EAS Journal of Parasitology and Infectious Diseases
Abbreviated Key Title: EAS J Parasitol Infect Dis
ISSN: 2663-0982 (Print) & ISSN: 2663-6727 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-4 | Issue-5 | Nov-Dec- 2022 | DOI: 10.36349/easjpid.2022.v04i05.002

Original Research Article

Efficacy of Antimalarial Drugs and Modulations of Human Intestinal Bacteria Flora

Ukwah B.N[1*]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

Article History
Received: 28.10.2022
Accepted: 03.12.2022
Published: 17.12.2022

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: ***Background & Objectives:*** Oral anti-malarial therapy has been associated with varied gastrointestinal disorders with limited information on the effects on intestinal microflora. This study investigated the effects of chloroquine, Amodiaquine and Artesunate-Amodiaquine therapies on human intestinal lactic acid bacteria flora along with the anti-malaria efficacies and bowel functions. ***Methodology:*** Venous blood samples were collected from 30 human subjects of both males and females, aged between 20 and 30 years. Malaria parasitemia was determined along with faecal lactic acid bacteria (LAB) population prior to and after 3 days of oral administration of anti-malaria drugs. ***Results:*** The three anti-malaria drugs showed significant ($P<0.05\%$) malaria parasite clearance with Artesunate-Amodiaquine being the most effectives at 67.3%, whereas chloroquine (32.9%) was the least. However, chloroquine showed highest stimulation of the intestinal LAB population (23.6%), whereas Artesunate-Amodiaquine significantly ($p<0.05$) reduced the mean LAB numbers by 87.3%. The Chi-Square (X^2) analysis showed significant ($p<0.05$) association between the increase in faecal volume and LAB population among subjects administered the drugs. ***Conclusion*:** The anti-plasmodia efficacies of chloroquine and amodiaquine with mild effects on intestinal probiotic population indicate the beneficial use as anti-malaria prophylaxis, whereas artesunate-amodiaquine with high anti-malaria and anti-probiotic activities would be best for treatment of acute malaria.

Keywords: Anti-Plasmodia Drugs, Lactic Acid Bacteria, Probiotics, Malaria Parasites, Bowel Function.

INTRODUCTION

Malaria is one of the highest global health challenges and has contributed immensely to disease burden worldwide especially in the tropical Africa (Adebola and Olugbemiro, 2014). In order to reduce the burden of malaria in the tropics especially sub-Saharan Africa, different control measures has been adopted including development of anti-malarial drugs with variable mode of actions. Studies have shown that one of the most important drawbacks to successful control of malaria is development of resistance by *Plasmodium* species to commonly used anti-malarial drugs (Asante *et al.,* 2009). Increase in resistance to anti-malarial monotherapies has led to increased use of Artemisinin-based combination therapy (ACT) for the treatment of malaria. ACT including artesunate-amodiaquine (AS+AQ) combination therapy has been shown to be highly effective in the treatment of acute uncomplicated malaria and reduces the risk of resistance by *Plasmodium* species and thus recommended by WHO as first line of drugs for treatment of malaria (Majori, 2004; Adjei *et al.,* 2008; Asante *et al.,* 2009).

Anti-malarial drugs have been associated with a lot of side effects including diarrhea, nausea, noisy stomach, cramps, bloating, flatulence etc (Lee *et al.,* 2017). The signs and symptoms experienced by patients taking anti-malarial drugs vary by individuals and the type of anti-malarial drug. Some of these signs and symptoms have been associated with increase or decrease in the population of intestinal microflora such as lactic acid bacteria (Canny and McCormick, 2008; Ezeonu and Ukwah, 2009). Lactic acid bacteria (LAB) otherwise called probiotics are beneficial bacteria that have been found to be normal flora in different parts of human body including the gut and have contributed greatly in revitalizing both human and animal health (Crittenden, 2003; Ezeonu *et al.*, 2016). The activities of the LAB have been found to improve the bowel

***Corresponding Author:** Ukwah B.N
Department of Medical Laboratory Science, Faculty of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

functions, boost immunity and prevent establishment of pathogens (Crittenden, 2003; Chakraborti, 2011).

