region.

The need to monitor the quality of antimalarial drugs imported into PNG cannot be overemphasized. The possibility that, in PNG, patients with malaria are not responding to treatment with any of the Artemisinin derivatives, which are currently our major option against malaria should be of major concern.

Eliminating poor drug quality as a possible cause of treatment failure is therefore of great priority. Malaria is a major cause of mortality and morbidity in the PNG population and any efforts to reduce this trend is of high priority. Poor quality antimalarial drugs are major impediment to improvement in the well-being of individuals in the tropics [13, 15, 17]. The health of people living in malaria endemic areas is critically dependent upon the availability of good quality antimalarial drugs. Ensuring that the antimalarial drugs are of good quality is as important as ensuring that they are available. In addition, drugs that are supplied after their expiry date should be considered as poor quality because degradation of the active ingredient may have occurred during the period of storage. The lack of knowledge about preventive measures together with poor dissemination of information among health workers and the public makes it difficult to identify and report the presence of counterfeit and substandard drugs in the markets. It is hoped that the outcome of this study will lead to increase access to quality assured Artemisinin-based combination therapies, which is the single most important intervention for reducing malaria mortality today. Furthermore, this study provides an appropriate evidence based data, which is the first step towards policy change. The next step should be to combine this evidence with effective advocacy, to ensure that the evidence produced by this project is translated into policy-relevant recommendations. The recommendations should include advocacy for improved training of public and private sector providers, strengthened regulations on drug monitoring, and consumer education.

It is hoped that the data obtained in this project will serve as the base line for a wider study to assess the quality of drugs used in the other provinces in PNG.

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REFERENCES:

1. Muller I. The epidemiology of malaria in Papua New Guinea. Trends in Parasitology Vol 19, No 6 June 2003;
   - Ung A.P. and Bennett N (1976) Chloroquine-resistant Plasmodium falciparum malaria in Papua New Guinea *Medical J Aust 1976; 2: 320-321*
2. Yung A.P. and Bennett N (1976) Chloroquine-resistant Plasmodium falciparum malaria in Papua New Guinea *Medical J Aust 1976; 2: 320-321*
3. Marfurt J, Müeller I, Sie A, Maku P, Goroti M, Reeder JC, Beck H, and Genton B; Low Efficacy of Amodiaquine or Chloroquine Plus Sulfadoxine-Pyrimethamine against *Plasmodium falciparum* and *P. vivax* Malaria in Papua New Guinea; Am. J. Trop. Med. Hyg., 77(5), 2007, pp. 947–954
4. Rieck Rieckmann, KH Plasmodium vivax resistance to chloroquine in *The Lancet, November 18, 1989*
5. Kuran Karunajeewa HA, Mueller Ivo, Senn M etc; A trial of combination antimalarial therapies in children form Papua New Guinea , N. England Journal of Medicine 2008; 359: 2545 – 57
6. Papua New Guinea National Health Plan 2001-2010; Program Policies and Strategies Vol II
7. WHO. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical diseases (1994-1995); The role of artemisinin and its derivatives in the current treatment of malaria; Report of an informal consultation convened by WHO in Geneva, Sept 1993 27-29
8. Guidelines for Field Surveys of the Quality of Medicines: A Proposal. Newton PN, Lee SJ, Goodman C, Fernández FM, Yeung S, Phanouvong S, Kaur H, Amin AA, Whitty CJM, Kokwaro GO, Lindegårdh N, Lukulay P, White LJ, Day NPJ, Green MD, White NJ; Wellcome Trust- Mahosot Hospital-Oxford, Tropical Medicine Research Collaboration, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao PDR, 2006, E-mail: paul@tropmedres.ac
9. Namdeo AG, Mahadik KR and Kadam SS. Antimalarial drug – Artemisia annua; Pharmacognosy Magazine Vol 2, Issue 6, 2006
10. Joint malaria meeting 2004 report, Edited by: Integrated cooperative research for malaria control (ICRMC) project between University of Papua New Guinea and Tokyo Women's Medical University; 7 – 9th June 2004, Port Moresby, Papua New Guinea
11. Olliaro, P. Drug resistance hampers our capacity to roll back malaria in *Clin Infect. Dis 2005 Aug 15; 41 suppl 4: S246-57*
12. Dean T, Jamison DT, Breman JG, Measham AR, Alleyne G, Disease Control Priorities in Developing Countries; 2nd edition, (2006) WDC & NY: Oxford University Press and World Bank; 1394 p. Available: http://files.dcp2.org/pdf/DCP/DCP.pdf.
13. Onwujekwe O, Kaur H, Dike N, Shu E, Uzochukwu B, Hanson K, Okoye V, Okonkwo P; Quality of anti-malarial drugs provided by public and private healthcare providers in south-east Nigeria; Malaria Journal, 2009, 8:22 Open Access www.malariajournal.com/content/8/1/22
14. United States Pharmacopeial Convention, Authorized USP SALMOUS Standards Version 1: Artemisinin, USP Artemether related compounds B RS, February 1, 2009. www.usp.org
15. Sahr F, Willoughby VR, Gbakim AA, Bockarie MJ, Apparent drug failure following Artesunate treatment of Plasmodium falciparum malaria in Freetown, Sierra Leone: four case reports; Annals of Tropical Medicine and Parsitology, 2001: Vol. 95, Issue 5: 445 – 449
16. Instructional manual Varian 920 HPLC operating system/ Manual, Waters Australia 2008.
17. Ofori-Kwakye K, Asantewaa Y, Gaye O, Quality of Artesunate tablets sold in pharmacies in Kumasi, Ghana; Tropical Journal of Pharmaceutical Res, 2008; 7 (4): 1179 – 1184.

Laboratory Evaluation of Traditionally Produced Coconut Oil as A Surface Larvacide Against *Anopheles Stephensi* Fourth Instar Larvae.

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ABSTRACT

Environmental concerns have resulted in the search for environmentally friendly natural oils for use as mosquito larvacide. Methylated coconut oil has been found to be toxic to mosquito larvae. However, the use of methylated coconut oil is limited by resource constraints in rural communities in Papua New Guinea and other Pacific Island countries where coconut oil is produced by traditional methods.

This study evaluated the toxicity of traditionally produced coconut oil to fourth instar *Anopheles stephensi* larvae. The results showed that traditionally produced coconut oil is toxic to fourth instar *Anopheles stephensi* larvae.

The results showed that coconut oil produced by traditional method is toxic to fourth instar *Anopheles stephensi* larvae. The coconut oil can be used as a larvacide for malaria vector control in community based programs utilizing community participation in the production and use of coconut oil for large scale use of coconut oil. However, a suitable surfactant needs to be identified.

Key Words: *Anopheles stephensi*, methylated coconut oil, *cocos nucifera*, larvacide.
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INTRODUCTION:

Mosquito-borne diseases can be controlled by reducing the larval stages of mosquito species. Larvae control methods include source reduction, use of biological control agents and applying chemicals to breeding sites. In the 1930s and 1940s petroleum-based products were used in malaria controlled programs in Brazil and Egypt [1]. Oiling was also useful where the larval sites were limited in size and number [1]. The use of dichlorodiphenyltrichloroethane (DDT) to control the adult mosquito during the global malaria eradication program resulted in a decreased use of oils as a form of larvacide.

Since then, costs, environmental concerns and insecticide resistance have increased, making environmental management within integrated control operations more attractive. The toxicity of a larvacide depends on its volatility [1]. Although pure plant and vegetable oils are too viscous to be used as a mosquito larvacide, their physical and chemical properties can be modified to form methyl and ethyl esters of fatty acids [1]. The spreading pressures of lipophilic products can also be increased by the addition of surfactants [1].

Methylated soy oil (MSO) mixed with polyoxyethylene (40) hydrogenated castor oil monopyroglutamate monoisostearate has been shown to be as effective as the petroleum-derived larvacide, Golden Bear Oil (GB-1111) in laboratory assays against *Culex pipiens* and *Anopheles stephensi [2]*. Field trials have shown that MSO with Pyroter to be comparable with *Bacillus thuringiensis* var. *Israelensis de Barjac* (Bti) in controlling *Anopheles quadrimaculatus* larvae [1]. Foley and Francis [1] evaluated the toxicity of methylated coconut oil (MCO) to *Anopheles farauti* and *Culex pipiens* and found MCO to be more toxic compared to GB-1111 after 24 hours. Furthermore, MCO without surfactant was also toxic to mosquito larvae [1]. However, for LD95 (lethal dose needed to kill 95% of test subjects), GB-1111 was more toxic than MCO for both *Anopheles farauti* and *Culex pipiens [1]*.

The coconut palm *Cocos nucifera* L. is a native plant and abundant in many tropical countries where malaria is endemic. Judging from the effectiveness of MSO and MCO, methylated form of coconut oil offers communities a local product for malaria vector control. However, in remote communities, even the simplest technology needed and the costs of producing MCO do not permit its use as a larvacide. Coconut oil is produced using traditional methods in the pacific for cooking and cosmetic uses. Based on previous studies [1] on MCO it can therefore be hypothesized that traditionally produced coconut oil will be

toxic to mosquito larvae. To evaluate its toxicity, traditionally produced coconut oil were bought from local markets in Solomon Islands and transported to Japan for toxicity studies. The objectives of the study were to evaluate the toxicity of traditionally made coconut oil by calculating its LD50 (lethal dose needed to kill 50% of test subjects) and to formulate a regression equation to calculate the amount of coconut oil that will be needed for field evaluation studies.

Anopheles stephensi is a known malaria vector in Asia [3]. Using the WHO protocol for testing new larvacides [4, 5], fourth instar *Anopheles stephensi* larvae were used to determine the toxicity of traditionally made coconut oil to mosquito larvae.

MATERIALS AND METHODS:

Fourth instar *Anopheles stephensi* larvae were used in the experiments. Larvae and adults were maintained using standard protocols in an insectary with temperature maintained at 26^{o}C and relative humidity 65 % with a 15 hours 8 hours day night cycle [1, 4, 5]. Light was provided by four 40-watt fluorescent light bulbs. Eggs were hatched in 250ml of de-chlorinated tap water in plastic cups (surface area = 95 cm^{2}). At the late second to early third instar stage, larvae were transferred to 33x 24 x7cm pans. Larvae were fed on ®Tetra Min baby fish food. Water was changed every other day. The traditionally produced coconut oil used in this study was purchased from local markets in the Solomon Islands. The trials were performed in an insectary with automated controls for room temperature and humidity. The experiments were conducted during daylight hours. The toxicity experiments were done following WHO protocol for laboratory evaluation of a new larvicide [4, 5]. A total of 25 fourth instar larvae were used per experiment with five larvae in a test cup. Each test had a corresponding control. Larvae were individually pipetted into 150ml of de-chlorinated tap water and then varying volumes of the coconut oil were added into the plastic cups. Water instead of coconut oil was added in the controls. Food was not added in the cups to prevent bacterial overgrowth. After 24 hours the number of dead larvae was counted. The same procedures were repeated on four different occasions using four different generations of larvae.

The data were collated and used to determine the mortality rate and toxicity. The surface area covered by the film of coconut oil on water was also determined.

The WHO recommends that the log-probit statistical model be used to calculate the LD50 or LD90 when assessing the toxicity of a new larvacide [4, 5]. The use of the benchmark dose (BMD) is now increasingly being preferred over the traditional no-observed-adverse-effect-level (NOAEL) or lowest-

observed-adverse-effect-level (LOAEL) approach in assessing the risk of chemicals to humans [6, 7]. The BMD is as an exposure due to a dose of a substance associated with a specified low incidence or risk, generally in the range of 1% to 10%, of a health effect; or the dose associated with a specified measure or change of a biological effect [8].

The extra risk of 1% or 10% is a function of the benchmark response (BMR). A BMR of 0.01 would account for 1% extra risk and BMR of 0.1 would give 10% extra risk. Therefore at BMR of 0.5, the BMD50 would be the dose at which the risk of incidence of the defined biological effect is 50% [8], in other words the LD50 and the 95% confidence lower one sided limit on the BMD50 (BMD LD50) calculated is the lower effective dose at which the defined biological effect is observed.

This criteria was used in this study to determine the toxicity of the traditionally produced coconut oil as a surface larvacide on fourth instar *Anopheles stephensi* larvae.

Log-probit analysis was done using the model for dichotomous data [8]. The bench mark dose software (BMDS) version 1.4.1 was used. The regression equation was formulated using the computational tools VassaStats [10].

RESULTS AND DISCUSSION:

Table 1 shows the percent mortality rates obtained after 24 hours exposures to the traditionally produced coconut oil. The 56% and the 92% mortality rates observed with 10ul and 80ul of the coconut oil respectively indicates effective toxicity to the fourth instar Anopheles stephensi larvae. Using the benchmark dose approach, the BMD50 (or LD50) was 7.54ul (Figure 1.0) although 50% mortality was already seen at 10ul. This difference is due to the data input requirements of the BMDS software which has to generate the line of best fit to calculate the BMD50. The line of best fit was calculated by using the dose of coconut oil and the mortality data from table 1. The lowest dose of the coconut oil at which toxic effect can be observed (BMD LD50) on *Anopheles stephensi* mosquito larvae was 4.44ul. The log-probit model was used because it is the recommended model for dichotomous data [7, 8]. Traditionally produced coconut oil has a higher LD50 compared to MCO and MSO [1, 2]. This difference may be due to the methylation of these two oils that makes them more volatile. Toxicity of natural oils depends on their volatility and the process of methylation increases the toxicity of coconut oil [1]. Traditionally produced coconut oil is not volatile thus it tends to kill only by suffocating mosquito larvae.

Table 1: *Anopheles stephensi* fourth instar larvae mortality rate after 24 hours of exposure to traditionally produced coconut oil.

Coconut oil (ul)	Dead *	Alive *	Mortality (%)
0	0	25	0
0.5	3.5	21.5	14
1	4.5	20.5	18
3	11.5	13.5	46
5	11	14	44
10	**14**	**11**	**56**
15	13	12	52
20	15	10	60
30	20	5	80
40	17	8	68
80	**23**	**2**	**92**
90	24	1	96

* Figures are the averages of four replicates of the experiment.

Figure 1: Illustration of BMD showing the BMD50 and the 95% confidence lower one sided limit (BMDS version 1.4.1) [8]. BMD50 = 7.54ul; BMDL = 4.44ul;

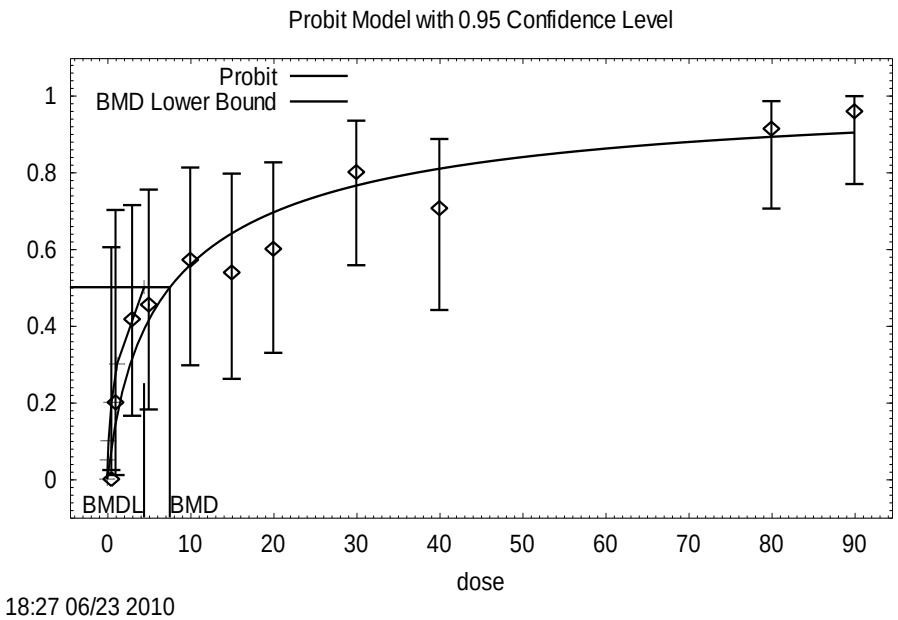

Figure 2: Amount of coconut oil needed in relation to surface treatment area. Regression equation: Y = -4.39+0.38x. r = 0.96. Confidence interval for r (rho), 95% = 0.46 – 0.99. Confidence internal for slope of regression, 95% = 0.16 – 0.59.

