African Journal of Pharmacy and Pharmacology Vol. 6(9), pp. 636-642, 8 March, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.841
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Quality survey of some brands of artesunate-amodiaquine in Lagos drug market

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Accepted 8 February, 2012

With the advent of Artemisinin Combination Therapy (ACTs) as the recommended treatment protocol for malaria by WHO, the menace of substandard and counterfeit anti-malaria drugs have been on the rise. Artesunate-amodiaquine, like other ACTs, has been widely implicated in this menace due to the market value and affordability. 13 representative brands of Artesunate/Amodiaquine were procured from different outlets in urban and peri-urban parts of Lagos, Nigeria. Quantitative and qualitative analysis were carried out on the different brands using HPLC. The results show all brands to contain the test APIs but in proportions varied about the USP specified limits. 30.8% of the test brands had artesunate within the USP specification. 30.8% of amodiaquine also had met the quality specification of USP. But only 15.4% of the sample had both amodiaquine and artesunate within the USP specification. 53.8% failed the active content test for both amodiaquine and artesunate.

Key words: Artemisinin combination therapy (ACTs), WHO, malaria, high-performance liquid chromatography (HPLC).

INTRODUCTION

Statistics have shown that malaria is the fifth leading cause of death worldwide, with 3.3 billion people at risk (World malaria report, 2010). Several centuries after its discovery, malaria remains a devastating human infection, totaling 300 to 500 million clinical cases and three million deaths every year (World Malaria Report, 2010), but many of these death would be avoided if anti-malaria drugs were effective (Bate et al., 2009). Since 2001, the World Health Organization has recommended that malaria-endemic African countries should consider changing to artemisinin derivative- based combination therapy (ACT) as first-line malaria treatment. Malaria is presently endemic in a broad band around the equator, in areas of the Americas, many parts of Asia, and much of Africa; however, it is in sub-Saharan Africa where 85 to 90% of malaria fatalities occur (Layne, 2006). The geographic distribution of malaria within large regions is complex, and malaria-afflicted and malaria-free areas are often found close to each other (Greenwood et al., 2002).

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ACTs have been largely successful in combating malarial in Africa. Successful drugs such as ACTs are at risk of being counterfeited or produced with insufficient quality control resulting in substandard products. Since more than 40% of the world's population is at risk of malaria, antimalarial drugs have become a favorite target of counterfeiters. For instance, counterfeit artemisinins are a significant problem in Southeast Asia (Singh, 2004) and are expected to become a serious problem in Africa where artemisinin combination therapy is being implemented (Dondorp et al., 2010). It has been estimated that the counterfeit medicine market is worth some US$ 35 to 44 billion per year (Newton et al., 2006). Hence, the need for an effective post-marketing surveillance of all medications especially those used in treating priority disease. Post Marketing Surveillance (PMS) is the practice of monitoring a pharmaceutical drug or device after it has been released on the market.

These substandard products contain active pharmaceutical ingredients below or above the range recommended by the USP. A lower dose than the labeled dose of the active medicament leads to a delivery of lower concentration to the blood plasma. This consequently

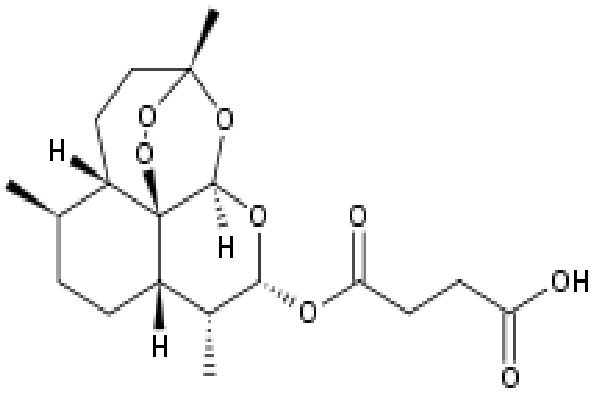

Figure 1. The chemical structure of artesunate.