There are has been several reports on these adverse effects of anti-malarial drugs on patients' bowel function but little/none has been associated with effects on the population of intestinal beneficial bacterial flora. A good knowledge of the effects of anti-malarial drugs on intestinal LAB population and malaria parasites clearance could help in the choice of drugs for treatment of malaria or prophylaxis.

This study evaluated the effects of commonly used mono-therapeutic and ACT-based anti-malarial drugs including chloroquine, amodiaquine and artesunate-amodiaquine on the population of LAB and the efficacies in malaria parasite clearance in human volunteers.

MATERIALS AND METHODS

Study Subject

The study was conducted by selecting volunteers within the environs of Ebonyi State University, Abakaliki, Nigeria (Presco campus Annex), who tested positive to malaria parasite prior to the commencement of the study. The subjects were made up of 15 males and 15 females, aged between 20 and 30 years divided into three groups (A, B and C). Each group was made up of 10 subjects (5 males and 5 females). The subjects weighed between 48 kg and 62 kg with mean body weight of 58.5 kg.

Drug Administration

The group A was given chloroquine (500 mg) supplied as 2-2-1 Nivaquine-forte (May and Baker Pharmaceuticals, Nigeria). Two capsules of these were administered once daily for two days and 1 capsule for another one day making a total of 5 capsules in 3 days. Group B was given amodiaquine supplied as camoquin (Pfizer, pharmaceutical company) (200 mg), administered as 3 tablets once daily for 3 days. Group C was given Artemisin-based Combination Therapy (ACT) consisting of Artesunate-Amodiaquine supplied as camosunate (Geneith Pharm) (200mg/600mg) respectively (four tablets were administered once daily for three days).

Sample Collection

Venous blood and faecal samples were collected two times in the course of this study. Prior to and 3 days after the administration of anti-malaria drugs, 4 ml of venous blood and 2 g of faecal samples were collected from each subject. The venous blood were immediately transferred into EDTA container and preserved at 4ºC prior to analysis. The faecal samples were collected with a clean and dry plastic pole and 2 g were transferred into clean-dry universal container.

Inclusion and Exclusion Criteria

All the subjects were asked to avoid intake of antibiotics and alcohol during the study period. Also volunteers that found to have taken antibiotics/antimalaria 1 month prior to the study were dropped. Volunteers that tested positive for malaria parasites with at least 1500 parasites per microliters (µl) of blood and within the age group of 20 to 30 years were selected for the study.

Data Collection

All inclusion and exclusion information was obtained by oral interview of the volunteers. Also, information on the bowel function was obtained from the subjects prior to drug administration and after treatment (i.e on the sampling days).

Ethical Consideration

Ethical approval for the study was obtained. Informed consents of the subjects were obtained and each subject was asked to sign the consent form as a confirmation of acceptance to participate in the study.

Laboratory Analysis of Samples

Determination of Malaria Parasitemia

Parasitemia assessment was done using a thick film stained with Fields A and B. A minimum of 200 WBC were counted per slide of a sample. Malaria parasite density was determined by dividing the number of parasites counted with the number of WBCs, then multiplied by 8000 WBCs = parasites/µl of blood. Blood films were considered negative if no parasites were seen in 100 oil-immersion fields in a thick blood film (WHO, 2004; Cheesbrough, 2005).

Identification and Enumeration of Faecal Lactic Acid Bacteria

All the faecal samples were processed within 3 h of collection. One gram of the faecal sample was homogenized in 9 ml of sterile phosphate buffered saline. The emulsified faecal samples were serially diluted in 10-folds in sterile phosphate buffered saline. Thereafter, 0.01 ml of the 10^{-5} dilution was inoculated onto two sets of replicate agar media; De Man, Rogosa and Sharpe agar (Oxoid, Germany). The media were prepared according to the manufacturers' instructions. The culture plates were incubated anaerobically at 37ºC for 48 h.

The isolated lactic acid bacteria were identified and enumerated by conventional microbiological methods including cultural morphology, Gram staining reaction, catalase and biochemical reactions including API 50 CH (Biomerieux, USA).