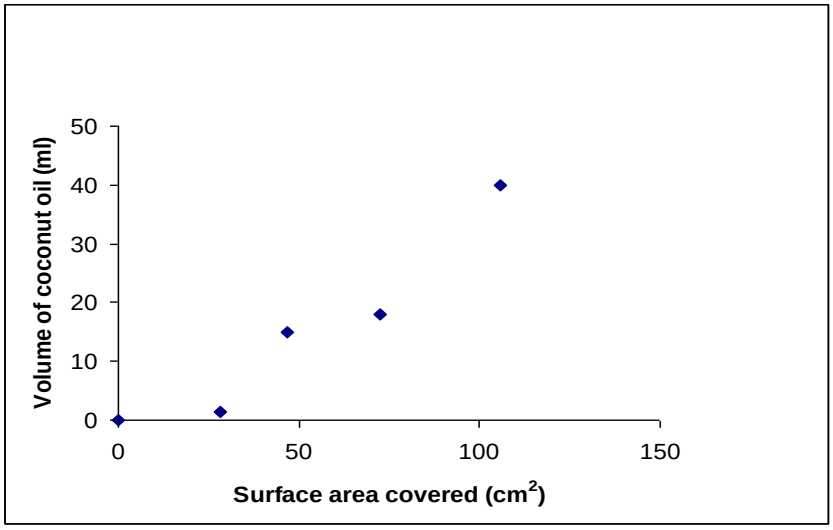

Figure 2 shows the regression equation (Y = -4.39+0.38x) obtained when our data were entered in the software VassaStats [10]. The regression equation indicated that as the treatment surface area increases, the amount of coconut oil needed to cover the treatment area increased exponentially. This behavior in the graph is due to the high viscosity and density of traditionally made coconut oil. As a result, as more and more coconut oil is added onto the water's surface, the coconut oil would begin to sink rather than spread. To overcome this chemical property of coconut oil, a suitable surfactant needs to be identified and added. The function of a surfactant is to reduce surface tension between the water molecules and the coconut oil molecules. Its addition would make coconut oil thinner and spread more easily over the treatment surface area. Thus a small amount of oil, pre-mixed with the suitable surfactant, would cover a large surface area.

Coconut oil can be produced cheaply using traditional methods in PNG. The technique and knowledge of producing coconut oil using traditional methods is well known, both in PNG and in other Pacific Island countries. Our study showed that traditionally produced coconut oil is toxic to *Anopheles stephensi* larvae. Methylated coconut oil (MCO) is also toxic to other *Anopheles* species but the cost and technology required to produce it makes it unsuitable for rural communities in PNG [1]. Furthermore, finance constraints and lack of technical expertise would make the production

and use of MCO unsustainable. Other natural oils have also been shown to have larvacidal properties and are now commercially available [1, 2]. Coconut is abundant in the Pacific and traditionally made coconut oil can be produced with simple technology and the methodology is common knowledge. It is commonly used for cooking and for cosmetic purposes but has a potential for use a larvacide for controlling malaria in rural communities in PNG. Using community based programs; coconut oil can be produced with the participation of the community and used to control malaria vectors. However, producing coconut oil using traditional methods is labour intensive (personal observation) and its production may not be cost-effective in terms of labour cost.

One of the main challenges of a community based program would be to keep people motivated to produce coconut oil. Since traditionally made coconut oil is a sought after product in PNG markets, people may feel that using coconut oil to kill mosquito larvae may be a waste of time and effort when they can easily sell the product and obtain an income to buy a mosquito net.

CONCLUSION:

Our study showed that traditionally made coconut oil is toxic to *Anopheles stephensi* fourth instar larvae. This finding agrees with current data that natural oils can be toxic to mosquito larvae. Although traditionally made coconut oil has a higher LD50 compared to commercially available oils, its advantage of using low cost technology to produce it makes it a good candidate for use in community based programs in rural communities to control malaria. However, its high viscosity will need to be overcome with the addition of a suitable surfactant.

Further studies are needed to continue to evaluate its effectives and find a suitable surfactant. The unavailability of a suitable surfactant makes this area of research more challenging.

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REFERENCES:

1. Foley. D. H and Frances. S.P. Laboratory evaluation of methylated coconut oil as a larvicide for *Anopheles Farauti* and *Culex Annulirostris*. *J Am Mosq Control Assoc.* 2005, 21(4): 477 - 479.
2. Lampman. R., Eckenbach. U., Seigler. D., Novak. R. Laboratory evaluations of methylated soy oil and monoterpenes as mosquito larvicides, *J Am Mosq Control Assoc.* 2000, 16(2):153-7.
3. Briet JTO, Galappaththy NLG, Amerasinghe PH, Konradsen F. Malaria in Sri Lanka: one year post-tsunami. *Malar J.* 2006:5:42.
4. Guidelines for laboratory and field testing of mosquito larvicides, WHO, 2005, [http://www.who.int/WHO/CDS/WHOPES/GCDPP/2005.13] (downloaded 02/06/07).
5. Larvicidial oil chemical property requirements and testing protocol, WHO,1999, [http://www.who.int/WHO/SIF/23.R1] (downloaded 05/06/07).
6. Filipsson. A. F and Victorin. K. Comparison of available benchmark dose softwares and models using trichloroethylene as a model substance, *Regulatory Toxicology and Pharmacology.* 2003, 37:343-355.
7. Foronda. N. M., Fowles. J., Smith. N., Taylor. M. A benchmark dose analysis for sodium monofluoroacetate (1080) using dichotomous toxicity data, *Regulatory Toxicology and Pharmacology.* 2007, 47:84-89.
8. US Environment Protection Agency (US EPA). 2007, Benchmark Dose Software version 1.4.1, [http://www.epa.gov] (downloaded 15/08/2008).
9. Sand. S. Filipsson. A. F., Victorin. K. 2002, Evaluation of the benchmark dose method for dichotomous data: model dependence and model selection, *Regulatory Toxicology and Pharmacology*. 2002, 36:184-197.
10. Lowry R. Vassa-Stats: Stat Computation, 2006 [http://faculty.vassar.edu/lowry/VassarStats.htm] (Last viewed April 2006).

Status of Iodine Nutrition among School-Age Children (6 – 12years) in Honiara Solomon Islands

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ABSTRACT

The effectiveness of the universal salt iodization strategy in the control and elimination of iodine deficiency in a community requires systematic monitoring of urinary iodine concentration, which is the key biochemical indicator recommended for assessing the impact of iodine deficiency control programs. Published data on the salt iodization programme for control of iodine deficiency in the

Solomon Islands is scanty. The aims of this study were to determine the iodine content of salt in the households and the urinary iodine concentrations (UIC) in schoolchildren, age 6 – 12 years, in Honiara, Solomon Islands. This was a prospective school-based study. Multistage cluster sampling method was used for selecting the study population. Simple random sampling technique was used to select 19 of the 28 primary schools in Honiara Solomon Islands. The iodine content in salt samples was measured using the single wavelength semi-automated WYD Iodine Checker Photometer. UIC was estimated using the Sandell-Kolthoff reaction.

Salt was available and used in 99.5% of the households. The mean iodine content in household salt samples was 55.2 $\pm$ 17.7ppm. The iodine content was $\geq$ 15.0ppm in salt samples from 99.1% of households. Data indicates successful implementation of universal salt iodisation strategy.

Median UIC for all the children was 328.0ug/L, Inter-quartile range was 210.38 – 437.0ug/L. UIC in 97.2% of all the children was $\geq$ 100ug/L and 0.7% had UIC below 50ug/L. Median UIC values for the male and female children were 337.0ug/L and 325.0ug/L respectively. UIC in 97.1% of male and 97.4% of female children was $\geq$ 100ug/L. Data indicate that iodine deficiency is not a public health problem among schoolchildren, age 6 – 12yrs, in Honiara.

Our findings indicate the need for an efficient, sustainable, and functional monitoring system to strengthen and improve on the achievements of the USI strategy in Honiara, Solomon Islands

Key Words: Solomon Islands, Honiara, Iodized salt, Urinary Iodine, Iodine deficiency.

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INTRODUCTION:

The trace element Iodine is required for the biosynthesis of the thyroid hormones (Thyroxin and Triiodothyronine), which are essential for normal growth and development [1, 2]. Low dietary intake of iodine or consumption of foodstuffs containing goitrogens can significantly impair the bioavailability of iodine leading to a spectrum of conditions known as iodine deficiency disorders (IDD) [1, 2].

According to the WHO/UNICEF/ICCIDD expert committee, the harmful effects of IDD are manifold, depending on the extent of iodine deficiency [1, 2, 3]. The prevalent forms of IDD in most developing countries are the more subtle degrees of mental impairment that occur in apparently normal children with low dietary intake of iodine [1, 4]. Its manifestations include poor performance of

the children in school and in psychometric tests, reduced intellectual ability and impaired motor functions [1, 4]. The World Health Assembly in 1990 recognized that iodine deficiency (ID) is a significant public health problem in most developing countries, and that it is the world's greatest single cause of preventable mental impairment [1, 3].

Universal salt iodization (USI), a policy of iodizing all salt for human consumption, is the agreed strategy for the control and elimination of IDD in affected populations [1 – 5]. Successfully and sustainable implementation of the USI strategy requires continuous monitoring of the process, impact and sustainable indicators in the affected communities [1, 3, 6, 7]. The recommended process indicator for monitoring and evaluating USI is the assessment of iodine content in salt in the households [1, 6]. The recommended principal impact indicator of USI is the assessment of Urinary Iodine concentration (UIC) among school-age children in the target population [1, 3, 7].

The sustainable indicator is used to assess whether iodine deficiency has been successfully eliminated and to judge whether achievements can be sustained and maintained for several years.

The sustainable indicator involves a combination of median UIC in the target population, availability of adequately iodized salt at the household level and a set of programmatic indicators that are regarded as evidence of sustainability [1, 3, 6, 7].

An important issue at present in some developing countries is the long-term sustainability of salt iodization programs, which require constant monitoring of the iodine status of the population [1, 3, 8]. Failure to monitor can cause a breakdown of IDD control programs. In addition, poorly monitored programs can result in excessive intake of dietary iodine which is associated with risks of adverse health consequences, such as, Iodine-Induced Hyperthyroidism (IIH) [1, 6 – 8].

A thorough search of the literature indicates that no published data is available on the status of iodine nutrition in the Solomon Islands [9]. There is scanty information on the salt iodization program for control of IDD in the Solomon Islands [9]. In addition, there are no data to indicate systematic monitoring of the iodine content of salt in the Solomon Islands [9]. Furthermore, no data is available on the median UIC in school-age children [9]. This study was prompted by the apparent lack of published data on the status of iodine nutrition in the Solomon Islands.

The aims of the current study were to determine the iodine content of salt in the

households and the UIC in schoolchildren, age 6 – 12 years, in Honiara, Solomon Islands.

MATERIALS AND METHODS:

The Solomon Islands is an Archipelago within the Southwest Pacific, located approximately 1,900 km Northeast of Australia, and about 485 km East of Papua New Guinea [10 – 12]. The Solomon Islands is made up of six large and numerus small islands with a total population of about 500.000 people [10 – 12]. The highest point is about 2.447m above sea level [10 – 12].

This study was carried out in Honiara, the national capital city and the biggest town in Solomon Islands. It is located on the Island of Guadalcanal, which is the biggest of the six big islands. Honiara is situated at latitude 9^0 28' South and Longitude 159^0 49' East, along the coastline of north Guadalcanal [13]. Honiara functions as a separate entity, with the Honiara city council responsible for many aspects of the health of the urban population of about 60,000 people [12].

This was a prospective school-based study. The study population consisted of School children in the age group 6 – 12 years. According to the primary school enrolment list prepared by the Ministry of Education and Human Resource Development (MEHRD) [14], there were 28 registered primary schools located in different areas in Honiara at the time of this study. The primary schools with a total population of 7737 children in the age range 6 – 12 years were officially in the Preparatory to Standard Six category [14].

Multistage cluster sampling method was used for selecting the study population. Simple random sampling technique was used to select 19 of the 28 primary schools that participated in this study.

Calculation of the sample size was based on a design effect of three, a relative precision of 10% and confidence level (CL) of 95%. As there was no available information on likely prevalence rate of IDD in Honiara, an assumed prevalence rate of 25% was used. With a predicted non-response rate of 10%, the sample size of 500 school-age children was obtained [1]. This number was ten times higher than the 50 recommended by the WHO/UNICEF/ICCIDD expert committee for school-based studies on the prevalence of IDD in an affected population [1]. The justification for selecting a larger sample size was the lack of data on the prevalence rate of IDD in Honiara [3].

The total enrolments for each of the 19 randomly selected primary schools were listed. In addition, the enrolments and ages for

children in each of the grades in the selected primary schools were also listed. All children below 6yrs of age and above 12yrs of age were identified and excluded from the study. The sample size for each school was calculated using the "proportionate to population size (PPS)" cluster sampling techniques [3]. For each school, each of the children in the age group 6 – 12yrs was assigned a computer-generated random number. The required number of children from each school was then selected by simple random sampling using the randomly generated number list.

Two cohorts of children were selected from each primary school using two separate randomly generated number lists. Those in the first cohort were involved in the sampling for household salts and the filling of the questionnaire, while those in the second cohort provided urine samples for analysis of urinary iodine. Fieldwork for this project was conducted between January and May 2007.

A total of 464 schoolchildren from the selected primary schools participated in the collection of salt samples and filling of questionnaires. The parent or guardian of each child was requested to complete the questionnaire and to put about three teaspoons (10 to 15g) of the salt available in the household into a zip-lock polythene bag provided for that purpose.

For the collection of urine samples a total of 500 schoolchildren from the selected primary schools participated. On the spot urine sample was collected from each of the consented children. Each urine sample was transferred into properly labelled plastic tube with tight fitting stopper that was further sealed with special plastic bands.

The urine and salt samples were packed into separate containers and transported by airfreight to Port Moresby Papua New Guinea (PNG) after detailed consultation with the Solomon Islands Quarantine Office in Honiara. On arrival in Port Moresby the packages were transported to the Micronutrient Laboratory (MNL) in the School of Medicine and Health Sciences (SMHS) University of Papua New Guinea (UPNG) and appropriately stored till required for analyses.

The iodine content in salt samples was measured using the WYD Iodine Checker [15]. Internal bench quality control (QC) for daily routine monitoring of performance characteristics of the WYD Iodine Checker was by the Westgard Rules using Levy-Jennings Charts prepared for iodized salt samples obtained from the National China Reference Laboratory. The percent coefficient of variation (CV) ranges from 2.5% to 5.0% throughout the analysis.

The spectrophotometric method of Sandell-Kolthoff reaction was used for the assay of urinary iodine after digesting the urine with Ammonium Persulfate in a water–bath at 100°C [1]. Internal bench QC characterization of the assay method was by the Levy-Jennings Charts and the Westgard Rules. External QC monitoring of the urinary iodine assay procedure was by Ensuring the Quality of Urinary Iodine Procedures (EQUIP), which is the External Quality Assurance Program (QAP) of the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA and also by the QAP of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Sydney, NSW, Australia.

Microsoft Excel Data Pack 2003 and SPSS-PC software (version 11) were used for the statistical analyses of the data.

Kolmogorov-Smirnov and Shapiro-Wilks tests were used to assess normality of the data. Mann Whitney U test was used for differences between two groups; Kruskal-Wallis and Friedman were used for comparison of all groups. Analysis of variance (ANOVA) was also used to compare differences between groups. Scheffe test was used for post-hoc analysis. $P < 0.05$ was considered as statistically significant.

The data were interpreted using the WHO/UNICEF/ICCIDD and other criteria [1, 7, 8, 15, 16]. Iodine deficiency was not a public health problem in the target population, if at least 90.0% of households were using salt with iodine content of 15.0ppm or more, the median urinary iodine concentration (UIC) was not below 100.0ug/L and the 20th percentile UIC was not below 50.0ug/L [1, 7, 8, 16]. Specific cut-off points for UIC were used for classifying status of iodine nutrition into different degrees of public health significance [1, 7, 8, 16].

Ethical clearance and permission for this study was obtained from the Ethical and Research Grant committee in the SMHS UPNG. Permission was also obtained from the Solomon Islands Medical Research and Ethical Committee and from appropriate authorities in Solomon Islands: the Ministry of Education and Human Resource Development, Honiara City Council (Education Section), and the Headmasters and Headmistresses of appropriate Primary Schools. Parental consent and approval were obtained from parents and / or Guardians of the children selected for the study. The verbal approval of each child with parental consent was also obtained at the time of urine collection. International ethical guidelines for epidemiological studies were implemented in this study [17].