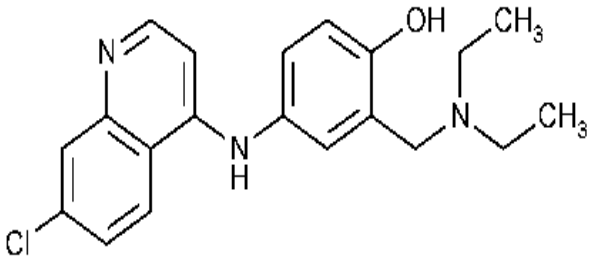

Figure 2. The chemical structure of amodiaquine.

leads to an improper treatment of the malaria infection and also increases the risk of the emergence of resistant species of the parasites. A higher dose than the labeled dose of the active medicament increases the risk of adverse and side effects, and may have fatal consequences on vital organs of the body system. In some counterfeited products, there is little or no active pharmaceutical ingredient, which is even more deadly. The total absence of a labeled active ingredient from a supposed drug will only increase the risk of mortality and morbidity in its users. Malarial parasites have developed resistance to several antimalarial drugs, most notably chloroquine (Wellems, 2002) and this is as a result of ineffective use of antimalarials and the prevalence of substandard and counterfeit drugs as well as quality-jeopardized antimalarials infiltrating the drug market. In most developing countries, substandard products are usually due to lack of robust quality control infrastructure. There is also a proliferation of brands and most times the manufacturers of these products are too small to handle the quality control. For instance, in Nigeria well over one hundred brands of antimalarials have been registered in the past five years.

Among the ACTs prevalent in the Nigerian drug market is Amodiaquine/Artesunate combined therapy, either co-formulated or co-administered. Amodiaquine is (4-(7 –Chloro-4-quinolyamino)-2-(diethylaminomethyl) phenol dihydrochloride dehydrate) while artesunate is (Decahydro-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano-[4, 3*j*]-1, 2-benzodioxepin-10-ol hydrogen succinate). They have been very useful in combating malaria. Amodiaquine belongs to the historically most important group of antimalarials drugs known as quinolines which mostly act during the blood stages of the parasite's lifecycle (Foley and Tilley, 1998) but some are also believed to target the hepatic stage (Baird et al., 2003). Quinolines act by inhibiting the dimerization of heme and/or prevention of the disposal of dimers from the food vacuole to the cytoplasm, where hemozoin is formed (Fitch, 2004). This leads to intra-plasmodial accumulation of free heme, which becomes highly toxic to the parasite (Cunha-Rodriguez et al., 2006). Artesunate is rapidly metabolized to the active metabolite dihydroartemisinin (Adewuyi et al., 2011). It acts by means of the prodrug, dihydroartemisin, which is active during the stage when the parasite is located inside the red blood cells (Krishna et al., 2004), with Cumming (1997), positing that the iron of the heme reduces the peroxide linkage in artemisinin, generating high-valent iron-oxo species and resulting in a cascade of reactions that produce reactive oxygen radicals that damage the falciparum and leads to its death.

In this study all the available brands of amodiaquine-artesunate in the Lagos Drug market were analyzed using high performance liquid chromatography in order to determine the quality strengths of the artesunate-amodiaquine combinations in this drug market.

MATERIALS AND METHODS

Sampling

To preserve the integrity of the study, mystery shoppers were employed in the sourcing and purchasing of the antimalarial formulations labeled to contain artesunate and amodiaquine from pharmacy outlets in urban and peri-urban parts of Lagos, Nigeria. All the samples purchased had an expiry date of not less than one year from the date of purchase. They were all properly registered by the appropriate regulatory agency.