Statistical Analysis

The data generated from the laboratory were analyzed using GenStat Discovery Edition 4 software.

The difference between the effects of the tested anti-plasmodial drugs on lactic acid bacterial population and malaria parasite clearance rate was determined using two-way ANOVA. Chi-Square test was used to determine the association between bowel function and increase in population of intestinal lactic acid bacteria using SPSS version 20. Significance was determined at 95% confidence level for all analysis. Standard deviation was used to determine the level of dispersion with each group of data.

RESULTS

Malaria Parasitemia of Subjects before Anti-Plasmodial Administration

Out of the 30 subjects that participated in the study, 10 subjects had parasitemia of 3000 to 4999 parasites/µl of blood with mild malaria effects, whereas 15 subjects had parasitemia between 5000 to 6999 parasites/µl of blood and was found to present severe malaria signs and symptoms, while the remaining 5 subjects that was presenting severe malaria signs and symptoms had parasites between 7000 to 8720 parasites/µl of blood. The drugs were administered to the subjects based on the previous history of non-adverse reactions to the anti-malaria drugs. All the subjects had significant recovery from the malaria with significant reduction in signs/symptoms and parasitemia after complete administration of the anti-plasmodial drugs.

Identified Intestinal Bacteria Flora (LAB)

Twenty-two subjects out of the 30 volunteers (73.3%) harbored LAB which increased in population after 3 days of treatment and were identified as *Lactobacillus* species in 9/30 subjects, *Enterococcus* species in (11/30) and the remaining 2 subjects harbored both *Lactobacillus* species and *Enterococcus* species in the guts. Eight of the subjects (8/30 representing 26.7%) had reductions in the total faecal LAB numbers and were identified as *Lactobacillus* spp and *Enterococcus* species in 5 subjects (5/30), whereas 3 of the subjects (3/30) harbored *Enterococcus* species only (Table 1).

Reported/Observed Effects of the Anti-Malarial Drugs on LAB and Gut Function

Out of the 10 subjects administered chloroquine, 9 subjects had increased LAB population and soft stool passage with increased frequency of faecal passage and volume. Of the 9 subjects, 2 reported noisy stomach in addition. One of the 10 subjects did not notice any observable change in bowel function, harbored *Enterococcus* spp in the gut which decreased after treatment (Figure 1). For those administered with amodiaquine, 7 subjects reported soft stool with increased LAB population, faecal frequency and volume. The remaining 3 subjects harbored *Enterococcus* spp with decreased LAB populations after treatment (Figure 1). In group administered AS+AQ, 4 subjects harbored predominantly *Lactobacillus* spp with non-significant increase in LAB numbers characterized with passage of soft stool in the first 2 days of treatment with no observable increase in faecal frequency. Of the 4 subjects, 3 reported constipation which lasted for a day during treatment period. Six of the subjects with significant decrease in LAB numbers after treatment harbored mainly *Enterococcus* species in the gut (Table 1 and Figure 1).

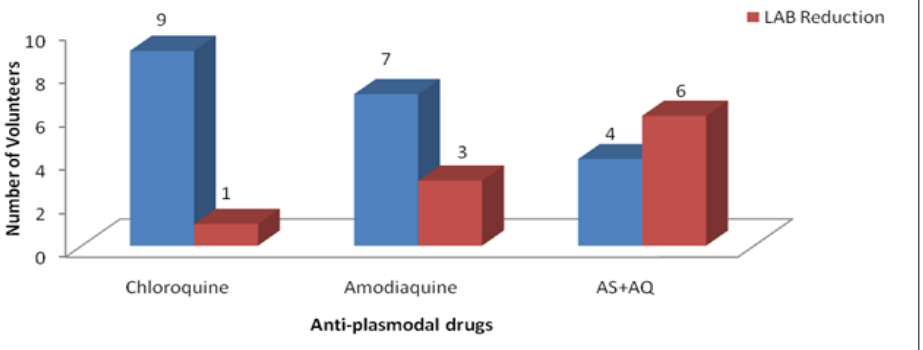

Figure 1: Effects of Anti-plasmodial drugs on Total LAB Population in the Gut of Human volunteers