RESULTS:

Each of the 464 children that consented to bring salt samples and complete the questionnaire was from a different household. A total of 432 salt samples (between 30 – 35g) in iodine-free polyethylene bags were received. This gave a response rate of 93.1%.

The frequency distribution of the iodine content (ppm) in the salt samples from the households indicates a wide scatter with a range of 10.0 to 99.0ppm. The mean iodine content in the salt samples was 55.17 ± 17.69ppm (Mean ± Standard Deviation). The median was 54.58ppm and the 95% confidence interval (95% CI) was 52.91 – 56.25ppm. The iodine content was equal to or greater than 15ppm in salt samples from 428 (99.07%) households.

The distribution of households according to the range of iodine content in salt samples is presented in Table 1.

A total of 431 (92.9%) of the 464 questionnaires distributed were returned and were suitable for analysis. The results indicated that salt was regularly used in 429 (99.5%) households. Respondents in 257 (59.6%) households indicated using iodised salt at home; 67 (15.5%) respondents indicated that they were not using iodises salt and 107 (24.8%) were not sure of using iodised salt at home.

In 225 (52.2%) households the respondents did not know the importance of iodised salt. Friends, relatives and neighbours were the major sources of information about the use of iodised salt as indicated by 227 (62.4%) of the respondents that indicated using iodised salt or nor sure of using iodised salt.

Of the 429 households that regularly use salt 390 (90.9%) of them usually purchase salt from supermarkets and trade stores, 33 (7.7%) purchase salt from local markets. When asked about the brand of salt commonly used at home, 164 (38.2%) of the respondents did not know the brand of salt commonly used in the households; however the most popular brand was Saxa salt used in 96 (22.4%) households, followed by Jumbo salt used in 61 (14.2%) households, Sky salt used in 54 (12.6%) households and others used in 54 (12.6%) households. Fine table salt was used in 369 (86%) households, coarse salt was used in 27 (6.3%) households and 33 (7.7%) were not sure.

Salt was kept in plastic containers in 298 (69.5%) households compared to 107 (24.9%) households using glass containers; both were used in 24 (5.6%) households. Salt was always kept in closed containers in 389

(90.7%) households. Sea-food were consumed frequently in 152 (35.4%) households, compared to 226 (52.7%) households that consume sea-foods once in a while and 51 (11.9%) households that did not remember eating seafood.

Of the 500 schoolchildren recruited for this section of the study only 462 (92.4%) gave consent. Assay for UIC was carried out in all the 462 urine samples.

The median UIC was 328.0ug/L, with a mean of 353.7 ± 189.57 ug/L. The Inter-quartile range was 210.38 – 437.0ug/L and the 95% CI of the UIC was 310.67 – 345.33ug/L.

A total of 97.18% (449) of all the children had UI concentration ≥ 100ug/L and only 0.65% (3) had UI concentration < 50ug/L. The 20th percentile UIC was 187.5ug/L.

Distribution of the children according to the range of UIC and status of iodine nutrition is presented in Table 2. None of the children had UI concentration below 20ug/L, and only 13 (2.81%) of them had UIC below 100ug/L.

A total of 257 (55.63%) of the children had UIC over 300ug/L, which indicates risk of developing adverse health consequences. Further analysis of the data indicates that 170 (36.8%) children had UIC in the 300 to 500ug/L range and 87 (18.83%) had UIC greater than 500ug/L.

For further interpretation, the UIC data was separated and analysed according to gender. There were 236 (51.1%) males and 226 (48.9%) females. Table 3 shows the UIC results for the male and female schoolchildren.

The median UIC for the male and female schoolchildren were 337.0ug/L and 325.0ug/L respectively. There was no statistically significant difference between the mean UIC of the male and female schoolchildren. A total of 229 (97.06%) male and 220 (97.35%) female schoolchildren had UIC greater than 100ug/L. The 20th percentile UIC for the male and female schoolchildren was 194.5ug/L and 186.0ug/L respectively.

Table 4 shows the distribution of the male and female schoolchildren according to the range of UIC and status of iodine nutrition. The UIC values in 134 (56.78%) male and 123 (54.42%) female schoolchildren were over 300ug/L. When these data were analysed further, 91 (38.56%) of the male and 77 (34.1%) of the female children had UIC in the 300 – 500ug/L range.

In addition, 43 (18.2%) of the male and 46 (20.4%) of the female children had UIC

greater than 500ug/L. No statistically significant differences were obtained in the number of male and female Schoolchildren in the various ranges of UIC.

The median UIC for all the schoolchildren and for the male and female children are in the range equal to or greater than 300ug/L, which indicates greater risk of developing Iodine-induced Hyperthyroidism (IIH).

Table 1: Iodine content of salt samples from the 432 households

Iodine content in salt samples	Households No. of salt samples (%)
< 15ppm	4 (0.93%)
≥15ppm	428 (99.07%)
15 – 30ppm	31 (7.18%)
> 30ppm	397 (91.9%)

Table 2: Number (%) of school-age children in the different ranges of urinary iodine concentrations (UIC) and status of iodine nutrition (n = 462)

Range of UIC (ug/L) [1]	Status of iodine nutrition [1]	No. of school-age children (%)
< 20	Severe	0
20 – 49	Moderate	3 (0.65)
50 – 99	Mild	10 (2.16)
100 – 199	Optimal	90 (19.48)
200 – 299	Risk of IIH*	102 (22.08)
≥ 300	Greater risk of IIH	257 (55.63)

*IIH: Iodine-Induced Hyperthyroidism

Table 3: Urinary iodine concentrations (UIC) in the male and female school-age children

	Males (n = 236)	Females (n = 226)
Median UIC (ug/L)	337.0	325.0
Interquartile Range UIC (ug/L)	217.5 – 432.1	205.8 – 443.8
Mean UIC (ug/L)	354.2	353.2
Std Dev	185.0	194.7
95% CI: UIC (ug/L)	330.5 – 377.9	327.7 – 378.7
20th Percentile UIC (ug/L)	194.5	186.0
Percent (n) with UIC ≥ 100ug/L	97.03 (229)	97.35 (220)
Percent (n) with UIC < 50ug/L	0.42 (1)	0.88 (2)

Table 4: Number (%) of male and female school-age children in the different ranges of urinary iodine concentrations (UIC) and status of iodine nutrition

Range of UIC (ug/L)	Status of iodine nutrition	Male (n = 236) No. (%)	Female (n = 226) No. (%)
< 20	Severe	0	0
20 – 49	Moderate	1 (0.42)	2 (0.88)
50 – 99	Mild	6 (2.54)	4 (1.77)
100 – 199	Optimal	43 (18.22)	47 (20.8)
200 – 299	Risk of IIH	52 (22.03)	50 (22.12)
≥ 300	Greater risk of IIH	134 (56.78)	123 (54.42)

DISCUSSION:

WHO/UNICEF/ICCIDD expert committee recommended the use of one of three variables for the assessment and monitoring of iodine nutrition in a population [1]. The three recommended variables are, the proportion households using adequately iodised salt, the UIC in an adequate sample size of schoolchildren (age 6 – 12 years) and the

prevalence of goitre in the population [1, 3]. In the present study two of the recommended variables were used to assess the status of iodine nutrition in Honiara, Solomon Island.

The iodine content of salt is the indicator of the salt iodization process. The principal indicator of the impact of salt iodization is the UIC among schoolchildren, age 6 – 12years [1, 2, 3].

School children in the 6 – 12 years age group are recommended for the assessment of iodine nutrition in a population because of their high vulnerability to iodine deficiency and easy accessibility in the community [1]. The school-based approach was used in this study because of the high enrolment and attendance of both male and female children in primary schools in Honiara, Solomon Islands [1, 14].
The non-response rate of 6.9% obtained in this section of the study was lower than the predicted 10.0% non-response rate used in calculating the sample size. The most common reason given by the 32 (6.9%) children that did not provide salt samples was because the salt in the house had just ran out or was not enough.

Testing of iodine content in household salt is the most important "process" indicator for monitoring progress in the implementation of USI, which is the most effective and sustainable long-term public health measure for the prevention and control of iodine deficiency [1, 6].

The mean (55.17ppm) iodine content in the salt samples from the households was higher than the values reported for household salt samples in various cities in PNG [18 – 21].

According to the WHO/UNICEF/ICCIDD criteria [1, 8, 16] at least 90.0% of households in the community should be using salt with iodine content of not less than 15.0ppm. Thus, adequately iodised salt was used in 99.07% of the households in Honiara (Table 1). This coverage of the use of adequately iodised salt indicates compliance with the current WHO/UNICEF/ICCIDD criteria that indicates implementation of the USI strategy [1, 8, 16]. Similar coverage has been reported for households in some cities in PNG [18 – 21].

In the present study, because of logistical reasons it was not possible to accurately assess the discretionary per capita consumption of salt in the households. The mean iodine content of the salt samples in the households was 55.17ppm. Assuming that the discretionary per capita intake of salt in the households was about 5 – 10g per day, the mean discretionary per capita intake of iodine should be between 275.85 to 551.7ug per day. Factoring in the about 20% loss of iodine in

salt during storage and preparation of food, the calculated discretionary per capita intake of iodine should be between 220.68 – 441.36ug per day. This indicates that the mean and median iodine content in salt samples used in the households are adequate for the prevention and control of iodine deficiency [1, 3, 8, 16].

The data, according to the WHO/UNICEF/ICCIDD criteria [1, 3, 8], indicates success in the implementation of the USI strategy in Honiara at the time of this study. The situation analysis of the status of the USI strategy in Honiara can be characterized as "existent but needing strengthening" [1, 8]. WHO/UNICEF recently proposed specific criteria for the categorization of salt iodisation programs in various areas within countries [8]. According to these criteria [8], salt iodization in Honiara should be in Group one, because over 90% of households have access to adequately iodised salt. Therefore, according to WHO/UNICEF guidelines [8], authorities in Honiara should strive to sustain the achievement of USI and periodically reassess the salt iodization programme and the iodine status of the population.

Despite the success in the implementation of USI there is need to enhance the advocacy and awareness on the importance and appropriate use of iodised salt which is already available and accessible to communities in Honiara. This is of significance because analysis of the questionnaires indicated that 52% of the respondents do not know the use of iodised salt and 15.5% indicates that they do not use iodised salt at home. Since 99.07% of households are using adequately iodised salt, there is the likelihood that people in these households, especially the elderly, may be consuming relatively large amount of iodized salt per day, putting them at risk of developing adverse health consequences in the long term.

Effective implementation of the USI strategy requires systematic monitoring of UIC, which is the key biochemical indicator recommended for assessing the impact of IDD control programs [1].

The 38 schoolchildren that did not provide the on-the spot urine samples represent a non-response rate of 7.6%, which is less than the 10% assumed non-response rate used in the calculation of sample size.

The median UIC obtained for all the schoolchildren was 328.0ug/L and the 20th percentile UIC was 187.5ug/L. In addition, 97.18% of all the children had UIC greater than or equals to 100ug/L.

Thus, according to the current WHO/ICCIDD/WHO criteria [1, 7, 8], iodine deficiency was not of public health significant among school children, age 6 – 12yrs, in Honiara, at the time of this study. This was strongly supported by the data in Table 2 indicating that mild to moderate status of iodine nutrition was present in only 2.8% of the school-age children.

The median and 20th percentile UIC for the male (337.0ug/L and 194.5ug/L) and female (325.0ug/L and 186.0ug/L) schoolchildren indicate optimal status of iodine nutrition.

The results also show that mild to moderate status of iodine nutrition was prevalent in 2.97% (7) of male and 2.65% (6) of female schoolchildren (Table 4), which indicates that iodine deficiency was not of public health significance.

The median UIC obtained for all the schoolchildren (328.0ug/L) and for the male (337.0ug/L) and female (325.0ug/L) schoolchildren indicate that the status of iodine nutrition can be classified as excessive iodine intake (range over 300ug/L).

These median UIC values were higher than those reported for school children, age 6 – 12yrs in Southern Highlands Province Papua New Guinea (48.0ug/L) [19], Honduras (287ug/L), Nicaragua (259ug/L) and El Salvador (251ug/L), but lower that those reported for schoolchildren in Chile (565ug/L), Ecuador (590ug/L), Brazil (1013ug/L) and Mexico (1150ug/L) [22].

Excessive intake of iodine, indicated by median UI concentration over 300ug/L, has been reported in many countries, particularly when salt iodization is excessive and poorly monitored [1, 7, 18, 21, 22]. Although most individuals can tolerate high intake of iodine per day without apparent problems, daily intake in excess of 1000ug per day can be potentially harmful to susceptible individuals [1, 7, 21, 23].

Ideally, intake of over 300ug/L iodine per day should be discouraged, particularly in communities with previous history of iodine deficiency [1, 7, 21, 23]. This is because, in such communities, individuals, particularly the elderly with autonomous nodules that were previously iodine deficient may develop iodine-induced hyperthyroidism (IIH) few years after exposure to either normal or high intake of iodized salt [1, 23].

However, in the community the problems that can be caused by excessive intake of iodine are minor compared to those that can be caused by inadequate intake leading to iodine deficiency.

Thus, the concept that "it is better to consume more iodine per day than to consume less, particularly for the vulnerable groups (women, children and infants) in the community [1, 4, 7, 8],. The best concept recommended by WHO/UNICEF/ICCIDD [1, 8] is to ensure the availability and appropriate use of adequately iodized salt in the community. This further emphasizes the notion that sustainable optimal iodine nutrition and consolidation of the current achievement of elimination of iodine deficiency, as a public health problem, among schoolchildren requires continuing effective monitoring and regular evaluation of the implementation of the USI strategy in Honiara, Solomon Islands.

CONCLUSION:

The present study indicates that over 99.0% of households in Honiara are using adequately iodized salt. Although this study did not evaluate other sources of iodine, the data strongly supports the use of salt as the major vehicle for iodine supplementation in Honiara Solomon Islands.

Iodine deficiency was not of public health significance among the schoolchildren age 6 – 12 years in Honiara. However, it is important for program planners to carry out intensive nutrition education, information, and awareness campaigns to advocate for appropriate use of iodised salt.

This process must be accompanied by effective monitoring to guard against continuous excessive consumption of iodine as indicated by the 56.78% of male and 54.42% of female school-age children in the over 300ug/L range of the UIC.

Our findings indicate the need for an efficient, sustainable, and functional monitoring system to strengthen and improve on the achievements of the USI strategy in Honiara, Solomon Islands.