Reagents

All solvents used were of HPLC grade, and were employed as supplied by manufacturers. Deionized water was used in all procedures, and deionization was carried out by means of a Millipore deionizer. A buffer solution comprising of Dipotassium Hydrogen Phosphate and Orthophosphoric was used. HPLC grade Methanol (Merck KGOA, 54271 darmstadt, Germany) and HPLC grade Acetonitrile (VWR int Ltd. Poole, BH15 ITD, England) were also used. The reference standards of artesunate (Decahydro-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano-[4, 3*j*]-1, 2-benzodioxepin-10-ol hydrogen succinate) (Figure 1) and amodiaquine (4-(7 –Chloro-4-quinolyamino)-2-(diethylaminomethyl) phenol dihydrochloride dehydrate) (Figure 2) were supplied by USP Rockville Maryland, USA.

Table 1. The uniformity of weight for the study samples.

	Mean ± Standard deviation	
Sample code	**Artesunate (mg)**	**Amodiaquine (mg)**
A	276.18 ± 4.89	298.85 ± 9.99
B*	1202.30 ± 22.13	
C	496.93 ± 6.77	870.77 ± 6.02
D	336.27 ± 5.19	971.11 ± 7.47
E	287.33 ± 4.16	509.82 ± 9.31
F	278.83 ± 3.33	557.93 ± 8.62
G	409.32 ± 10.66	495.73 ± 6.49
H*	582.45 ± 9.31	
I	302.19 ± 15.91	317.56 ± 13.05
J	217.58 ± 23.80	515.88 ± 6.92
K	356.00 ± 6.32	922.93 ± 16.80
L	289.68 ± 6.67	319.25 ± 15.51
M	296.41 ± 8.72	260.592 ± 4.19

*: Co-formulated samples.

Instrumental and analytical conditions

The HPLC analyses were carried out on an Agilent 1100 system composed of a quaternary pump, autosampler, a UV detector and HP chemstation software. The column used for the analyses was an X Bridge TM C18 (150×4.6 mm i.d.; 5um particle size). UV detection was performed at 220 nm, while the injection volume was maintained at 20 µl. An isocratic mobile phase containing a 0.025 M K_2HPO_4 and acetonitrile (30:70, v/v) adjusted to pH 3.1 was employed at a flow rate of 1.0 ml/min and run time of 3 min (Gandhi et al., 2010). All chromatographic conditions were carried out at ambient temperature.

Reference standards preparation

2 mg artesunate and 6 mg amodiaquine reference standards were weighed and dissolved in 6 ml of HPLC grade methanol to produce concentrations of 333 and 1000 µg/ml respectively. The solutions were gently agitated to ensure complete solubilization. Serial dilutions of (166.5 µg, 500 µg/ml); (88.25 µg, 250 µg/ml); (41.16 µg, 125 µg/ml) and (20.51 µg, 62.5 µg/ml) were prepared for artesunate and amodiaquine respectively by first diluting 1ml of a stock with 1ml of HPLC grade methanol to obtain half the original concentration. The different concentrations prepared were transferred into clean sample vials and labeled appropriately for HPLC analysis.

Drug samples preparation

At least three tablets per brand were weighed on an analytical balance and the average tablet weight per brand determined. A tablet from each brand was pulverized in a clean dry porcelain mortar with the aid of a clean dry porcelain pestle. 1 mg each of artesunate and amodiaquine was weighed out on an analytical balance respectively and dissolved in 2 ml of methanol to obtain a drug stock solution of concentrations of 500 µg/ml. The stock solution prepared was then filtered with a Whatman filter paper No 42 (125 mm) to remove insoluble excipients in order to obtain a clear solution. The filtrate was transferred into clean sample vials and labeled appropriately for HPLC analysis.

Buffer solution preparation

2.175 mg Dipotassium Hydrogen Phosphate was weighed and transferred into a 500 ml volumetric flask and made up to mark with distilled water. With the aid of a pH meter, the pH of the buffer solution was adjusted from 5.4 to 3.1, by adding few drops of Orthophosphoric acid solution.

Analysis

The 13 different brands of artesunate-amodiaquine combinations were analyzed using the method of Gandhi et al. (2010). Each sample was run in triplicate and quantified by relating the peak area with those of the serial concentrations of the reference standards. Calibration curves for peak areas versus concentrations of the reference standards were plotted and the obtained data were subjected to regression analysis using the least squares method.

