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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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