Table 1: Distribution of Intestinal Bacteria (LAB) species among Subjects Administered Anti-malarial Drugs (n=30)

LAB isolated from subjects	*Lactobacillus* species	*Enterococcus* species	*Enterococcus/ Lactobacillus* species
Anti-malarial			
Chloroquine	5	3	2
Amodiaquine	5	5	0
Artesunate-Amodiaquine	2	3	5

Anti-Plasmodia and Probiotic Effects of Chloroquine, Amodiaquine and Artesunate-Amodiaquine

Ten subjects including males and females with mean age of 27.6±2.21 years and body weight of 57.4±2.01 kg were administered chloroquine. The chloroquine significantly ($p<0.05$) cleared the malaria parasites after 3 days of treatment with mean difference (2005) > $LSD_{0.05}$ (1046.2) at 32.9% malaria parasite clearance rate. However, the intestinal lactic acid bacteria numbers significantly ($p>0.05$) increased by 56.7% with mean difference (0.76) > $LSD_{0.05}$ (0.375) (Table 2 and 3). The subjects that were administered amodiaquine had mean body weight of 58.1±1.8 kg with mean age of 28.05±1.2 years. The amodiaquine significantly ($p<0.05$) cleared malaria parasites load by 36.3% with mean difference (2101) > $LSD_{0.05}$ (1046.2). On the other hand, the population of intestinal lactic acid bacteria increased non-significantly by 18.1% among the group administered amodiaquine with mean difference (0.278) < $LSD_{0.05}$ (1.375) (Table 2 and 3). The group C was administered artesunate-amodiaquine combination therapy. The subjects in this group had a mean age of 27.74±2.31 years with average body weight of 58.05±1.05 kg. This group had a significant ($p<0.05$) reduction in malaria parasites load (67.3%) after three days of treatment as compared to the pre-treatment period with mean difference (3669) > $LSD_{0.05}$ (1046.2). The lactic acid bacteria numbers in this group also decreased significantly ($p<0.05$) after treatment by 62.3% with mean difference (2.62) >$LSD_{0.05}$ (1.375) (Table 2 and 3).

Comparative evaluation of the anti-plasmodia efficacies of chloroquine and amodiaquine showed that chloroquine (CQ) and amodiaquine (AQ) differed non-significantly ($p>0.05$) in malaria parasite clearance [mean difference (323 < $LSD_{0.05}$ (739.8)]. Also, the two drugs encouraged increase in population of LAB and differed significantly ($p<0.05$) with mean difference (4.04) > $LSD_{0.05}$ (0.972) (Table 2 and Figure 1). However, AS+AQ showed significant ($p<0.05$) higher efficacy in malaria parasite clearance as compared with chloroquine (CQ) [mean difference (1474) > $LSD_{0.05}$ (739.8)] and amodiaquine mono-therapy (AQ) [mean difference (1151) > $LSD_{0.05}$ (739.8)]. The population of LAB was significantly ($p<0.05$) decreased when AS+AQ was administered as compared with chloroquine [1.18 > $LSD_{0.05}$ (0.972)] and amodiaquine [1.22 > $LSD_{0.05}$ (0.972)] which encouraged increase in LAB numbers (Table 3 and 4).

Table 2: **Anti-plasmodia Effects of Chloroquine, Amodiaquine and Artesunate-Amodiaquine**

Anti-plasmodia Drugs	Parameters	Pre-treatment effects	Post-treatment effects	Mean Difference	Percentage Change	Significance (p-value)
Chloroquine	M. Parasites (μl^{-1})	6090±1484.57	4085±1349.61	-2005**	-32.9%	P<0.05
Amodiaquine	M. Parasites (μl^{-1})	5815±857.16	3714±953.69	-2101**	-36.1%	P<0.05
Artesunate-Amodiaquine	M. Parasites (μl^{-1})	5449±1503.44	1780±396.12	-3669**	-67.3%	P<0.05

Key: **Significance=LSD<Mean Difference; - Decrease in number; + Increase in number