RESULTS AND DISCUSSION

This study is an attempt to establish the quality profile for the different brands of artesunate-amodiaquine combination in the Lagos drug market via comparing the label contents of different brands of Artesunate/ Amodiaquine combination therapy of antimalarials with the actual content as observed upon analysis with HPLC. Table 1 gives the summary of the uniformity of weight for the different brands while Table 2 gives the summary of the potency of the drugs. Calibration curves for the standards were obtained using a series of concentrations of these compounds. The calibration curves for the two standards were linear. The regression coefficients were 0.9935 and 0.9965 for artesunate and amodiaquine respectively. Figure 3 shows a representative chroma-togram for the mixture of the standards of artesunate and amodiaquine. The same separation parameters were

Table 2. Summary of the assay results.

Sample	Labeled active ingredients	Label weight of active (mg)	Actual weight of active (mg)	Percentage composition (%)
Sample A	Amodiaquine	153	117.32	76.68
	Artesunate	50	18.45	36.89
Sample B	Amodiaquine	600	347.34	57.89
	Artesunate	200	239.44	119.72
Sample C	Amodiaquine	600	764.10	127.35
	Artesunate	200	120.06	60.03
Sample D	Amodiaquine	600	614.04	102.32
	Artesunate	200	200.84	100.42
Sample E	Amodiaquine	300	366.06	122.02
	Artesunate	100	65.91	65.91
Sample F	Amodiaquine	300	296.37	98.79
	Artesunate	100	71.44	71.44
Sample G	Amodiaquine	306.3	410.44	134.00
	Artesunate	100	115.4	115.40
Sample H	Amodiaquine	270	186.38	69.03
	Artesunate	100	126.63	126.63
Sample I	Amodiaquine	153	149.21	97.52
	Artesunate	50	48.94	97.87
Sample J	Amodiaquine	300	299.58	99.86
	Artesunate	100	69.61	69.61
Sample K	Amodiaquine	600	531.36	88.56
	Artesunate	200	324.46	162.23
Sample L	Amodiaquine	153.1	115.48	75.43
	Artesunate	50	47.03	94.05
Sample M	Amodiaquine	150	117.03	78.02
	Artesunate	50	49.14	98.27

employed for the analysis of the samples. The compounds were identified using retention time match against those of the calibration standards while quantification was performed by means of peak area match against those of the standards.

The percentage composition of the artesunate and amodiaquine present in the different brands varied grossly. According to the USP specifications, artesunate and amodiaquine should be within the range of 90 to 110%. In this study, 30.77% had artesunate concentrations higher than the upper limit, 38.46% of the brands had artesunate concentrations lower than the lower limit of the USP specifications while only 30.77% fell within the 90 to 110% composition range. In the same vein, 23.08% had amodiaquine concentrations higher than the upper limit, 46.15% of the brands had amodiaquine concentrations lower than the lower limit of the USP specifications, while only 30.77% fell within

