International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:10, No:3, 2016

Quantitative Assessment of Different Formulations of Antimalarials in Sentinel Sites of India

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***Abstract*—**Substandard and counterfeit antimalarials is a major problem in malaria endemic areas. The availability of counterfeit/ substandard medicines is not only decreasing the efficacy in patients, but it is also one of the contributing factors for developing antimalarial drug resistance. Owing to this, a pilot study was conducted to survey quality of drugs collected from different malaria endemic areas of India. Artesunate+Sulphadoxine-Pyrimethamine (AS+SP), Artemether-Lumefantrine (AL), Chloroquine (CQ) tablets were randomly picked from public health facilities in selected states of India. The quality of antimalarial drugs from these areas was assessed by using Global Pharma Health Fund Minilab test kit. This includes physical/visual inspection and disintegration test. Thin-layer chromatography (TLC) was carried out for semi-quantitative assessment of active pharmaceutical ingredients. A total of 45 brands, out of which 21 were for CQ, 14 for AL and 10 for AS+SP were tested from Uttar Pradesh (U.P.), Mizoram, Meghalaya and Gujrat states. One out of 45 samples showed variable disintegration and retension factor. The variable disintegration and retention factor which would have been due to substandard quality or other factors including storage. However, HPLC analysis confirms standard active pharmaceutical ingredient, but may be due to humid temperature and moisture in storage may account for the observed result.

***Keywords*—**Antimalarial medicines, counterfeit, substandard, thin layer chromatography.

I. Introduction

MALARIA is one of the major public health problems of the all over world. India reports around one million malaria cases annually [1]. In India, *P. falciparum* and *P. vivax* are the most common species causing malaria, their proportion being around 50% each. *P. vivax* is more prevalent in the plain areas, while *P. falciparum* predominates in forested and hilly areas [2]. According to national drug policy, *P. vivax* cases should be treated with chloroquine (25 mg/kg) followed by primaquine (0.25 mg/kg) for 14 days. Artemisinin Combination Therapy (ACT) should be given to all the confirmed *P. falciparum* cases [3]. ACT consists of an artemisinin derivative combined with a long acting antimalarial (amodiaquine, lumefantrine, mefloquine, piperaquine or sulfadoxine-pyrimethamine). Combination antimalarial therapy is advocated to improve treatment efficacy and limit selection of drug-resistant parasites. The artesunate plus sulfadoxine-pyrimethamine (AS+SP) ACT is recommended as the first line antimalarial for treatment of P. falciparum malaria in the National Programme all over the country except northeastern states, where fixed dose combination (FDC) of Artemether-lumefantrine (AL) is being recommended since May 2013 [4]. Patients who are considered to have severe malaria are treated with parenteral antimalarial therapy or Quinine, depending upon the availability at the health facility. In public health facilities, due to its availability, quinine remains the best choice of drug for severe malaria. However, artesunate injections have been used for the treatment of malaria with high severity.

For complete cure of the disease, correct dosage as well as duration is essential. However, sometimes, chemical break-down of some drugs can occur due to poor storage conditions, especially in warm and humid tropical climates. The amount of the active ingredient can also vary due to lack of regulations and poor quality control practices. Poor quality drugs are vital public health problem [5]. Previous reports have identified the problem of fake antimalarials particularly in South East Asia regions [6]. It has also been reported that in Ghana, only 3 (17.6%) of the samples of artesunate met the European Pharmacopoeia (Ph. Eur.) content requirements while 14 (82.4%) were found to be of substandard quality [7].

Counterfeit drugs are category of substandard drugs that are deliberately and fraudulently mislabeled for profit. Counterfeit medicines may include products with correct ingredients but fake packaging with the wrong ingredients or insufficient active ingredient [5]. These medicines may be ineffective and potentially dangerous. Such medicines could be circulating in the market. Hence, it is essential to monitor the quality of antimalarials circulating in public and private health system.

The availability of counterfeit antimalarial drugs is a major hurdle in effective disease controlling strategies in malaria endemic areas. The aim of this study was to determine the prevalence of poor quality antimalarials drugs in different malaria endemic areas of India and rapid assessment of these combinations i.e. Artesunate+Sulphadoxine-Pyrimethamine (AS+SP), Artemether-Lumefantrine (AL), Chloroquine (CQ) by using global pharma health fund (GPHF) minilab kit. The drugs those found to be fake was quantitatively analyzed by screening methods (High Performance Liquid Chromatography).

II. Materials and Methods

The survey was conducted in different geographical regions of Uttar Pradesh (U.P.), Mizoram, Meghalaya and Gujrat. Antimalarial samples of ACT (Artesunate+Sulphadoxine-pyremethamine), (Artesunate+Lumefantrine), Chloroquine were collected for qualitative analysis. Collection of samples was done during the period of July 2015 to Jan 2016, spanning a time period of more than six months in a year from all the

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above mentioned regions. All the details of sample procurement, storage and labeling were maintained.