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REFERENCES

1. World Health Organization / United Nations Children's Fund / International Council for Control of Iodine Deficiency Disorders. Assessment of Iodine Deficiency Disorders and monitoring their elimination: A guide for program mangers, 2nd Edition, WHO Pub, Geneva, WHO/NHD/01.1, 2001: 1 – 107.
2. Delange F. Hetzel B. In: The thyroid and its diseases. Braverman and Utiger eds. Lippincott Williams &Wilkins publ. Philadelphia: 2004. Chapter 20: The iodine deficiency disorders.
3. WHO. Iodine deficiency; WHO Executive Board 103rd Session, Provisional Agenda item 8. EB103/27, Nov. 1998; 1 – 3.
4. Delange F, Burgi H, Chen ZP and Dunn JT. Word status of monitoring iodine deficiency disorders control programs. Thyroid, 2002; Vol. 12; No. 10: 915 – 923.
5. Van den Briel, West CE, Bleichrodt N, Van de Vijver JR, Ategbo EA, Hautvast GAJ. Improved iodine status is associated with improved mental performance of schoolchildren in Benin. Am J Clin Nutr 2000; 72: 1179 –1185.
6. Azizi F. Salt iodization, monitoring and evaluation (SIME): An effective replacement for universal salt iodization. Int. J. Endocrinol Metab. 2003; 2: 47 .
7. Delange, F. B. de Benoist, Hans Burgi, & the ICCIDD working group: Determining median urinary iodine concentration that indicates adequate iodine intake at population level. Bulletin of WHO 2002, 80: 633 – 636.
8. World Health Organization / United Nations Children's Fund Joint statement by WHO and UNICEF: Reaching optimal iodine nutrition in pregnant and lactating women and young children, WHO Pub, Geneva, 2007: 1 – 2.
9. WWW.indorgs.virginia.edu/iccidd/mi/idd 154.htm IDD Prevalence and control program data: Solomon Islands, August 2007.
10. WWW.wpro.who.int/contries/04/sol/. WHO Regional office for the Western Pacific, Solomon Islands, 2005
11. WWW.travel.state.gov. Bureau of East Asian and Pacific affairs April 2007 Background note: Solomon Islands
12. WWW.spc.in/prisin/sb test about %20 us/welcome.htm; 2006; Population summary, Solomon Islands national statistical office (SINSO).
13. WWW.http://en.wikipedia.org/wiki/Honiara: visited in August 2007.
14. Primary schools enrolment list of Honiara. Ministry of Education and Human Resource Development; Solomon Islands, 2006: 1 – 8.
15. WYD Iodine Checker. Users manual Salt research institute. China National Salt Industry Corporation: 1 – 10.
16. UNICEF. The roadmap towards achievement of sustainable elimination of iodine deficiency; Operational Guidance: 2005 and beyond. UNICEF, New York, 2005.
17. CIOMS International guidelines for ethical review of epidemiological studies. Council for International Organization of Medical Sciences, Geneva, 1991.
18. Amoa B, Pikire T and P Tine Iodine content in salt in Lae city of Papua New Guinea Asia Pac J. Clin Nutr 1998; **7**:128 – 130.
19. Temple VJ, Mapira P, Adeniyi KO, Sims P. Iodine deficiency in Papua New Guinea (Sub-clinical iodine deficiency and salt iodization in the highlands of PNG). Journal of Public Health, 2005, 27: 45 – 48.
20. Temple VJ. "Progress towards elimination of IDD in PNG"

International Council for Control of Iodine Deficiency Disorders. IDD Newsletter, Vol. 22, No 4, Nov. 2006; 11 – 13.
21. Temple VJ, Oge R, Daphne I, Vince JD, Ripa P, Delange F and Eastman CJ. "Salt Iodization and Iodine Status among Infants and Lactating Mothers in PNG" AJFAND, Vol 9, No. 9, Dec 2009, 1807 - 1823.
22. Pretell EA, Delange F, Hostalek U, Carigliano S, Barreda L, Higa MA, Altschuler N, Barragan D, Cevallos JL, Gonzales O, Jara JA, Medeiros-Neto G, Montes JA, Muzzo S, Pacheco M, and Cordero L. Iodine Nutrition Improves in Latin America. Thyroid, Vol. 14, No. 8, 2004; 590 – 599.
23. Dunn JT. "Iodine should be routinely added to complementary foods" In: Nutrient composition of fortified complementary foods. J. Nutr. 133: 2003; 3008S – 3010S.

Characterisation of Methicillin-resistant *Staphylococcus aureus* isolated from blood cultures in South East Asian hospitals.

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ABSTRACT

Methicillin-resistance *Staphylococcus aureus* (MRSA) are common in hospitals in many countries, including South East Asia. Nosocomial MRSA is characteristically resistant to multiple antibiotics but can be treated with vancomycin. More recently MRSA have emerged in the community (CMRSA) and these characteristically are not multiply resistant like the hospital strains. MRSA have been found to spread within and between hospitals, thus are referred to as epidermic MRSA (EMRSA). It is therefore important to characterise isolates so that pathogenic and epidemic strains of MRSA can be identified.

For this study 309 MRSA isolated from blood cultures between June 1998 and December 1999 were provided by the Sentry Program Centre in Adelaide, South Australia. The isolates were from hospitals in Australia, South Africa, Singapore, China, Hong Kong, Taiwan, the Philippines and Japan. Isolates were characterised by phenotypic and molecular methods. The methods used were extended antibiograms and resistograms, bacteriophage typing, countour-clamped homogeneous electric field (CHEF) electrophoresis, plasmid profiling and analysis of the *mec*

complex. Most of the isolates were resistant to the majority of the antimicrobials tested although all were susceptible to vancomycin.

Three isolates from Hong Kong and seven from Royal Perth hospitals had resistance profiles similar to those of CMRSA. The majority of isolates were not susceptible to the International Bacteriophage Set (IBS). There was a predominant CHEF pattern amongst the South African isolates which had 88% similarity with the Australian CHEF pattern 1. However, the isolates were not related in other respects and had different plasmid profiles and *mec* complexes. The results indicated that the South African isolates are different from those from other hospitals and that Taiwanese and Japanese isolates are generally more diverse than those in the other countries. Hospitals in Australia, Singapore and Hong Kong appear to have many different strains but do not have predominance of a particular strain.

This study has provided a basis for additional studies to further characterise isolates from various countries and to understand the epidemiology of MRSA in the hospitals.

Key Words: MRSA, CHEFF, Methicillin, Staphylococcus, Bacteriophage, Plasmids, Typing, Antibiograms, Resistograms, Hospitals

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INTRODUCTION:

Staphylococcus aureus (*S.aureus*) is a Gram-positive coccus, found in approximately 30% of healthy individuals especially on the skin, nose and upper respiratory tract [1]. It is also found in hospitals, on the clothes, bedding and instruments. It is a pathogen that causes a range of infections from superficial skin infections to severe and life threatening infections. It is a major cause of nosocomial infections [1]. It has been reported that *S.aureus* is the most frequent cause of bacteraemia in the hospital environment [1]. *S. aureus* has become increasingly resistant to antibiotics [2, 3].

The antibiotic methicillin was introduced in 1959 to control the strains of *S.aureus* that had become resistant to penicillin and other antibiotics [4]. Shortly after its introduction, strains of *S.aureus* were isolated that were resistant to methicillin [5, 6]. These strains were referred to as methicillin-resistant *Staphylococcus aureus* (MRSA). In South East Asian countries, the isolation rates for MRSA have increased and have caused outbreaks of infections in hospitals [7]. The strains have been referred to as "Southeast Asian MRSA"(SEA-MRSA) because of their

origin [8]. MRSA isolated from blood cultures has been reported to be a significant pathogen causing bacteraemia in Asian countries [6, 8].

The high percentage of MRSA reported from bacteraemias in recent years may have occurred because of failure of the immune system to contain the infection at the focal site, prolong use of antibiotics and indwelling devises [9].

The aims of this study were to use conventional methods to type MRSA isolates from cases of bacteraemia in South East Asian hospitals. To use the typing results obtained to determine the particular strains of MRSA that are responsible for bacteraemia within and between hospitals.

MATERIAL AND METHODS:

MRSA isolates:

A total of 309 MRSA isolated from blood cultures between June 1998 and December 1999 were provided by the Sentry Program Centre in Adelaide, South Australia. The isolates were from three hospitals in Australia, One in South Africa and eleven hospitals from six Asian countries (Singapore, China, Hong Kong, Taiwan, Philippines and Japan).

Typing methods:

Susceptibility tests (Antibiogram and Resistogram): Antibiogram typing was performed on Mueller-Hinton agar (Oxoid) by the single disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines [10] against a range of 21 antimicrobial agents (Oxoid): amikacin (Ak-30µg), fusidic acid (Fd-10µg), kanamycin (K -30µg), minocycline (Mh-30µg), mupirocin (Mup-5µg), neomycin (N-30µg), spectinomycin (Sh-25µg), streptomycin (S-25µg), sulphafurazole (Sf-300µg), lyncomycin (My-15µg), tobramycin (Tob-10µg) and trimethoprim (W-5µg). Resistogram typing was performed on Mueller-Hinton agar (Oxoid) by the single disc diffusion method against a range of 6 chemicals [10]: cadmium acetate (Cd-0.1 M) from Chem-Supply limited Australia, ethidium bromide (Eb-15mM) from Sigma Chemical limited Australia, mercuric chloride (HgCl- 0.4µM), phenylmercuric acetate (Pma-0.17%) from Ajax Chemical limited Australia, propamidine isethionate (Pi -1%) from May and Baker limited United Kingdom and sodium arsenate (Ars-0.2µM) from BDH Chemical limited United Kingdom.

Bacteriophage typing:

Bacteriophage typing was performed on 3CY agar by using the appropriate propergating strain of *S. aureus* against the international basic set (IBS) of 26 phages: Group I - 29, 52, 52A, 79, 80; Group II - 3A,

3C, 55, 71; Group III - 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85; Group IV - 94, 96; Miscellaneous - 81, 96; Experimental phage - 187,90,88 and the international MRSA set (IMS) of 10 phages: MR8, MR12, MR25, F30, F33, F38, M3, M5, 622, 56B. Both sets were prepared to 100 times routine test dilution (RTD) according to the method of Blair and Williams [11].

Contour-clamp homogeneous electric field electrophoresis (CHEF):

CHEF method used was adapted from Wei and Grubb [12] and O'Brien *et al*., [13] with the CHEF DR III System (Bio-Rad Laboratories Pty Ltd, Regents Park, New South Wales). Chromosomal banding pattern were examined visually, scanned with a Fluor-S Multimager (Bio-Rad Laboratories) and digitally analysed with Multi-Analyst/PC (Bio-Rad Laboratories). The CHEF patterns were grouped according to Tenover *et al*., [14] and using the dendogram similarities of 80% to assign strain relatedness. *S. aureus* NCTC 8325 was used as the size standard.

Plasmid profiles:

Plasmid typing was performed by isolating the plasmid DNA by the cetyltrimethylammoniumbromide (CTAB) method [15] and separated by horizontal-gels electrophoresis in 0.6% (wt/vol) molecular biology-grade agarose (Promega Corporation, United State of America). Plasmid DNA pattern were examined visually, scanned with a Fluor-S Multimager (Bio-Rad Laboratories) and digitally analysed with Multi-Analyst/PC (Bio-Rad Laboratories). Plasmid sizing were determined by comparing the migration of the covalently closed circular (CCC) forms with the plasmid of WBG 4483 that had the standard sizes of 44.3 kb, 22.5kb, 4.4kb, and 3.5kb using the QWBASIC Corona PC Basic Version 1.04 Corona Data System to calculate the sizes. Transfer of plasmids from selected isolates to WBG1876 was attempted using the Mixed-culture transfer (MCT) as described previously [16]. WBG1876 transipients were selected on media containing fusidic acid (5µg/ml) and rifampin (25µg/ml) (Sigma Chemical Company) with a range of antimicrobial agents for selection plates. Transferrants were analysed for plasmids as previously described [15]. Isolates were examined for conjugative plasmids using the polyethylene glycol (PEG) method of Townsend *et al*., [16] using WBG541 as recipient. Transferred conjugative plasmids were analysed as previously described [15].

Restriction endonucleases *Eco*RI and *Hind*III (Promega) analysis of transferred plasmids was digitally analysed according to the manufacturer's directions. The

fragments are separated by horizontal-gels electrophoresis in 0.8% (wt/vol) molecular biology-grade agarose (Promega Corporation, United State of America) with Multi-Analyst/PC (Bio-Rad Laboratories). The DNA sizing was determined by comparing the sizes of plasmid digested fragments with those of *Eco*RI/*Hind*III digested Lambda DNA that has fragments of 21,227 bp, 5148 bp, 4268 bp, 3530 bp, 1904 bp, 1584 bp, 1375 bp, 947 bp, 831 bp and 564 bp using the QWBASIC Corona PC Basic Version 1.04 Corona Data System to calculate the sizes.

Analysis of *mec*A complex:

Chromosomal DNA was prepared to the method of Sambrook *et al*., [17]. The *mec*A complex of the isolates was analysed digitally using primers designed to target specific areas of the *mec* region and the PCR amplification of the *mec*A gene was performed as described previously [17]. Chromosomal sizing of the amplification products were measured using the QWBASIC Corona PC Basic Version 1.04 Corona Data System to calculate the sizes. Restriction enzymes (*Eco*RI and *Hind*III) analyses for amplicons of *mec*A and *mec*I2 primers were digested with *Cla*I in order to detect deletions in the membrane-spanning binding region of *mec*R1.

The sizes of amplication products were measured using the QWBASIC Corona PC Basic Version 1.04 Corona Data System to calculate the size.

RESULTS:

Susceptibility tests (Antibiogram and Resistogram): MRSA isolates were resistant to the majority of the antimicrobial agents and chemicals tested. However, susceptibility to vancomycin alone was found in all isolates from the fifteen hospitals studied. Bacteriophage typing: Variable bacteriophage lysis patterns were found amongst the isolates. However of the common bacteriophage patterns identified (Table 1), only seven bacteriophage patterns belonging to Group III were found in more than one country. With 80 isolates belonging to the untypable pattern. Contour-clamp homogeneous electric field electrophoresis (CHEF):

Fifty CHEF patterns as illustrated in figure 1 were identified by PFGE, although prominent CHEF patterns as shown in Table 2 (CHEF pattern 1, CHEF pattern 7, CHEF pattern 8, CHEF pattern 17 and CHEF pattern 44) were identified in six countries (Hong Kong, Australia, Singapore, South Africa, Japan and Taiwan). Within these countries several MRSA strains were identified. Although individual strains

prominent in some countries, only two strains (CHEFF pattern 7 and 8) were isolated in several Asian countries. CHEF patterns 7 was common in Hong Kong 38 [71%] and Taiwan 7 [13%] but not very common in Singapore 4 [8%], China 3 [6%] and Australia 1 [2%].

The other CHEF pattern 8 was isolated in five countries and was common in Singapore 31 [76%] and less common in Taiwan 5 [12%], Australia 4 [10%] and China 1 [2%].

Plasmid profiles: Some of the isolates (82/309) did not carry any plasmids, however those isolates that carried plasmids varied in sizes with smaller plasmids (2.0-4.0 kb) being disseminated in several countries (Table 3). When transfer of representative plasmids into suitable recipient WBG 1876 by mixed culture transfer and WBG541 and polyethylene glycol respectively, almost all isolates plasmid did not or fail to transfer. Analysis of *mec*A complex: Majority carried Class A *mec* complex (Table 4), the strains from South Africa carried Class B *mec* complex and the variant Class A1 *mec* complex was identified in one isolate from Hong Kong.

The results from the epidemiological methods described demonstrated that the multiresistant MRSA causing bacteraemia, many belonging to bacteriophage Group III, carried small plasmids, have prominent CHEF pattern and carried Class A *mec* complex appear similar in several hospitals in Southeast Asia and confirm the spread of MRSA causing bacteraemia between Southeast Asia and Australia.

While some strains differ considerably in their genomic diversity in the *mec complex* and are found to be present in hospital such as Japan and South Africa. Three different *mec* complex classes were found in the randomly selected prominent CHEF patterns isolates (twenty-seven) tested.

DISCUSSION:

MRSA is an important cause of infection in hospitalised patients. It is the third most common pathogen in bacteraemias (43.1%) and the second most common in respiratory tract infections (56.9%) and urinary tract infections (57%) in hospitals [7]. The prevalence of MRSA in blood cultures differs in the countries in this study. Bell *et al.*, [7] reported that the percentage of MRSA isolates from blood cultures varied from 11.8% in the Philippines, 22.4% in Australia, 26.9% in China, 40.4 in South Africa, 46.7% in Taiwan, 58.2% in Hong Kong, 60.6% in Singapore to 66.8% in Japan. In our present study various methods were used to characterize and type MRSA isolated from bacteraemias in hospitals in these countries.

Antibiogram typing of the 309 MRSA isolates from 15 hospitals, revealed that most of the isolates with the exception of isolates from Australia 203 RPH, and three from Hong Kong 204 QMH had multiple-resistant as defined by Pearman *et al*., [18]. Multiple-resistance is typical of nosocomial MRSA and the resistance pattern in our study is consistent with the findings reported by other researchers [7, 19].

There are some variations in the resistance patterns of the isolates between hospitals and this may reflect the use of antibiotics in these hospitals. Most of the multiple-resistant isolates were resistant to most of the aminoglycosides, macrolides and lincosamide, as well as others such as neomycin, sulphamethoxazole and trimethoprim. Similar patterns of resistance to the aminoglycosides have been reported by others for Australia [20], Hong Kong [6], Japan [19], France [21], Singapore [22] and Kuwait [23]. There were a few notable differences between the isolates from the different countries in the present study. Resistogram typing revealed that most of the isolates were resistant to cadmium acetate. Resistance has been reported as being either chromosomal [24], on ψTn*554* inserted in the SCC*mec* [25] or on a plasmid [13]. Except for the Japanese isolates most of the isolates were sensitive to arsenate. It is interesting that earlier, Classic MRSA were found to be arsenate resistant whereas the later MRSA, EA MRSA, were arsenate sensitive [26].

In most cases the isolates were resistant to both mercury ion (mercuric chloride/HgCl) and organic mercury (phenylmercuric acetate/Pma). Most of the Japanese isolates were sensitive to Pma. The reasons for these differences are not known, but it is unlikely to be due to the use of agents containing these chemicals. Although mercurochromes had been used widely in the past they are rarely used today. There were no clear differences between the isolates from the different countries in their patterns of resistance to ethidium bromide and propamidine isethionate.