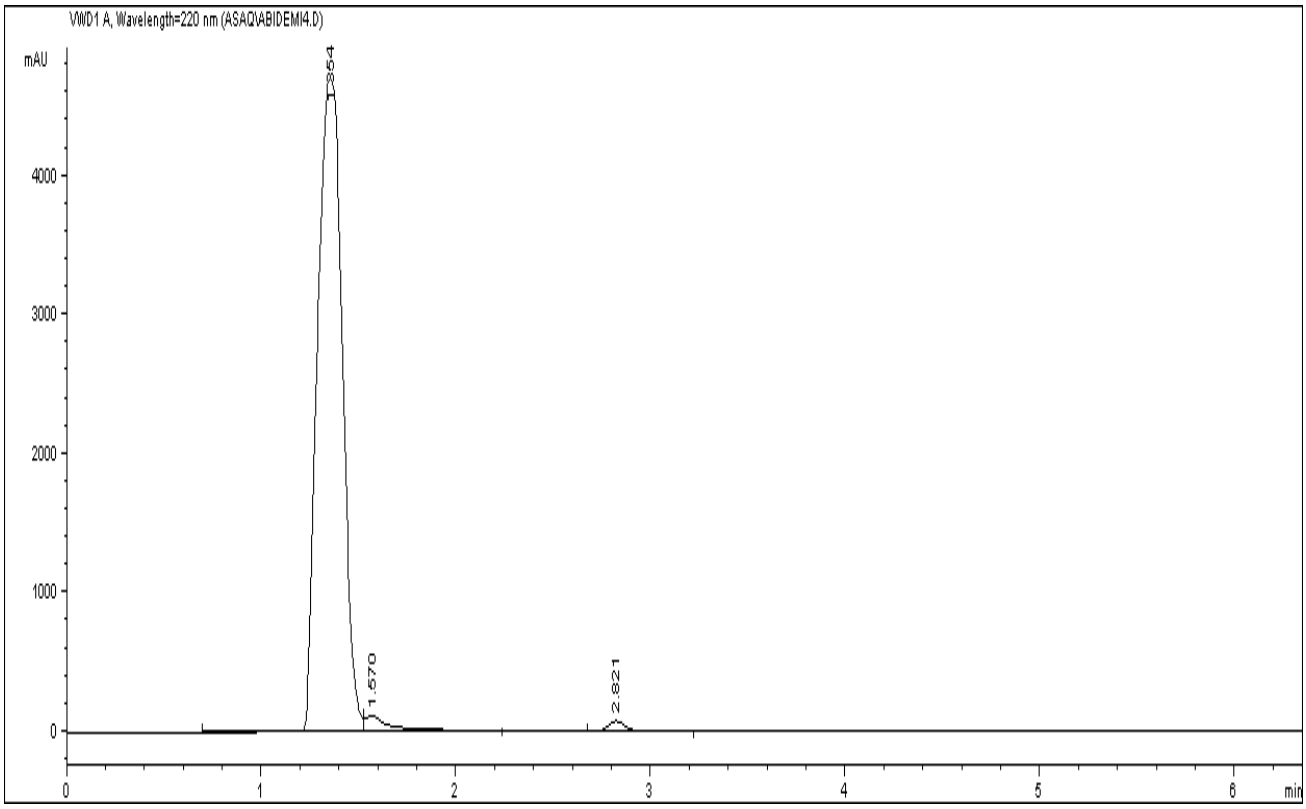

Figure 3. chromatogram of amodiaquine and artesunate respectively.

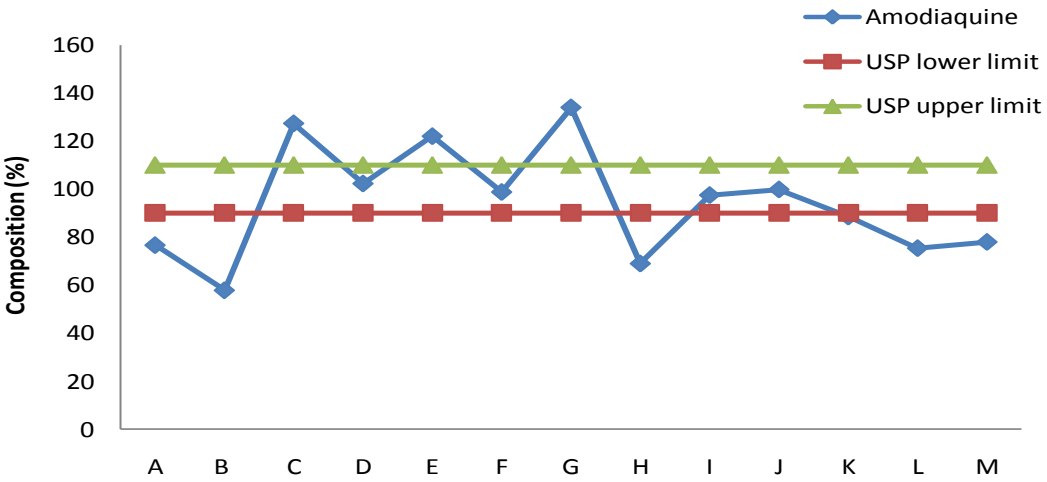

Figure 4. Percentage composition of amodiaquine in the different samples compared with USP limits.

the 90 to 110% composition range. However, only 15.38% had both artesunate and amodiaquine content within USP limits while 84.62% fell outside the 90 to 110% (Figures 4, 5 and 6).