Table 3: **Effects of Chloroquine, Amodiaquine and Artesunate-Amodiaquine Therapies on Intestinal bacteria flora (LAB) population**

Anti-plasmodia Drugs	Parameters	Pre-treatment effects	Post-treatment effects	Mean Difference	Percentage Change	Significance (p-value)
Chloroquine	LAB No ($x10^5/g$)	1.34±0.375	2.1±0.827	+0.76**	+56.7%	P<0.05
Amodiaquine	LAB No ($x10^5/g$)	1.54±0.337	1.818±0.452	+0.278	+18.1%	P>0.05
Artesunate-Amodiaquine	LAB No ($x10^5/g$)	4.21±3.535	1.58±0.681	-2.63**	-62.5%	P<0.05

Key: **Significance=LSD<Mean Difference; - Decrease in number; + Increase in number.

Table 4: **Comparison of malaria parasite clearance rates and probiotic effects of the anti-plasmodia drugs**

Anti-plasmodia Drugs	Chloroquine	Amodiaquine	Artesunate-Amodiaquine	Mean Difference	Significance (p-value)
Parameters					
Clearance of Malaria Parasites (%)	-32.9%	-36.1%	NC	3.2	P>0.05
	-32.9%	NC	-67.3%	34.4**	P<0.05
	NC	-36.1%	-67.3%	31.2**	P<0.05
Effects on bacterial population (%)	+56.7%	+18.1%	NC	38.6**	P<0.05
	+56.7%	NC	-62.5%	5.8**	P<0.05
	NC	+18.1%	-62.5%	44.4**	P<0.05

****Significance=LSD<Mean Difference; - Decrease in number; + Increase in number; NC=Not compared**

DISCUSSION

Anti-malaria drugs such as chloroquine, artemesin-based products are by-products of plant or derivatives of plant products such as phenols or polyphenols (Rawe and McDonnell, 2020). Studies have shown that phenolic compounds have antimicrobial, anti-parasitic, anti-malaria effects, and can selectively stimulate increase in the population of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* (Azegbeze *et al.,* 2015; Evandro *et al*., 2019).

Findings of this study showed that all the malaria drugs administered including chloroquine, amodaquine and amodaquine+artesunate effectively eliminated malaria parasites within the 3 days of treatment (Tables 2 & 4). On the contrary, chloroquine and amodaquine increased the population of intestinal beneficial bacterial flora with a significantly ($p<0.05$) higher increase in the population of *Lactobacillus* and *Enterococcus* spp among subjects administered chloroquine. The finding of this study is in agreement with the reports of Asante *et al.*, (2009), which showed that anti-malaria drugs such as amodaquine+artesunate is highly effective in malaria treatment including complicated ones. It also agrees with the report of Evandro *et al.*, (2019), which showed that population and functionalities of probiotics such as *Lactobacillus* can be enhanced by phenolic compounds or its derivatives like amodaquine or chloroquine in order to improve health. In the same vein, the study of Rawe & McDonnell (2020) revealed that chloroquine and amodaquine are derivatives of cinchona alkaloids with anti-malaria effects and intestinal bacteria modulatory potentials. The ability of chloroquine and amodaquine to stimulate and increase the population of beneficial bacteria flora in the subjects is probably because they are derivatives of phenolic compounds from plants according to the report of Rawe & McDonnell (2020). Most phenolic compounds have oligosaccharides or carbohydrates side chains making it easier for possible selective breakdown by probiotic organisms such as *Lactobacillus* and *Enterococcus* spp as seen in this study. The combination of artesunate and amodaquine in this study significantly cleared the malaria parasites as well reduce the population of the intestinal bacteria flora. The effective clearance rate is probably due to its double mechanisms of actions which enhances oxidative destruction of parasitic and bacterial cells. The efficacy of the combined therapy as seen in this study is in line with the reports of Adjei *et al*., (2008) and Asante *et al*., (2009).

CONCLUSION

The findings of this indicated the importance of chloroquine and amodaquine in treatment of uncomplicated malaria and underscores the usefulness as prophylaxis in malaria prevention.