A total of 45 brands of which, 21 were of CQ, 14 for AL and 10 for AS+SP were collected from the study sites. The details of samples include product name, manufacturers, manufacturing & expiry dates, dosage form, package size, packaging material & strength etc which were recorded separately.

Samples of antimalarials were collected from all study sites and were transported to the reached in testing lab for further processing where their qualitative analysis was performed by mini lab kit which included visual inspection, disintegration time and color reaction followed by TLC. These procedures were primarily used as screening methods. The GPHF-Minilab kit relies on a combination of accessible techniques for simple, fast and reliable detection of falsified and substandard drugs. Kit contains all the lab ware, reagents and standards for comparison of running antimalarials with the standards given in the kit.

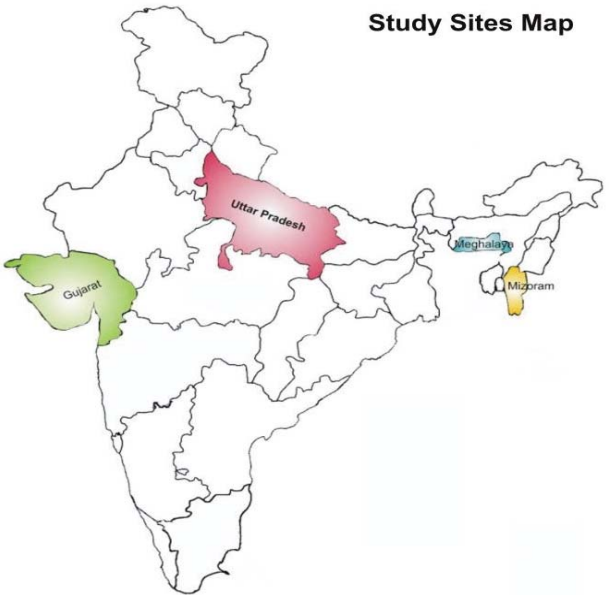

Fig. 1 Map depicting the sample collection sites

A. Visual Inspection

Firstly, visual inspection was performed for all collected samples. In this packaging, holograms, barcodes of drugs were observed and under magnification, lettering on a legitimate or fake blister pack became clear. Colour, shape, and size of samples were also observed. Visual inspection alone is not an adequate technique to test the quality of drug [8].

B. Disintegration Test

Secondly, each collected sample underwent disintegration test - means measures how rapidly solid dosage forms disintegrate in solution i.e. distilled water at (37°C) and is quite simple. Following this, a color reaction test was performed for each sample to check the presence of ingredients /salts mentioned on the blister pack.

C. Colorimetric Test

Colorimetric test is one such technique which relies on chemicals that undergo color change when reacted with certain compounds to provide qualitative data about a drugs identity. A- Fast Red TR for further identification: Turning yellow colour of sample indicates the presence of artesunate. B- turning reddish brown color of sample indicates the presence of Artemether + lumefantrine and Chloroqunine.

D. Thin Layer Chromatography (TLC)

After checking the presence of compound in corresponding drug by FTR, each sample was analyzed by TLC. Minilab attempts to simplify the analysis by providing reference tablets which can be used to prepare 100% and 80% dosage strengths for comparison [10].

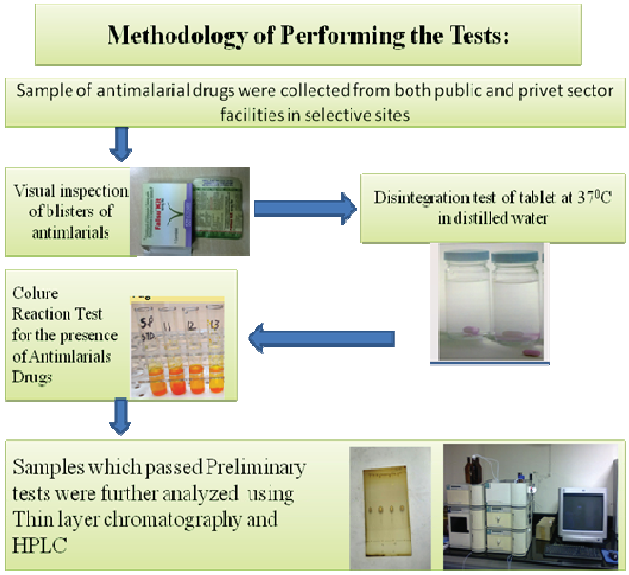

Fig. 2 Procedure used for qualitative and quantitative assessment of antimalarials

TLC was performed on a sheet of glass/plastic/aluminium foil and (a thin layer of adsorbent) usually silica gel, aluminium oxide or cellulose was coated on it in the form of thin layer which act as stationary phase. After this, standards of 100% and 80% were applied to the plate at a distance of 1.5" from bottom of TLC plate. 100% was applied on left edge and 80% on right edge of plate and in between these two samples were applied. A development chamber having solvent mixture in it acts as the mobile phase. When plate was kept in the chamber, the solvent mixture was drawn up to the plate via capillary action. It was allowed to run up to ¾ of the plate. Following this, the plate was removed out of the chamber and then was allowed to dry and observations were taken and recorded for the movement of sample (drug to be analyzed) on the stationary phase [2].