Resistance to these two compounds is known as NAB-I [27] or *qac*A resistance. In a few cases isolates were resistant to one of these but not the other. The reason for this is not known but it has been observed before. Bacteriophage susceptibility demonstrated that the majority of the isolates were not typable with the IBS phages. This corresponds with the finding of others reported in China [28], Australia [29], Egypt [30], Israel [31] and the United Kingdom [32]. Although common phage lysis patterns were identified in some countries a comparison of the result with the CHEF

results shows that phage typing is not as discriminating as the CHEF gel electrophoresis. For example, the 88//M3 phage type is quite common in the Hong Kong isolates and mostly corresponds to CHEF pattern 7 but not all pattern 7 isolates are this phage type and some 88//M3 have quite different CHEF patterns. Also some 88//M3 is found in the Singapore isolates and some of these have CHEF pattern 7. However, some Singaporean CHEF Pattern 7 isolates do not have the 88//M3 phage type. Similarly, some of the Taiwanese isolates have the 88//M3 phage type and are CHEF pattern 7, some CHEF patterns 7s have greatly extended phage lysis patterns and some 88//M3 phage patterns have different CHEF patterns.

Fifty CHEF patterns (Figure 2) were obtained and some were found in more than one country. For example, CHEF pattern 7 was found in five countries and CHEF pattern 8 in four (Table 2). CHEF pattern 1 was found only in the isolates from Australian hospitals and was found in 32 of the 67 (47%) isolates.

This pattern corresponds to the US EMRSA 2 that has been found in Sydney and Adelaide hospitals. CHEF patterns 41 and 42 were only found in Singapore isolates and were found in only a few isolates, three and one respectively. Although some isolates from hospitals did not carry any plasmids the majority carried one or more plasmids.

The absence of plasmids in multi-resistant MRSA is uncommon; however, the absence of plasmids has been reported in isolates from Hong Kong [6], China [28] and Portugal [33]. Generally isolates from a hospital contained similar sized plasmids as shown in Table 3, even if their profiles were different, suggesting that plasmids are moving between strains in a hospital. In some cases, as already mention, plasmids of the same size were found in more than one hospital in more than one country.

Small plasmids ranging from 1.7 kb to 3.5 kb were found to be common in isolates from all hospitals. Although isolates may have the same genetic background it has now become apparent that the isolates with the same genetic background can have different SCC*mecs* (*mec* regions).

Analysis of the *mec* complex which consists of an IS*431*, the *mec*A gene and its regulatory genes, *mecR1* and *mecI*, have been useful in comparing the relatedness of MRSA [34]. At the time this work was done the *mec* complexes that had been described in MRSA, were Class A, Class B, Class C

and Class E [35]. Other *mec* complexes Class A and B are the most common complexes found in MRSA [36].

In this study three different *mec* complexes (Class A, Class B and Class A1) were detected. The majority of the isolates (23 of 27) had the Class A *mec* complex. Eleven were CHEF pattern 7, nine CHEF pattern 8 and three CHEF pattern 1. The isolates were from China, Singapore, Taiwan, Hong Kong and Australia. In addition one isolate from Hong Kong had a deletion in the membrane spanning domain of *mecR1* and presumably was the same as Class A1. The widespread occurrence of Class A *mec* complex in the isolates from these countries is similar to that reported for epidemic strains isolate in England and Australia [38]. Although these results may reflect the overall picture for MRSA in Southeast Asian and Australian hospitals they need to be interpreted with caution because not all isolates were tested; only representatives from the predominant CHEF patterns were tested. However, the results are consistent with other results. Lim [37] found that many of the Malaysian isolates were the same as the predominant strain in Singapore and that they all had *mec* Class A with a single base nonsense mutation at nucleotide 202 in *mecI*. The EA MRSA that was examined had Class A1, that is, it had the same *mecI* but in addition had a 166 bp deletion in the membrane-spanning domain of *mecR1* [37].

Chongtrakool *et al.* [39] examined isolates from many countries including isolates from Singapore, the Philippines, China and Japan. They found that the majority of the isolates had the Class A *mec* complex. In fact, with the exception of one isolate from the Philippines, all the isolates from Singapore, the Philippines and China had Class A.

The one isolate from the Philippines that was different had the Class B *mec* and 5.1% of the Japanese isolates were also Class B. Five (3.6%) of the Japanese isolates were found to belonged to an additional *mec* complex. The three isolates representative of the predominant CHEF pattern 44 in the South African isolates that were isolated had a type B *mec* complex.

To fully characterize the *mec* region (SCC*mec*) it is now necessary to also type the ccr genes and the J (junk) regions, particularly the J1 region, which is now referred to as *mec* left extremity polymorphism (MLEP) by Chongtrakool *et al.,* [39].

In their recent paper Chongtrakool *et al.,* [39] proposes a new nomenclature for

SCC*mec* typing. It is possible to speculate that in our present study if the isolates were further typed for the *ccr* genes and by MLEP typing the isolates may have been found to belong to the additional SCC*mec* types.

Chongtrakool *et al.,* [39] found that most of the Korean and all of the Japanese Class A *mec* complexes had type two *ccr* genes whereas the isolates from Singapore, the Philippines and China had type three *ccr* genes [39]. In addition the isolates could be further divided based on MLEP typing.

Table 1: Common bacteriophage patterns in eight countries

	Common bacteriophage patterns									
Countries	No	A	B	C	D	E	F	G	H	I
China	5				1					1
Hong Kong	59			15		2			1	15
Japan	91	8	7		1		5	15	2	47
Philippines	2				1					1
Singapore	43		26	2						6
Taiwan	21		4	5						1
Australia	67	1		1	4	1				5
South Africa	21									3
Total	309	9	37	23	7	3	5	15	3	80

Bacteriophage patterns: A (MR8/M3), C (88/M3), D (56B), E (88), F (MR8/MR12) and I (non-typable)

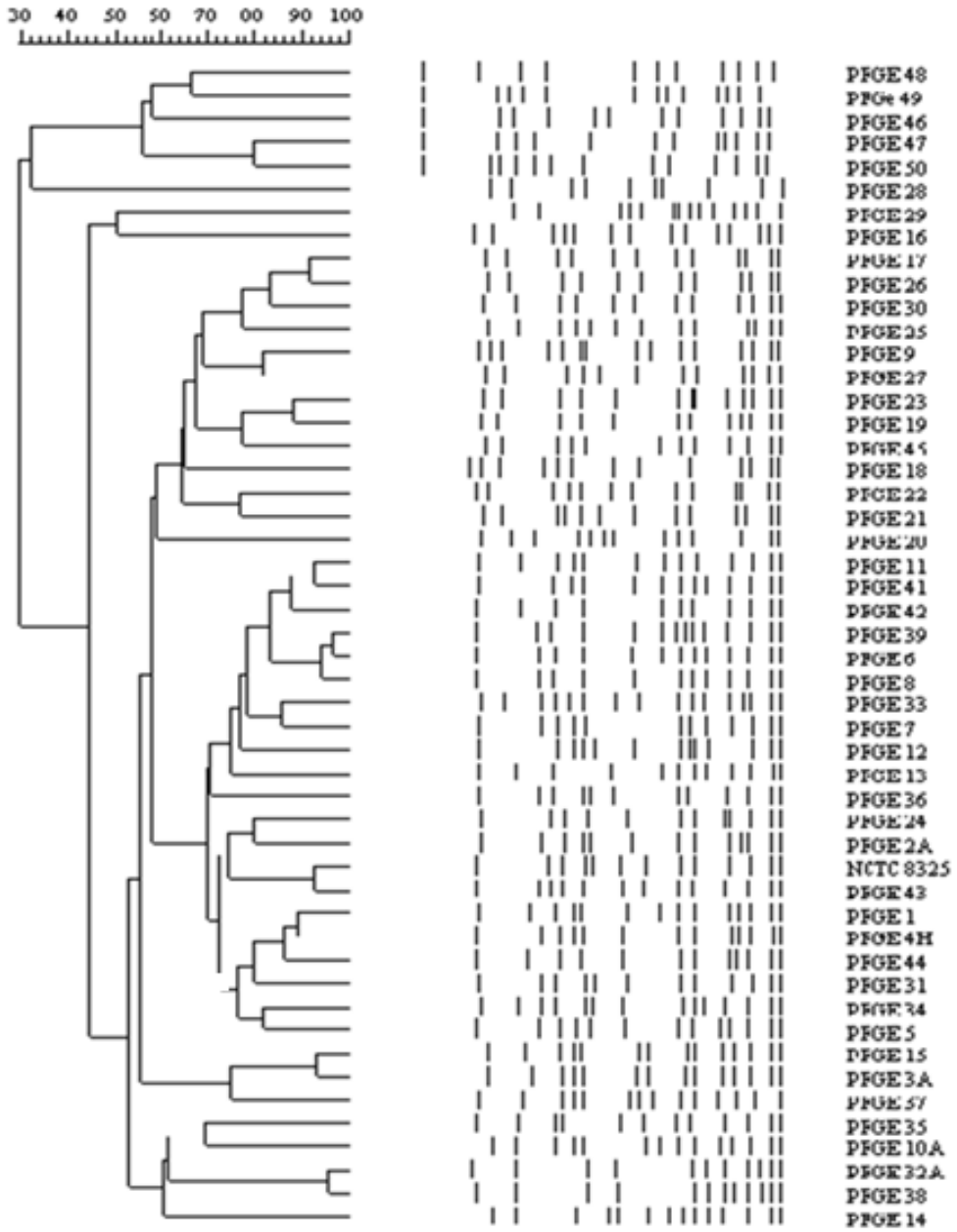

Figure 1: Dendrogram of 50 pulsotypes CHEF patterns for the MRSA

Table 2: Predominant CHEFF pattern

Predominant CHEFF Pattern		1	7	8	17	44
Country	MRSA 309	(32)	(53)	(40)	(15)	(18)
Isolates						
China	5	--	3 [6%]	1 [2%]	--	--
Hong Kong	59	--	38 [71%]	--	--	--
Japan	91	--	--	--	15 [100%]	--
Philippines	2	--	--	--	--	--
Singapore	43	--	4 [8%]	30 [76%]	--	--
Taiwan	21	--	7 [13%]	5 [12%]	--	--
Australia	67	32 [100%]	1 [2%]	4 [10%]	--	--
South Africa	21	--	--	--	--	18 [100%]

Table 3: Predominant plasmid size patterns (kb)

Country	Size (kb)	Predominant plasmid size patterns (kb)
China	18.5 and 3.3	3.2
Hong Kong	32.0 and 2.6	3.2
Japan	40.3 and 1.6 35, 23	2.6
Philippines	Did not carry any plasmid	
Singapore	31.6 and 2.0	3.0
Taiwan	25.5 and 1.7	2.2, 1.9, 1.7
Australia	39.5 and 2.0	3.2, 2.7, 2.4
South Africa	39.1 and 3.0	35.1

Table 4: Results for the amplification of the *mec* complex

Isolate		CHEF pattern	*mecA*	*mecR1* MS	*mecR1* PB	*mecI*	IS*1272*
Class A *mec* complex							
AR19	Australia	8	+	+	+	+	-
S1	Singapore	8	+	+	+	-	
S2	Singapore	8	+	+	+	-	
S28	Singapore	8	+	+	+	-	
S17	Singapore	8	+	+	+	-	
S5	Singapore	8	+	+	+	-	
S21	Singapore	8	+	+	+	-	
CG1	China	8	+	+	+	+	-
TV1	Taiwan	8	+	+	+	+	-
AR1	Australia	1	+	+	+	+	-
AA1	Australia	1	+	+	+	+	-
AP2	Australia	1	+	+	+	+	-
AA6	Australia	7	+	+	+	+	-
S7	Singapore	7	+	+	+	-	
S22	Singapore	7	+	+	+	-	
HK16	Hong Kong	7	+	+	+	+	-
HK3	Hong Kong	7	+	+	+	+	-
HK19	Hong Kong	7	+	+	+	+	-
HK11	Hong Kong	7	+	+	+	+	-
HK47	Hong Kong	7	+	+	+	+	-
HK57	Hong Kong	7	+	+	+	+	-
TN3	Taiwan	7	+	+	+	+	-
TC3	Taiwan	7	+	+	+	+	-
Variant Class A *mec* complex							
HK7	Hong Kong	7	+	-	+	+	-
Class B *mec* complex							
SA1	South Africa	44	+	+	-		+
SA4	South Africa	44	+	+	-		+
SA18	South Africa	44	+	+	-		+

Abbreviation: MS, membrane spanning domain; PB, penicillin binding domain; -, not amplified; +,amplified

CONCLUSION:

Most of the MRSA were resistant to multiple antimicrobials which is a problem for treatment because only vancomycin is use to reliably treats staphylococcal infection without a laboratory report where MRSA is endemic. Few Hong Kong isolates were non-multi-resistant and were suggestive of community MRSA. Most of the isolates were not typable with IBS phages in contrast to IMS phages. Although some Hong Kong and few Taiwan as well as Singapore isolates have IMS phage pattern 88/M3 that have CHEF pattern 7, there was generally little correlation with phage typing. PFGE is not suitable for comparing isolates between different hospitals and countries and over long time periods but the value of this test is suitable for studying outbreaks in hospitals. It was not possible to characterise many of the plasmids isolated and generally the plasmid profiles of the isolates were characteristic of a particular country. Except for the South African isolates, which had

type B *mec* complex, all isolates tested had type A *mec* complex while the Hong Kong isolate had type A1 *mec* complex.

Our results indicate that there is no particular strain that was responsible for bacteraemias in the countries studied. Each country appears to have its own predominant strain or strains.

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REFERENCE:

1. Boyce JM. Are the epidemiology and microbiology of methicillin-resistant *Staphylococcus aureus* changing? Journal of American Medical Association. 1998; 279 (8):623-624.
2. Levy SB. Multi-drug resistance – a sign of times. New England Journal of Medicine. 1998; 338:1376-1378.
3. Schmitz F-J, Fluit AC, Hafner D, Beeck A, Perdikouli M, Boos M, Scheuring S, Verhoef J, Kohrer K and vonEiff C. Development of resistance to ciprofloxicin, rifampin, and mupirocin in methicillin- susceptible and resistant *Staphylococcus aureus* isolates. Antimicrobial Agents and Chemotherapy. 2000; 44; (11):3229-3231.
4. Jevons MP. "Celbenin"-resistant staphylococci. British Medical Journal. 1961; 1: 124-125.
5. Barber M. Methicillin-resistant Staphylococci, Journal of Clinical Pathology. 1961;14: 385-389.
6. Cheng AF and French GL. Methicillin-resistant *Staphylococcus aureus* bacteraemia in Hong Kong. Journal of Hospital infection.1988; 12: 91-101.
7. Bell JM, Turnidge JD and Sentry APAC Participant. High prevalence of Oxacillin-resistant *Staphylococcus aureus* isolates from hospitalised patients in Asia-Pacific and South Africa: Results from SENTRY antimicrobial surveillance program, 1998-1999.Antimicrobial Agents and Chemotherapy.2002; 46 (3):879-881.
8. Grubb WB, Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*, in *Molecular biology of Staphylococci*, Novick,R.P. (ed.), VCH Pub, New Yolk, N.Y., 1990;pp.597-606.
9. Karchmer AW. Nosocomial bloodstream infections: organism, rick factors, and implication. Clinical Infectious Disease. 2000;31 (Suppl 4):S139-143.

10. National committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests; approved standard-seventh edition. NCCLC document M2-A7, 2000; vol.20 (1), Villanova, Pa: NCCLS; January 2000.

11. Blair JE and Williams REO. Phage typing of Staphylococci. Bulletin of World Health Organization.1961;24: 771-184

12. Wei M-Q and Grubb WB. Typing of methicillin-resistant *Staphylococcus aureus* strains by pulsed-field gel electrophoresis. Journal of Microbiology.1992; 37: 187-191.

13. O'Brien FG, Pearman JW, Gracey M, Riley TV and Grubb WG. Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J of Clinical Microbiology.1999; 37 (9): 2858-2862.

14. Tenover FC, Arbeit RD, Goering R, Mickelson PA, Murray BE, Persing HD and Swaminathan B. Interpreting chromosomal DNA restriction patterns produces by pulsed-field gel electrophoresis: criteria for bacterial strain typing. Journal of Clinical Microbiology.1995; 33 (9):2233-2239.

15. Townsend DE, Ashdown N, Bolton S and Grubb W. The use of cetytrimethlammonium bromide for rapid plasmid isolation from *Staphylococcus aureus* of relaxeable plasmid for the in vitro manipulation. Letter in Applied Microbiology. 1985; 1: 87-89.