Various health implications may arise if the quality of anti-malaria drugs available in drug market does not meet the required specifications. Such health implications includes: resistance, (Wellems, 2002) recrudescence and relapse of the disease. In severe cases death could result following exposures of the patient to sub-clinical doses or overly high doses of fake and counterfeit drugs. Hence, the need for an effective post-marketing surveillance of

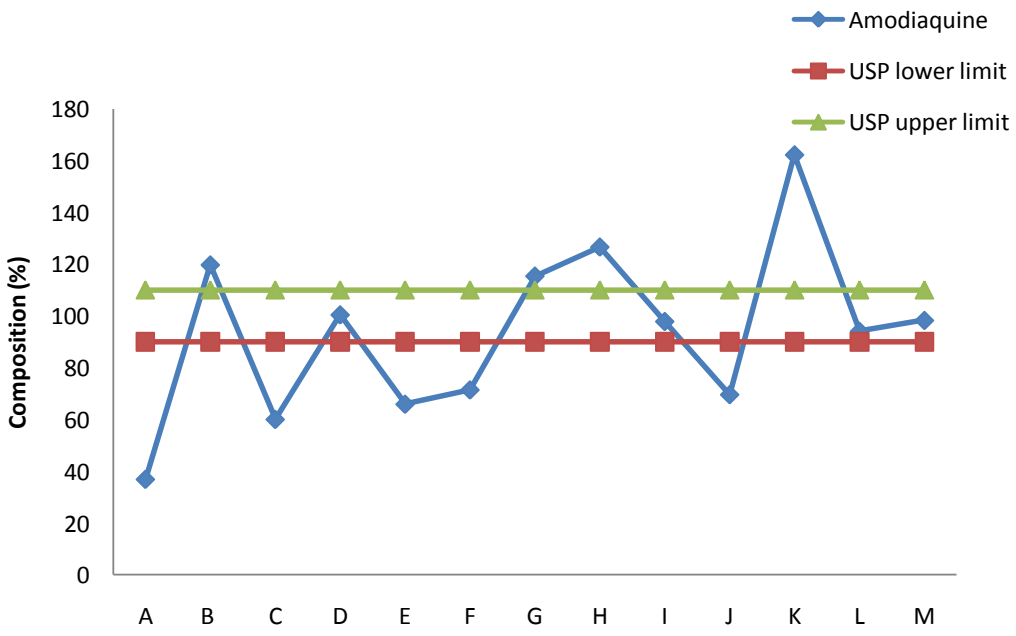

Figure 5. Percentage composition of artesunate in the different samples compared with USP limits.