E. High Performance Liquid Chromatography (HPLC)

Further, samples showing discordant results were sent for further analysis using sophisticated technique like High

Performance Chromatography (HPLC). The HPLC was used to verify the results obtained using Mini lab kit (HPLC was outsourced to Shri Ram Institute of Industrial Research Institute, New Delhi).

III. Results and Discussion

The collected samples were qualitatively analyzed by GPHF minilab kit. All the samples passed for their physical test, disintegration test and colour tests except one sample. 44 samples passed preliminary Qualitative TLC test when compared with 100% and 80% of the standards but for further reassurance one sample which showed improper disintegration and decreased retention factor was further analyzed using sophisticated techniques like High performance liquid chromatography. Results obtained from HPLC showed that the quality met with the standard and claims the limit. This shows the problem of storage as high humidity or variable storage temperature might have changed property of the excipients used in the tablet. The HPLC data in accordance with the previous reports (Table III).

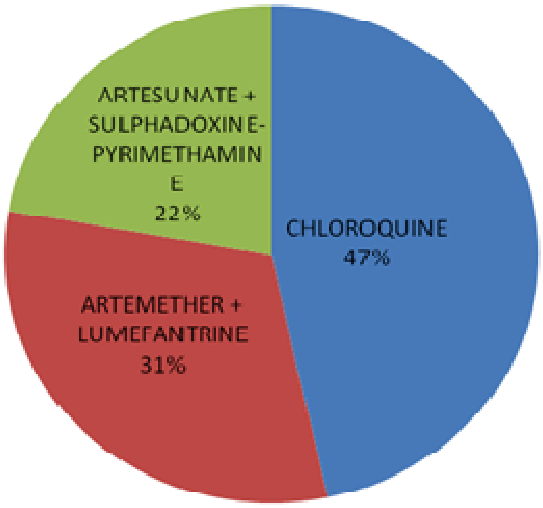

Fig. 3 Percentage of anti-malarial drugs collected

TABLE I
Categories of Collected Antimalarial Medicine

Name of drug	Number of samples analyzed	Sample from manufacturing companies / brands
Chloroquine	21	7
Artemether + Lumefantrine	14	4
Artesunate + Sulphadoxine-Pyrimethamine	10	4
Total	45	15

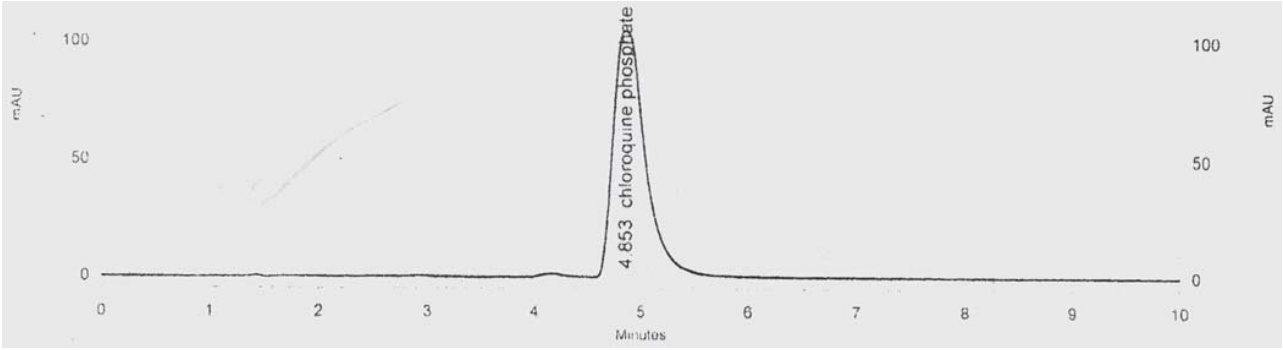

Fig. 4 HPLC Chromatogram

TABLE II
Chloroquine Tablet Showing Retension Time

Name	Retension Time	Area	Area%	Height
Chloroquine phosphate	4.853	4573303	100.00	219292
Total		4573303	100.00	219292

TABLE III
HPLC Data Showing the Amount of API Present in the Tablet

Assay	Result	Claim	Limit
Each film coated tablet on an average contains Chloroquine	237 mg (complies)	250 mg	231.3-268.8 mg

IV. Conclusion

The results suggest likely existence of variable storage conditions for antimalarials. This may further lead to deterioration of active pharmaceutical ingredient used in the tablets. However, more number of samples and detailed analysis using more sophisticated techniques like LCMS including HPLC is required to confirm the quality assessment of different antimalarials circulating in the market.

Acknowledgment

We are highly thankful to Indian Council of Medical Research (ICMR) for providing Post-Doctoral fellowship (PDF).

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