16. Townsend DE, Bolton S, Ashdown N, Taheri S and Grubb W. Comparison of phage-mediated and conjugative transfer of staphylococcal plasmids *in vitro* and i*n vivo*. Journal of Medical Microbiology.1986;22:107-11

17. Sambrook J, Fritsch EF and Maniatis T. *Molecular cloning:A laboratory manual,second edition*.Cold Spring Harbor Laboratory press,New York.1989.

18. Pearman JW, Coombs GW, Grubb WB and O'Brein F. A British epidemic strain methicillin-resistant *Staphylococcus aureus* (UK EMRSA-15) in Western Australia. Medical Journal of Australia.2001;174: 662.

19. Aires de Sousa M, de Lancastre H, Santos Sanches I, Kukuchi K, Totsuka K. and Tomasez A. Similarity of antibiotic resistance patterns and molecular typing properties of Methicillin-resistant *Staphylococcus aureus* isolates widely spread in hospitals in New York City and in the hospital in Tokyo, Japan. Microbial Drug Resistance.2000; 6 (3):253-258.

20. Grubb WB, Townsend DE, Greed LG, Ashdown N. and Momoh M. Characteristics of methicillin-resistant *Staphylococcus aureus* endemic in Australian hospitals, in *Proceedings of the 13th international congress of chemotherapy*.Spitsz, K.H. and Karrer K. (ed), Verlag H.Egemann,Vienna Part,1983; 83:23-24.

21. Bertrand X, Thouveres M and Talon D. Antibiotic susceptibility and genotypic characterization of methicillin-resistant *Staphylococcus aureus* strains in Eastern France. Journal of Hospital Infection.2000; 46: 280-287.

22. Grubb WB, Townsend DE, Ashdown N and Taheri S. A genetic comparison of methicillin-resistant *Staphylococcus aureus* isolated from different countries, in *Resent advances in chemotherapy, Ishigami Journal* (ed), University of Tokyo Press Tokyo, 1985; pp.401-402.

23. Al-Haddad AM, Udo EE, Mokados EM, Sanyal SC and Grubb WB. Persistence of a clone of methicillin-resistant *Staphylococcus aureus* in a burns unit. Journal of Medical Microbiology.2001;50: 558-564.

24. Townsend DE, Grubb WB and Ashdown N. Genetics of drug resistance in methicillin-resistant *Staphylococcus aureus* from Australian hospitals. Journal of Hospital Infection. 1983;4:331-337.

25. Dubin DT, Chikramane SG, Inglis B, Mathews PR and Stewart PR. (1992) Physical mapping of the *mec* region of an Australian methicillin-resistant *Staphylococcus aureus* lineage and a closely related American strain. Journal of General Microbiology.138:169-180.

26. Townsend DE, Ashdown N, Annear DI, Pearman JW and Grubb WB. Genetics analysis of methicillin-resistant *Staphylococcus aureus* from Western Australian hospital. Journal of Hospital Infection. 1984;5: 417-424.

27. Emslie KR, Townsend DE, Bolton SA and Grubb WB. Two distinct resistance determinants to nucleic acid-binding compounds in *Staphylococcus aureus*? Fed of European Med Society (FEMS) Microbiology letters.1985;27: 61-64.

28. Wei M-Q. Epidemiology and typing of Asian methicillin-resistant *Staphylococcus aureus. PhD Thesis,* School of Biomedical Science, Curtin University of Technology, Perth, Western Australia.1993.

29. Vickery AM, Bead-Pegler MA and Stubbs E. Phage-typing patterns and lysogenicity of methicillin strains of *Staphylococcus aureus* from Sydney Australia, 1965-1985. Journal of Medical Microbiology. 1986;22: 209-216.

30. Khalifa KI, Heibe AA and Hancock G. Nontypeable bacteriophage patterns of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. Journal of Clinical Microbiology.1989;27 (10):2249-2251.

31. Samra Z, Gadb R and Ofir O. Antibiotic susceptibility and phage typing of methicillin-resistant and –sensitive *Staphylococcus aureus* clinical isolates at three periods 1991-1997. European Journal of Clinical Microbiology and Infectious Diaease.2001;20:425-427

32. Richardson JF, Rosdahl VT, van Leeuwen WJ, Vickery AM, Vindel A and Witte W. Phages for methicillin-resistant *Staphylococcus aureus*: an international trail. Epidemiology Infection. 1999;122 (2): 227-233.

33. Cristino Melo JAC and Torres Pereira A. Plasmid analysis of 219 methicillin-resistant *Staphylococcus aureus* strains with uncommon profiles isolates in Lisbon. J. of Hospital Infection. 1989;13: 133-141.

34. Kobayasi N, Taniguichi K, Kojima K, Urasawa S, Kishi N, Yagihashi A, Kurokawa I and Watanabe N. Genomic diversity of mec regulator genes in methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Epidemiology and infections.1998;117:289-295.

35. Ito I, Arakawa Y, MA X, Tatewaki K, Nagasawa M, Totima H, Tanimoto K and Fujimoto S. Structural comparison of three types of Staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus* N315. Antimicrobial Agents and Chemotherapy.2001;45 (5):1323-1336.

36. Hiramatsu K, Asada K, Suzuki E, Okonogi K and Yoyota T. Molecular cloning and nucleotide sequence determination of the regular region of *mecA* gene in methicillin-resistant *Staphylococcus aureus* (MRSA). Federation of European Biochemical Societies (FEBS) 1992;298: 133-136.

37. Lim TT. *PhD thesis*, Curtin University of Technology, Perth, Western Australia.2006.

38. Sim HC. Molecular characterisation of Singapore methicillin-resistant *Staphylococcus aureus*. *PhD thesis*, Curtin University of Technology, Perth, Western Australia.2003.

39. Chongtrakool P, Ito T, Ma XX, Kondo Y, Trakulsomboon S, Tiensasitorn C, Jamklang J, Chavalit T, Song J-H and Hiramatsu K. Staphylococcal cassette chromosome (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian Countries: a protocol for a new nomenclature for SCC*mec* elements. Antimicrobial Agents and Chemotherapy.2006; 50: 1001-1012.

Using Thyroid Stimulating Hormone (TSH) Levels in Cord Blood to Assess the Iodine Status of Neonates

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ABSTRACT:

Neonatal Thyroid Stimulating Hormone (TSH) level in blood is one of the indicators recommended for assessing iodine deficiency control programs in a population. This study evaluates the TSH level in cord blood as a way of assessing the iodine status of neonates in the National Capital District, PNG.

Assay of TSH in 150 cord sera was by enzyme immunoassay (EIA 96 Microwell plates) using the sensitive EIA kit provided by LiNEAR Chemicals, S.L.
The median TSH level in the sera for all the neonates was 2.17mIU/L, the interquartile range (IQR) was 1.53 – 3.48mIU/L. The TSH level in only 2 (1.3%) cord serum samples was greater than 10.0mIU/L. The lower limit (2.5th) and upper limit (99.0th) of the TSH percentile cut-off levels in all the cord sera were 0.76mIU/L and 11.16mIU/L.

The median TSH level in the cord sera of the male neonates was 1.98mIU/L and the IQR was 1.55 – 3.38mIU/L. For the female neonates the median TSH level was 2.22mIU/L and the IQR was 1.52 – 3.81mIU/L.

The data indicates normal iodine and thyroid status and zero prevalence of congenital hypothyroidism among the neonates in NCD.

Key Words: Cord Serum, Neonates, TSH, Iodine deficiency, Papua New Guinea
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INTRODUCTION:

Hypothyroidism caused by inadequate intake and proper utilization of iodine can result in a spectrum of diseases collectively referred to as Iodine Deficiency Disorders (IDD) [1-3]. Maternal iodine deficiency during pregnancy and lactation may compromise the thyroid status of the foetus and neonate [1 – 6]. Maternal T4 is particularly required for neurodevelopment during the first half of gestation [1, 3, 5 – 7]. Maternal iodine deficiency affects neurophysiologic development and functional abnormalities of the foetus and also indirectly impairs foetal brain development by causing hypothyroidism in both mother and foetus [1, 7]. Maternal iodine deficiency also impairs foetal thyroid function, causing increase TSH level in the neonate [1, 5 – 7].

Increase in neonatal TSH level indicates sub-optimal supply of thyroid hormones to the developing foetus and the neonate [7 – 10]. Neonatal TSH level in blood is one of the indicators recommended for assessing IDD control programs in a population [1, 4, 7 – 11]. Iodine deficiency in a population is indicated if the frequency of neonatal TSH level is above 5.0mIU/L in whole blood or above 10.0mIU/L in serum is found in more than 3% of the blood samples obtained from Cord Blood or 3 days old neonates [1, 7 – 11]. The cut-off points for mild, moderate and severe IDD are indicated by neonatal TSH frequencies of 3 – 19.9%, 20 – 39.9% and greater than 40%, respectively [1, 7 – 11]. Congenital Hypothyroidism (CH) has been recognized as a preventable cause of mental impairment, thus routine screening programs have been implemented in most developed countries [1].

According to several experts there are difficulties in the diagnosis of CH at birth by physical examination of neonates, in addition CH is usually asymptomatic for several months, thus if neonatal screening is not carried out, most cases of hypothyroidism may be missed at birth and treatment may be delayed [7 – 14]. CH is a relatively common condition with a frequency of about 1:400 in Southern Australia and other countries [7 – 14].

Early diagnosis and treatment is vital for normal physical and mental development of affected neonates. The use of TSH as the primary screening test for neonates is recommended because it can also detect compensated or transient primary hypothyroidism caused by iodine deficiency with a frequency of about 1 in 10 neonates in some countries [12 – 14].

In some countries blood samples for screening are collected from 3 to 5 days old

neonates [7 – 13]. Because of logistical reasons cord blood samples have been recommended as the most practical specimens for neonatal screening in most developing countries [12 – 17].

According to recent reports by WHO/UNICEF/ICCIDD [4, 15], there is growing evidence that iodine deficiency may be reappearing in some countries, where it was previously under control. This statement underscores the need for continued monitoring and evaluation of the iodine status and thyroid function of populations that have been at risk in the past.

Recent data indicates prevalence of mild to moderate status of iodine deficiency in some areas in Papua New Guinea [18 – 20]. However, there are limited data on iodine status of neonates in Papua New Guinea (PNG). There are no indications of neonatal screening for CH in any of the hospitals in PNG. No data is available on the prevalence of compensated or transient primary hypothyroidism, which can be caused by maternal iodine deficiency, and whose incidence can be as high as 1 in 10 neonates.

The principal aims of this study were to determine the thyroid stimulating hormone (TSH) levels in cord blood as a way of assessing the iodine status of neonates and to assess the prevalence of Congenital Hypothyroidism among the neonates in the National Capital District (NCD), PNG.

SUBJECT AND METHODS:

This cross-sectional study was conducted in the NCD, which is the incorporated area around Port Moresby the capital of PNG. The Obstetrics and Gynaecology unit in Port Moresby General Hospital (PMGH) was selected as the sampling site because of the difficulty in obtaining blood samples from healthy neonates in NCD.

The study population included consecutive deliveries at the PMGH from May to September, 2009 of women resident in the NCD. In this period of time, 5000 deliveries occurred. Two hundred (200) of the deliveries were randomly selected for study. The sample size was based on a design effect of one, relative precision of 10%, confidence level of 95%, predicted non-response rate of 25% and assumed prevalence rate of 10% [1]. Selection of the women was by simple random sampling. Cord blood was collected from cords of neonates whose mothers had given informed written consent before delivery. Cord blood samples were collected into sterile properly labelled containers. The neonates' side of the cord was clamped and cut, blood was collected with a plain

vacutainer from the placental cord just before delivery of the placenta. Serum was prepared from each blood sample and stored frozen at – 20^{o}C till required for assay. Before delivery the following information was recorded for each consented pregnant mother, age, parity, thyroid status, blood pressure, diabetic status and use of iodine antiseptics. After delivery the gender, weight and length of the neonate were recorded. Consenting mothers with history of thyroid disease or medications that affect thyroid status including those with systemic illness were excluded from the study.

Assay of TSH was by enzyme immunoassay (EIA 96 Microwell plates) using the sensitive EIA kit provided LiNEAR Chemicals, S. L. [21]. Assay of TSH in cord sera, standard sera and quality control sera were carried out as indicated in the instructional protocol of the manufacturer [21].

Statistical analysis of data was by the SPSS-PC software (version 11). Kolmogorov-Smirnov and Shapiro-Wilks tests were used to assess normality of the data. Mann Whitney U test, Kruskal-Wallis and Friedman were used as appropriate. Scheffe test was used for post-hoc analysis. $P < 0.05$ was considered as statistically significant.

In the present study the data were interpreted using the WHO/UNICEF/ICCIDD recommended criteria [1, 7, 11, 22]. Iodine deficiency is not indicated if less than 3.0% of all the cord sera have TSH level greater than 10.0mIU/L. In addition, the severity of IDD was classified using the proportion of cord sera with frequency of TSH > 10.0mIU/L: frequency of 3% – 19.9% indicates mild IDD; Frequency of 20% – 39.9% indicates moderate IDD and Frequency above 40% indicate severe IDD [1, 7, 11, 22]. Cord Serum TSH level > 20.0mIU/L indicates Congenital Hypothyroidism [1].

Ethical clearance and approval for the study was obtained from the SMHS Ethics and Research Grant Committee and the PNG Medical Research Advisory Committee (PNG MRAC). Permission for the study was also obtained from the appropriate authorities in PMGH.

RESULTS:

A total of 200 pregnant mothers were randomly selected, but enrolment occurred just before they were prepared for delivery. Verbal consent was obtained from 172 of them, but signed informed consent was obtained from only 150, which gave a total response rate of 75.0%. Assay for TSH was

carried out in the 150 sera obtained from the 150 cord blood samples collected.

The mean (± standard deviation) age of all the pregnant mothers was 25.2 ± 5.3 years and the median age was 25.0 years. The diabetic status and thyroid status of all the mothers were normal. The blood pressures of the women that participated in the study were all normal. The gestational age was within the range 38 – 42 weeks. All the mothers had normal vaginal delivery. Gender distribution of the 150 neonates delivered indicated 81 (54%) males and 69 (46%) females.

The mean and median birth weights of the neonates were 3.1± 0.46 kg and 3.1kg respectively. The range was 2.0 – 4.2kg, and the 95% CI was 3.0 – 3.2kg. The mean and median birth weights for the male neonates were 3.1 ± 0.43kg and 3.2kg respectively, with a range of 2.0 to 4.2kg and 95% CI was 3.0 – 3.2kg.

For the female neonates the mean birth weight was 3.1 ± 0.49kg, the median was 3.0kg, the range was 2.1 – 4.0kg and the 95% CI was 2.9 – 3.2kg. There was no statistically significantly different (p = 0.21) between the birth weights of the male and female neonates. Distribution of the neonates according to the cut-off points for classification of birth weights is presented in Table 1. The birth weight was ≥ 2.5kg in 139 (92.7%) of all neonates, among 78 (96.3%) male and 61 (88.4%) female neonates. None of the neonates was classified as having very low birth weight.

The Kolmogorov-Smirnov test for normality indicated that the TSH levels (m IU/L) in all the cord sera and in the male and female cord sera were not normally distributed. This is confirmed by the several outliers shown on the Box-plots (Fig. 1) of the TSH levels in the cord sera of all the neonates and of the male and female neonates. Thus further analysis of the TSH data was by non-parametric statistics. Table 2 shows the TSH levels in the cord sera for all the neonates and for the male and female neonates. The median TSH level in the sera for all the neonates was 2.17mIU/L the interquartile range (IQR) was 1.53 – 3.48mIU/L. The TSH level in only 2 (1.3%) cord serum samples of all the neonates was greater than 10.0mIU/L. The TSH level in all the cord serum samples of all the neonates was less than 20.0mIU/L.

The percentile cut-offs of the TSH levels in the cord sera of all the neonates are presented in Table 3. The lower limit (2.5^{th}) and upper limit (99.0^{th}) of the TSH percentile cut-off levels in the cord serum samples for all the neonates were 0.76mIU/L and 11.16mIU/L.

The median TSH level in the cord sera of the male neonates was 1.98mIU/L and the IQR was 1.55 – 3.38mIU/L. For the female neonates the median was 2.22mIU/L and the IQR was 1.52 – 3.81mIU/L. The Kruskal Wallis (p = 0.55) and Chi-Square (p = 0.42) tests indicated that there was no statistically significant difference between the TSH levels in the cord sera of the male and female neonates. The Mann-Whitney and Wilcoxon tests also indicated no statistically significant (p = 0.55) difference between the mean TSH levels in the cord sera of the male and female neonates. The TSH level was greater than 10.0mIU/L in 1.2% and 1.4% of the cord serum of the male and female neonates respectively.