REFERENCES

- Adjei, G. O., Kurtzhals, J. AL, Rodrigues, O. P., Alifrangis, M., Hoegberg, L. C., Kitcher, E. D, Badoe, E. V., Lamptey, R., & Goka, B. Q. (2008). Amodiaquine-artesunate vs artemether-lumefantrine for uncomplicated malaria in Ghanaian children: A randomized efficacy and safety trial with one year follow-up. *Malaria Journal*, *7*(1), 127.
- Asante, K. P., Owusu, R., Dosoo, D., Awini, E., Adjei, G., Amenga Etego, S., Chandramohan, D., & Owusu-Agyei, S. (2009). Adherence to Artesunate-Amodiaquine Therapy for Uncomplicated Malaria in Rural Ghana: A Randomised Trial of Supervised versus Unsupervised Drug Administration [Review of *Adherence to Artesunate-Amodiaquine Therapy for Uncomplicated Malaria in Rural Ghana: A Randomised Trial of Supervised versus Unsupervised Drug Administration*]. *Journal of Tropical Medicine*, *2009*, 1–7.
- Azebaze A.G.B., J.E.M. Teinkela, E.L. Nguemfo, A.Valentin, A. B. Dongmo, J.C. Vardamides (2015). Antiplasmodial activity of some phenolic compounds from Cameroonians Allanblackia. *African Health Sciences,* 15(3): 835–840. *doi: 10.4314/ahs.v15i3.18*
- Canny, G. O., & McCormick, B. A. (2008). Bacteria in the Intestine, Helpful Residents or Enemies from Within? *Infection and Immunity*, *76*(8), 3360–3373.
- Chakraborti, C.K. 2011. The status of synbiotics in colorectal cancer. Life Science Medical Research, 2011: 1-13.
- Cheesbrough, M. 2005. District Laboratory Practice in Tropical Countries. Tropical Health Technology and Butterworth – Heinemann Ltd Cambridge Low price ed. Vol. 2, pp 63 70.
- Crittenden, R. G., Martinez, N. R., & Playne, M. J. (2003). Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria. *International Journal of Food Microbiology*, *80*(3), 217–222.
- Ezeonu, I., & Ukwah, B. (2009). In *vivo* studies on the prebiotic effects of *Vernonia amygdalina* leaf extracts. *Bio-Research*, *7*(1).
- Ezeonu, I. M., Asuquo, A. E., Ukwah, B. N., & Ukoha, P. O. (2016). IMMUNO-MODULATORY PROPERTIES OF PREBIOTICS EXTRACTED FROM Vernonia amygdalina. *African Journal of Traditional, Complementary and Alternative Medicines*, *13*(6), 11–17.
- Evandro L.S., Thatyane M.R. A., Aldeir S.D.S., Nayara M.L.M., Jose L.B.A (2019). Potential Interactions among Phenolic Compounds and Probiotics for Mutual Boosting of their Health-Promoting Properties and Food Functionalities-Review *Critical Review in Food Science and Nutrition,* 59(10): 1645-1659.

- Lee, S. J., ter Kuile, F. O., Price, R. N., Luxemburger, C., & Nosten, F. (2017). Adverse effects of mefloquine for the treatment of uncomplicated malaria in Thailand: A pooled analysis of 19, 850 individual patients. *PLoS ONE*, *12*(2).
- Majori, G. (2004). Combined antimalarial therapy using artemisinin. *Parassitologia*, *46*(1–2), 85–87.
- Orimadegun, A., & Sodeinde, O. (2014). Features and outcomes of malaria infection in glucose-6-phosphatedehydrogenase normal and deficient Nigerian children. *Journal of Vector Borne Diseases*, *51*(1), 33.
- Rawe, S. L., & McDonnell, C. (2020). The cinchona alkaloids and the aminoquinolines. *Antimalarial Agents*, 65–98.

Cite This Article: Ukwah B.N (2022). Efficacy of Antimalarial Drugs and Modulations of Human Intestinal Bacteria Flora. *EAS J Parasitol Infect Dis, 4*(5), 40-45.