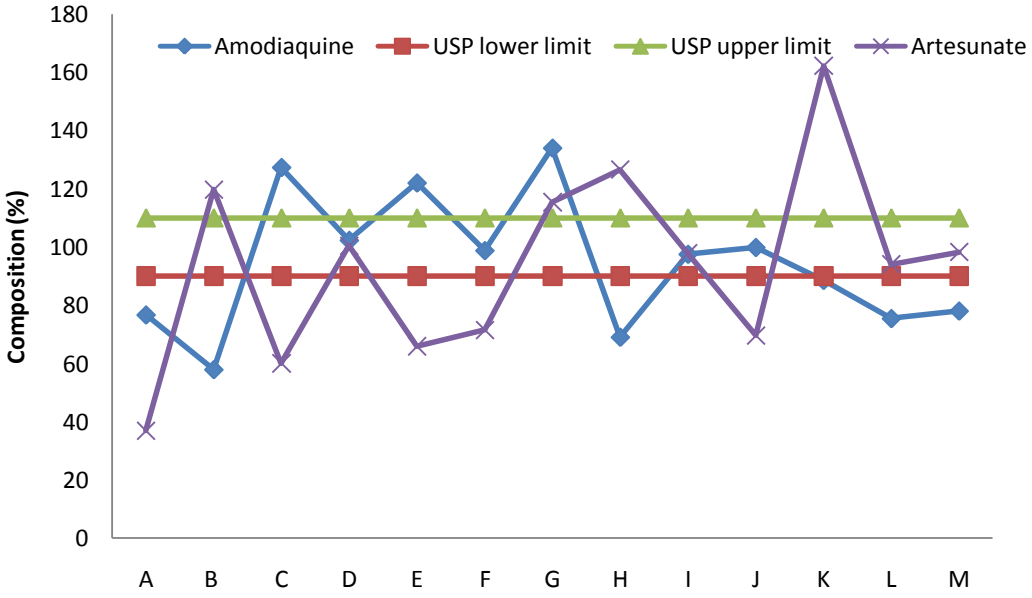

Figure 6. Percentage composition of artesunate-amodiaquine in the different brands compared with USP limits.

all medications especially those used in treating priority disease. Worldwide proliferation of poor quality and counterfeit drugs not only affects the quality of healthcare unfavourably, but it also confounds studies determining the effectiveness of commonly used pharmaceuticals to potentially resistant micro-organisms.

Observed treatment failure may result from either actual drug resistance or sub-therapeutic levels. If treatment failure is not a direct result of resistance, then exposure of organisms to suboptimal levels of the drug will certainly contribute to developing resistance. Systemic drug levels are influenced by metabolism,

absorbance, compliance or drug quality. For example, age, genetics, drug interactions, diet and disease state affect metabolism and absorption rates, while high drug costs, inconvenient dosing regimen and side-effects have an adverse influence on compliance (Green, 2006). Also, administration of poor-quality or counterfeit drugs can expose a patient to insufficient levels of active ingredients. Although pharmacokinetic parameters such as absorption and metabolism are difficult to control, diligent monitoring of drug quality can ensure that the correct dose of active ingredient is being administered.

The trend of low quality of drugs that was found in this study has implications in the effort to combat malarial scourge in sub-Sahara Africa. It is a herculean task to monitor every single batch of every single brand in these poor countries due to lack of quality control (QC) infrastructures and limited expertise and manpower in the relevant agencies in these countries. Several other studies have corroborated the result from this study. For example, in Tanzania, there was availability of low quality sulphadoxine-pyrimethamine and amodiaquine tablets in wholesale pharmacies (Minzi et al., 2003). Similarly in Cambodia, it was found that out of 133 drug vendors, 71 and 60% had counterfeit artesunate and mefloquine respectively (Newton et al., 2006; Rozendal, 2000). A search of the medical literature yielded only 43 primary published research reports concerning counterfeit drugs in the world (Newton et al., 2006). Failing products more often originated or were claimed to originate from poorer parts of the world with weaker regulatory systems (Bate, 2008). Some researchers found 21 peer-reviewed articles and 3 reports on the quality of anti-malarial drugs in Africa with poor quality artemisinin monotherapies such as dihydroartemisinin been reported from formulations tested in Kenya (Amin, 2007). This study recommends a more frequent monitoring of the quality of products in the market.

Even though there is no case of outright counterfeiting from the results obtained in the study, the potency of the drugs vary considerably. Only 15.4% of the samples studied had required amount of active ingredients for the two drugs. It is clear that the quality of most brands of artesunate amodiaquine combined therapy in circulation in Lagos metropolis is less than adequate. This result can be extrapolated for other cities in the sub-Saharan Africa. This poses a great threat to the global effort to combat the scourge of malaria.

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