The Percentile cut-offs for the TSH levels in the cord sera of both male and female neonates are presented in Table 3. The lower limits (2.5th) of the TSH percentile cut-offs in the cord sera for the male and female neonates were 0.83mIU/L and 0.71mIU/L respectively. The upper limits (99.0th) TSH percentile cut-offs for male and female neonates were 9.28mIU/L and 10.70mIU/L respectively. The Spearman's rho coefficient of correlation (r = - 0.048, p = 0.668) indicated a weak inverse non-significant relationship between the TSH level in the cord serum of the male neonates and the birth weights of the male neonates. For the female neonates the Spearman's rho coefficient of correlation (r = 0.069, p = 0.575) indicated a weak non-statistically significant linear relationship between the TSH level in the cord serum samples and the birth weights.

Table 1: Distribution (%) of neonates according to classification of birth weights

Birth Weights	Classification	All neonates (n = 150)	Males (n = 81)	Females (n = 69)
< 2.0kg	Very Low Birth Weight	0	0	0
2.0 – 2.49kg	Low Birth Weight	11 (7.3%)	3 (3.7%)	8 (11.6%)
≥ 2.5kg	Normal Birth Weight	139 (92.7%)	78 (96.3%)	61 (88.4%)

Figure 1: Box-plots for TSH levels (m IU/L) in cord sera of all neonates and of the male and female neonates

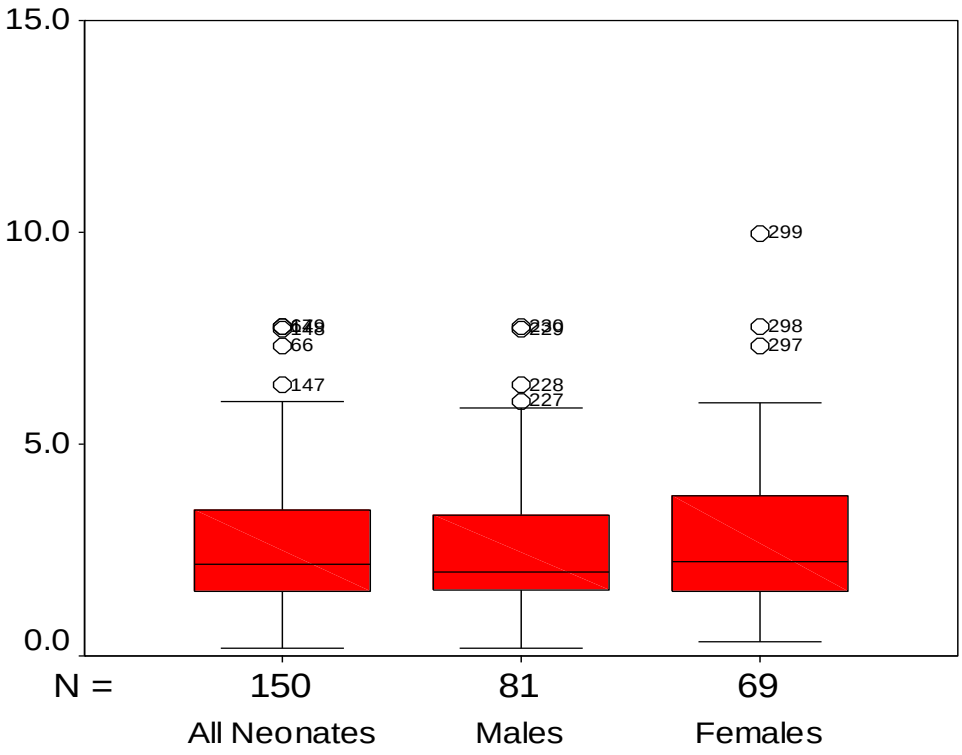

Table 2: TSH levels (m IU/L) in cord sera of all neonates and for male and female neonates

Parameters (TSH m IU/L)	All Neonates (n = 150)	Males (n = 81)	Females (n = 69)
Median	2.17	1.98	2.22
Interquartile Range (IQR)	1.53 – 3.48	1.55 – 3.38	1.52 – 3.81
Mean	2.83	2.75	2.93
95% CI	2.49 – 3.18	2.28 – 3.22	2.42 – 3.44
Std Dev	2.13	2.13	2.13
Range	0.19 – 15.3	0.19 – 15.30	0.35 – 12.3
TSH >10.0mIU/L	2 (1.3%)	1 (1.2%)	1 (1.4%)
TSH ≥ 20.0mIU/L	0	0	0

Table 3: Percentile cut-offs for TSH levels (m IU/L) in cord sera of all neonates and of male and female neonates

Percentile	All Neonates	Males	Females
2.5th	0.76	0.83	0.71
5th	0.9	0.94	0.86
10th	1.23	1.11	1.25
20th	1.45	1.46	1.41
25th	1.53	1.55	1.52
50th	2.17	1.98	2.22
75th	3.48	3.38	3.81
90th	5.45	5.43	5.62
95th	6.81	6.36	7.55
97.5th	7.77	7.72	8.43
97.8th	7.77	7.72	8.88
99.0th	11.16	9.28	10.70

DISCUSSION:

In most developing countries the use of TSH in cord serum for assessing the thyroid status of neonates is well documented and supported by WHO/UNICEF/ICCIDD [1, 12 – 16, 22]. In addition, mixed cord blood is a good sampling technique that has proved to be an attractive neonatal TSH screening tool for CH, because it is easier to obtain from consented mothers [11 – 14]. Sera from mixed cord blood samples were used in the present study.

The non-response rate of 25% was similar to the predicted non-response rate used in the calculation of the sample size. Some authors have reported non-response rates over 25.0% in research studies involving collection of biological samples from "apparently" healthy infants [20, 23].

The proportion of female neonates (11.6%) with low birth weight was higher than that of the male neonates (3.7%). This difference was not statistically significant (p = 0.43). After routine examinations each of the pregnant mothers was satisfied as "apparently" healthy before delivery. The gestational age of between 38 to 42 weeks was satisfied as normal in each case.

The non-Gaussian distribution of the TSH levels for cord sera obtained in the present study is similar to that reported by others [1, 10 – 14]. In the present study, the mean TSH level in the cord sera of all the neonates (2.83 ± 2.13mIU/L) was within the reference ranges of 1.0 to 20.0mIU/L and 2.4 – 20.6mIU/L reported in the literature [22, 24]. Our mean TSH level was lower than the mean TSH levels of 6.13 ± 5.29mIU/L and 9.6 ± 7.8mIU/L reported by Manglik et al [13] and Feleke et al [10] in the cord sera of neonates in India and Ethiopia respectively.

The TSH level was greater than 10.0mIU/L in only two (1.3%) cord serum samples. This according to the WHO/UNICEF/ICCIDD criteria indicates normal iodine status among the neonates. The TSH levels in the two cord serum samples were 12.3mIU/L and 15.3mIU/L, both of which are lower than 20.0mIU/L that represents the lower limit of the cut-off point indicating CH. Thus, none of the neonates in our study population was recalled for repeat testing. Our data indicates zero prevalence rate of CH among the neonates that participated in our present study. This indicates normal thyroid status among the neonates. This finding should, however be interpreted with care because of the small sample size (150 cord sera) used in our present study.

The 97.8th and 99.0th percentiles TSH levels obtained for all the neonates were 7.77mIU/L and 11.16mIU/L respectively. These TSH levels were lower than the 97.8th (14.98mIU/L) and 99.0th (25.8mIU/L) percentiles reported for neonates in India [13]. The median TSH level in the cord sera of the male (1.98mIU/L) and female (2.22mIU/L) neonates were similar to the TSH values reported by Mekonnen et al [14] for male (1.94mIU/L) and female (1.45mIU/L) neonates in Ethiopia. The mean TSH levels for both male (2.75 ± 2.13mIU/L) and female (2.93 ± 2.13mIU/L) neonates were also similar to the TSH levels for male (2.71 ± 2.42mIU/L) and female (2.34 ± 2.26mIU/L) neonates in Ethiopia [14].

Our mean cord sera TSH values were however lower than the 6.48 ± 5.2mIU/L and 5.75 ± 4.16mIU/L reported by Manglik et al [13] for male and female neonates respectively in India. TSH level was greater than 10.0mIU/L in only one (1.2%) of the male cord serum samples and one (1.4%) of the female cord serum samples. The TSH levels in the two cord serum samples were below 20.0mIU/L as indicated earlier.

Our data indicate normal iodine status and normal thyroid status among the male and female neonates in our study population. The 2.5th and 97.5th percentile cord sera TSH cut-off levels obtained for both the male

and female neonates can be used tentatively as reference ranges for apparently healthy male and female neonates in NCD, until a larger and more extensive study is conducted.

In conclusion, our data indicates normal iodine and thyroid status and zero prevalence of congenital hypothyroidism among the NCD neonates in the study population. Despite these findings there is need to advocate for the implementation of routine screening test for primary hypothyroidism among neonates in PNG.
The use of cord blood TSH level for screening of neonates is more practical, cost-effective and relatively simple. It has the highest detection rate compared to other methods.

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REFERENCES:

1. World Health Organization / United Nations Children's Fund / International Council for Control of Iodine Deficiency Disorders. Assessment of Iodine Deficiency Disorders and monitoring their elimination: A guide for program mangers, 2nd Ed., WHO Pub, Geneva, WHO/NHD/01.1, 2001: 1 – 107.
2. Delange F. Hetzel B. In: The thyroid and its diseases. Braverman and Utiger eds. Lippincott Williams &Wilkins publ. Philadelphia: 2004. Chapter 20: The iodine deficiency disorders
3. Delange F. Optimal iodine nutrition during pregnancy, lactation and neonatal period. Int J Endocrinol Metab. 2004; 2: 1-12.
4. World Health Organization / United Nations Children's Fund Joint statement by WHO and UNICEF: Reaching optimal iodine nutrition in pregnant and lactating women and young children, WHO Pub, Geneva, 2007: 1 – 2.
5. Zimmermann M, Delange F. Iodine supplementation of pregnant women in Europe: a review and recommendations. Euro Journal of Clinical Nutrition. 2004; 58, 979 – 984.
6. Ikaros J, Pickard M, Evans I, Sinha A, Hardiman P, Ekins R, Thyroid hormone receptor gene expression in first trimester human fetal brain. J. of Clinical Endocrinology and Metabolism 2000, 85, 7: 2620 – 2623.
7. McElduff A, McElduff P, Gunton JE, Hams G, Wiley V and Wilcken B; Neonatal thyroid-stimulating hormone concentration in northern Sydney: further indications of mild iodine deficiency? MJA Vol 176, 2002; 317 – 320.
8. Delange F. Neonatal screening for congenital hypothyroidism: Results and

perspectives. Hormones Research 1997; 48: 51 – 61.
9. Delange F. Screening for congenital hypothyroidism used as indicator of iodine deficiency and its control. Thyroid; 1998; 8: 1185 – 1192.
10. Feleke Y, Enquoselassie F, Deneke F, Abdulkadir J, Hawariat GW, Tilahun M, et al. Neonatal congenital hypothyroidism screening in Addis Ababa, Ethiopia. East Afr Med J 2000; 77:377-381.
11. Sullivan KM, May W, Nordenberg D, Houston R and Maberly GF; Community and International Nutr: Use of thyroid stimulating hormone testing in newborns to identify iodine deficiency: J. Nutr. 1997; 127: 55 – 58.
12. Ward LS, Iida.S, and Maciel RMB. Thyroid Stimulating Hormone Levels in cord blood are not influenced by non-thyroidal mothers' diseases. In: Sao Paulo Medical Journal. Sao Paula Med J/Rev Paul Med. 2000; 114- 118.
13. Manglik AK, Chatterjee N and Ghosh G; Umbilical cord blood TSH levels in term neonates: A screening tool for congenital hypothyroidism. Indian Pediatrics 2005; Vol 42; 17: 1029 – 1032.
14. Mekennon Y.Gizachew WH, Chamiso B, Raue F. Thyroid Stimulating Hormone values in cord blood in neonates Ethiop J Health Dev 2003; 17: 125-130.
15. Andersson M, Takkouche B, Egli I, Allen, H E, de Benoist B. Current global iodine status and progress over the last decade towards the elimination of iodine deficiency. Bull WHO 2005; 83: 518-525.
16. Wu LL, Sazali BS, Adeeb N, Khalid BAK. Congenital hypothyroid screening using cord blood TSH. Singapore Med J 1999; 40: 23-26.
17. Desai MP, Colaco MP, Ajgao AR, Mahadik CV, Rege C, Shirodkar VV,et al. Neonatal Screening for congenital hypothyroidism in a developing country: problems and strategies. Indian Journal of Paediatrics, 1987; 54: 571-581.
18. Temple VJ, Mapira P, Adeniyi KO, Sims P. Iodine deficiency in Papua New Guinea (Sub-clinical iodine deficiency and salt iodization in the highlands of PNG). Journal of Public Health, 2005, 27: 45 – 48.
19. Temple VJ, Haindapa B, Turare R, Masta A, Amoa AB, Ripa P. "Status of Iodine Nutrition in Pregnant and Lactating Women in National Capital District, PNG. Asia Pac J Clin Nutr: 2006; Vol. 15 (4): 533 – 537.
20. Temple VJ, Oge R, Daphne I, Vince JD, Ripa P, Delange F and Eastman CJ. "Salt Iodization and Iodine Status among Infants and Lactating Mothers in PNG" AJFAND, Vol 9, No. 9, Dec 2009, 1807 - 1823.
21. Thyroid Stimulating Hormone (TSH) Enzyme Immunoassay Test kit: Enzyme Immunoassay for the quantitative determination of TSH in human serum. LiNEAR Chemicals. S.L. Barcelona Spain; Cromatest.
22. Delange F; Screening for Congenital Hypothyroidism used as an indicator of the degree of iodine deficiency and of its control; Thyroid, 1998, 8; 12: 1185 – 92.
23. Skeaff SA, Ferguson EL, Mc Kenzie JE, Valeix P, Gibson RS and CD Thomson Are breast-fed infants and toddlers in New Zealand at risk of iodine deficiency? *Nutrition* 2005; 21: 325 – 331.
24. Henry JG, Sobki SH, Al Beshara NM, Harkonen ME and Miller HR; Thyroid function in cord blood. Saudi Med. J. 2000; Vol. 21 (1): 36 – 39.

SYNOPSIS OF PAPERS PRESENTED AT THE JOINT DIVISIONAL SEMINAR SERIES: MARCH TO JULY 2010: DIVISIONS OF BASIC MEDICAL SCIENCES, HEALTH SCIENCES & DENTISTRY IN SCHOOL OF MEDICINE AND HEALTH SCIENCES

Cancer in Papua New Guinea

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SYNOPSIS:

Cancer remains a relatively infrequent cause of admission to health facilities in PNG, other than the cancer wards in Lae and Port Moresby. However, much malignant disease remains undiagnosed and untreated in villages and measures of cancer incidence are underestimated. Late presentation and limited treatment facilities results in much patient suffering;

Amongst adults, cancer of the mouth, skin and digestive system predominate. Betel nut chewing and smoking are dose-related to mouth cancer incidence. Chronic skin ulcers and untreated burns precede malignant change in the skin. Amongst female cancers, malignancy of the cervix predominates and breast cancer may be increasing in incidence. In children, lymphomas are common with a high frequency of Burkitt lymphoma. Leukaemia is also common in children and survival is very poor, even following treatment.

Several reviews of cancer services in PNG have highlighted the need for improvement in diagnostic and treatment facilities, including staff training. The cancer unit in Lae remains the only functioning radiotherapy service in the country and most medical and surgical management remains the work of the general physician and surgeon, with few trained specifically in oncology. Access to anti-cancer drugs and effective analgesics remains fragile.

Cancer prevention strategies remain ineffective. Legislation on tobacco sale and use was enacted in 1987 but remains without specific regulations that would enable enforcement of smoking-free areas. Regulation of betel nut sale in Port Moresby has proven unsustainable. The future use of HPV vaccine remains to be researched but will likely result in a decrease in cancer of the cervix in women.

Assessment of Hyperhomocysteinemia among Cardiovascular Patients in Port Moresby General Hospital: A Prospective Study

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INTRODUCTION

Cardiovascular disease remains the leading cause of mortality in industrialized countries and is becoming increasingly prevalent in developing countries [1]. In the last four decades tremendous gains have been made in understanding the effect of the risk factors for cardiovascular disease. Well-known (or traditional) risk factors such as abnormal cholesterol levels, increased blood pressure, hereditary, advancing age, diabetes mellitus, obesity, lack of physical activity and smoking are helpful in predicting the likelihood of heart attacks or strokes [2, 3]. Modification of some of these risk factors can reduce the risk of having a heart attack or stroke [3]. These traditional risk factors do not fully account for all the cardiovascular disorders reported in hospitals and clinics worldwide [3]. Correlation between Hyperhomocysteinemia and vascular diseases was observed by clinicians and scientists as early as the 1960's [4]. Since then the number of studies have greatly increased and the trend continues to support the Hyperhomocysteinemia as an important risk factor for coronary atherosclerosis, coronary artery disease (CAD), myocardial infarction, stroke, thromboembolism and peripheral vascular disease [4, 5]. It is now widely accepted that an elevated level of Homocysteine (>15umol/L) is an independent risk factor for cardiovascular disease [3, 6]. According to the American Heart Association (AHA) advisory statement [5], the total Homocysteine concentration in blood can be characterized as normal (5 – 15umol/L), intermediate (31 – 100umol/L) or severe (> 100umol/L). In 2002 the Centers for Disease Control and Prevention (CDC) Environmental Health division proposed normal reference ranges for total plasma homocysteine levels according to gender and age groups (Table 1) [7].

In addition, the CDC also published the cut-off points for homocysteine concentration in blood plasma that indicates moderate (16 – 30 umol/L), intermediate (31 – 100umol/L) and severe (> 100umol/L) Hyperhomocysteinemia [7]. Homocysteine is a non-standard, non-protein sulphur-containing (thiol) amino acid with the molecular formula $C_4H_9NO_2S$ [8, 9, 10].

Homocysteine is produced in the liver as a metabolic intermediate in biosynthesis of cysteine from Methionine [8, 9]. In humans, the metabolism of Homocysteine occurs at the intersection of the Trans-sulfuration and Remethylation pathways [8, 9]. In the Trans-sulfuration pathway Homocysteine condenses with Serine to form Cystathionine, catalyzed by Cystathionine β-synthetase (CBS) that requires the cofactor Pyridoxal-phosphate (B_6-Phosphate) [8, 9]. Hydrolysis of Cystathione to Cysteine and α-Ketobutyrate is catalyzed by γ-Cystathionase [8, 9].

Table 1: Homocysteine Reference Ranges from CDC Environmental Health [7]

	Total Homocysteine (umol/L)	Age	Gender
Normal	4.6 - 8.1	< 30 years	All
Normal	4.5 - 7.9	30 - 59 years	Females
Normal	6.3 - 11.2	30 - 59 years	Males
Normal	5.8 - 11.9	> 60 years	All

The Remethylation pathway involved methylation of Homocysteine to Methionine [8, 9]. The methyl group is obtained from either N-5-Methyltetrahydofolate or Betaine.

N-5-methyl-tetrahydofolate is formed from 5-10-methyltenetrahydofolate by 5-10-methyl-tenetrahydofolate reductase (MTHR).

The reaction that uses N-5-methyl-tetrahydofolate occurs in all tissues and is catalysed by Methionine synthase (MS) that utilizes Vitamin B_{12} as cofactor [8, 9]. The Remethylation reaction involving Betaine occurs in the liver; it is catalyzed by Betaine-Homocysteine methyl-transferase (BHMT) [8, 9].

Some causes of Hyperhomocysteinemia include the following: Vitamin B_6 (Pyridoxine phosphate) and Vitamin B_{12} (Cobalamin) are cofactors and Folic Acid is a co-substrate needed for Homocysteine metabolism [9, 10]. Deficiencies in the B-complex vitamins may cause accumulation of Homocysteine in the body [9, 10, 11];

Genetic defects (mutations) in genes encoding for enzymes involved in the Homocysteine metabolic pathways can result in Hyperhomocysteinemia. Some of the enzymes are 5,10-Methylene-Tetrahydrofolate (MTHFR); Cystathionine β-synthetase (CBS); Methionine synthase

(MS); Betaine-Homocysteine Methyl-Transferase (BHMT) [8, 9, 10, 11, 12]; Renal failure may cause accumulation of Homocysteine in blood because of the less efficient renal clearance of Homocysteine by the kidneys. [4, 8, 11, 20]; certain drugs such as Methotrexate and Cyclosporine A can cause elevation of Homocysteine [11, 18]; Homocysteine levels increase with age [13]. On average Homocysteine levels are higher in males than females [13]; Stress, physical inactivity, smoking, and coffee drinking cause elevation of Homocysteine. [18, 19, 20]. The link between blood Homocysteine level and cardiovascular disease was first suggested by McCully [14], who encountered patients with Homocystinuria. This is an autosomal recessive disorder characterized by abnormalities of the long bones, ocular lens dislocation, mental retardation and venous thromboembolism that can cause aggressive vascular disease [9, 14].

Patients with Homocystinuria usually have Hyperhomocysteinemia [4, 9, 14]. Children with Homocystinuria usually do not thrive because of the complications of arteriosclerosis [4, 9, 14]. Untreated patients who are homozygous for Homocystinuria may have Homocysteine concentration in the blood about five times above the normal level for unaffected people [16]. They may also have about 50% chance of developing vascular events by age 30 years [16]. Earlier studies [4, 17] indicated that Homocysteine is an independent risk factor for vascular disease, similar to that of smoking or hyperlipidemia and that the risk was more pronounced in smokers and in those with hypertension. In a recent review by Wald et al [15] meta-analyses of cohort studies show significant positive associations between serum Homocysteine concentrations and Ischemic heart disease events and stroke. According to the authors [15, 16] a 3.0umol/L decrease in serum Homocysteine (achievable by utilizing 0.8mg/day of Folic acid) lowers the risk of myocardial infarction by 15% and stroke by 24%. According to Mann and Green [16] a 5.0umol/L increase in total Homocysteine level in blood can significantly increase the risks of having coronary heart disease and cerebrovascular disease.

Several recent studies [18, 19, 20] have linked Hyperhomocysteinemia to birth defects, Down Syndrome, Diabetes Mellitus, Alzheimer's, Osteoporosis, Renal Failure and some cancers. Despite several well structured research and clinical studies on Hyperhomocysteinemia, the exact mechanism(s) by which it can cause vascular injuries remains unclear. There are however a number of hypotheses that have

been proposed. Some of these hypotheses include the following: The direct toxic effect of Homocysteine that damages the cells lining the intima of arteries [21, 22, 23]; Oxidation and modification of low-density lipoproteins by Homocysteine [24, 25, 26]; Interference of Homocysteine with the blood clotting factors [27, 28, 29]. There are several ongoing research studies to elucidate the mechanism(s) of action of Hyperhomocysteinemia in Atherosclerosis and cardiovascular diseases.

Despite counter arguments by some authors [30] there are evidence indicating strong correlation between Hyperhomocysteinemia and cardiovascular diseases.

Proposed prospective research project:

Hyperhomocysteinemia is a risk factor for cardiovascular diseases [4, 5, 9, 16, 17]. All confirmed cardiovascular cases in Papua New Guinea (PNG) are reported to the Sir Buri Kidu Heart Institute in the Port Moresby General Hospital (PMGH), which is the major specialist and referral hospital in PNG. Most of the cardiovascular cases have been successfully managed and have been related to traditional risk factors.

There are, however no published data indicating the involvement of Homocysteine in the aetiology of cardiovascular disease in PNG. The need to investigate the possible role of Homocysteine in cardiovascular diseases in PNG is strongly supported by the Chief consultant cardiologist and other specialists in PMGH.

The aim of the study will be to assess the Homocysteine level in the blood of patients with cardiovascular diseases admitted to the PMGH. The data obtained will be used to evaluate the relationship (if any) of Homocysteine to cardiovascular diseases among patients in PMGH. The Sir Buri Kidu Heart Institute in PMGH will serve as the sampling site for this collaborative cross-sectional study.

Signed informed consent will be obtained from the relatives of patients that are enrolled in the study.

Fasting blood samples will be collected from patients with heat attack and stroke admitted in the institute.

Assay of plasma Homocysteine levels will be carried out using the HPLC procedures and protocol designed by Immundiagnostiks [32, 33]. Blood samples will also be collected from healthy age-matched controls. Parameters such as age, gender, health status, current and past history of illnesses such as renal failures and those affecting the CVS especially diabetes mellitus will be recorded. Data obtained will be statistically analysed and interpreted using approved standards and cut-off points.

Ethical clearance and permission for this project will be obtained from the relevant authorities. It is hoped that this project will set the stage for more detailed study to investigate the causes of Homocystinemia in PNG. Hopefully this could also lead to studies of nutritional factors affecting Homocysteine levels namely the B vitamins (vitamin B_6, B_{12} and folic acid) and to develop genetic screening of patients and individuals who may be at risk of having cardiovascular disease [2, 6, 10, 11].

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REFERENCES

1. Dierkes J, Luley C and Westphal S. Effect of lipid-lowering and anti-hypertensive drugs on plasma homocysteine levels. Vascular Health and Risk Management 2007, 3 1: 99-108
2. Mann J and Green T. Hyperhomocysteinaemia: time to screen and treat? Journal of the New Zealand Medical Association 2002, 115: 1163
3. Barrett, S., Homocysteine: A Cardiovascular Risk Factor worth Considering. (28/10/2009) http://www.quackwatch.com/03HealthPromotion/homocysteine.html
4. Finkelstein JD. Homocysteine: A History In Progress. Nutrition Reviews 2001, 58 7: 193-204
5. Coffey M, Crowder GK and Cheek DJ. Reducing Coronary Artery Disease by Decreasing Homocysteine Levels. Critical Care Nurse 2003, 23: 25-30
6. Cortese C and Motti C. MTHFR gene polymorphism, homocysteine and cardiovascular disease. Public Health Nutrition 2001, 4(2B): 493-497
7. CDC Environmental Health Laboratory Procedure Manual for Total Homocysteine in Plasma 2002
8. Selhub J. Homocysteine Metabolism. Annual Review of Nutr. 1999, 19: 217-246
9. Robinson, K. (2000) Homocysteine, B vitamins, and risk of cardiovascular disease. Heart 83:127–130
10. Jacobsen DW. Homocysteine and vitamins in cardiovascular disease. Clinical Chemistry 1998, 44:1833–1843
11. Maron BA and Loscalzo J. The Treatment of Hyperhomocysteinemia. Annu. Rev. Med. 2009, 60: 39–54.
12. Tsai MT, Welge BG, Hanson NQ, Vessey J, Schwichtenber K, Yang Bullemer, FE, Rasmussen R and Graham KJ. Genetic causes of mild hyperhomocysteinemia in patients with premature occlusive coronary artery diseases. Atherosclerosis 1999, 143 1: 163-170
13. Stanisławska-Sachadyn A, Woodside JV, Brown KS, Young IS, Murray L, McNulty H, Strain JJ, Boreham CA, Scott JM, Whitehead AS, Mitchell LE. Evidence for sex differences in the determinants of homocysteine concentrations. Molecular Genetics and Metabolism 2008, 93 4: 355-362
14. McCully KS. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. American Journal of Pathology 1969, 56: 111-128.
15. McCully KS. "The Homocysteine Revolution," Keats Pub, New Canaan, CT 1997.
16. Wald DS, Morris JK, Law M and Wald NJ. Folic acid, homocysteine, and cardiovascular disease: Judging causality in the face of inconclusive trial evidence. BMJ 2006, 333: 1114–7
17. Graham IM, Daly LE and Refsum HM. (1997) Plasma homocysteine as a risk factor for vascular disease. The European

Concerted Action Project. Journal of the American Medical Assoc 277: 1775–81

18. Carmel R and Jacobsen DW. Homocysteine in Health and Disease. Cambridge University Press, Cambridge 2001

19. Piyathilake CJ, Johanning GL, Macaluso M, Whiteside M, Oelschlager DK, Heimburger DC. Localized folate and vitamin B-12 deficiency in squamous cell lung cancer is associated with global DNA hypomethylation. Nutrition Cancer 2001, 37: 99-107

20. Sánchez-Margalet V, Valle M, Ruz FJ, Gascón F, Mateo J and Goberna R. Elevated plasma total homocysteine levels in hyperinsulinemic obese subjects The Journal of Nutritional Biochemistry 2002, 13 2: 75-79.

21. Rodríguez-Nieto S, Chavarría T, Martínez-Poveda B, Sánchez-Jiménez F, Quesada AR and Medina AM. Anti-angiogenic effects of homocysteine on cultured endothelial cells. Biochemical and Biophysical Research Communications 2002, 293 1: 497-500

22. Jakubowski H, Zhang L, Bardeguez A and Aviv A. Homocysteine Thiolactone and Protein Homocysteinylation in Human Endothelial Cells: Implications for Atherosclerosis. Circulation Research 2000, 87: 45-51

23. Heydrick SJ, Weiss N, Thomas SR, Cap AP, Pimentel DR, Loscalzo J and Keaney JF. Homocysteine and l-homocystine stereospecifically induce endothelial nitric oxide synthase-dependent lipid peroxidation in endothelial cells. Free Radical Biology and Medicine 2004, 36 5: 632-640

24. Cavalca V, Cighetti G,Bamonti F,Loaldi A, Bortone L,Novembrino C, de Franceschi M, Belardinelli R and Guazzi MD. Oxidative Stress and Homocysteine in Coronary Artery Disease. Clinical Chemistry 2001, 47 5: 887–892 (2001)

25. Pfanzagl B, Tribl F, Koller E and Möslinger T. Homocysteine strongly enhance metal-catalyzed LDL oxidation in the presence of cystine and cysteine. Atherosclerosis 2003, 168 1: 39-48

26. Olszewski AJ and McCully KS. Homocysteine metabolism and the oxidative modification of proteins and lipids. Free Radical Biology and Medicine 1993,14 6: 683-693

27. Kuch B, Bobak M, Fobker M, Junker R, von Eckardstein A, Marmot M and Hense HW. Associations between Homocysteine and Coagulation Factors — A Cross-Sectional Study in Two Populations of Central Europe. Thrombosis Research 2001, 103 4: 265-273

28. Luo F, Liu X, Wang S and Chen H. Effect of homocysteine on platelet activation induced by collagen. Nutrition 2006, 22 1: 69-75

29. Sauls DL, Lockhart E, Warren ME, Lenkowski A, Wilhelm SE and Hoffman M. Modification of Fibrinogen by Homocysteine Thiolactone Increases Resistance to Fibrinolysis: A Potential Mechanism of the Thrombotic Tendency in Hyperhomocysteinemia. Biochemistry including Biophysical Chemistry and Molecular Biolgoy 2006, 45 8: 2480–2487

30. Shamsara J, Ramezani M, and Mohammadpoor AH. Why homocysteine-lowering therapy does not have beneficial effects on patients with cardiovascular disease? Bioscience Hypotheses, 2009, 2 1: 13-15

31. Solomon BP and Duda CT. Homocysteine Determination in Plasma. Current Separations 1998, 17:1

32. Fricka B, Schro¨cksnadela K, Neurautera G, Wirleitnera B, Artner-Dworzaka E and Fuchsa D. Rapid measurement of total plasma homocysteine by HPLC. Clinica Chimica Acta 2003, 331: 19–23

33. “Immundiagnostik HPLC Homocysteine Kit”. Immundiagnostik www.Immundiagnostik.com

34. Ashavaid TF, Eghlim FF, Shalia KK and Nair KG. Standardisation of Homocysteine on HPLC using DTT as Reductant. Indian Journal of Clinical Biochemistry 2003, 18 2: 106-110

35. “Formosa Biomedical Technology Total Homocysteine Biochemical Assay Kit”. Formosa Biomedical Technology Corporation

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Acknowledgements:

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***Book*:**

2. Gillett JE. The health of women in Papua New Guinea. PNGIMR: Kristen Press, 1991

Chapter in a Book:

3. Chaney SG. Principles of nutrition II: Micronutrients. In: Delvin TM, editor. Textbook of Biochemistry with Clinical Correlations, 4th ed. Brisbane: Wiley-Less, 1997: 11–36.

Published proceedings paper:

4. Kruse-Jarres JD. Basic principles of zinc metabolism. *In*: Kruse-Jarres JD, Scholmerich J, editors. Zinc and diseases of the digestive tract. Proceedings of the International Falk workshop, UK, 1996: 3 – 15.

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