Microbial Antibiotics as Antimalarials

Krishna Murthy P¹ and Vathsala PG²*

¹Department of Animal Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India
²Undergraduate Programme, Indian Institute of Science, Bangalore, Karnataka, India

*Corresponding Author: Vathsala PG, Principal Research Scientist, Undergraduate Programme, Indian Institute of Science, Bangalore, Karnataka, India.

Abstract

Malaria affects 250 - 300 million people in the world most of them are children below 5 years and pregnant woman and the mortality stands second among infectious diseases. With a vaccine not being available against malaria and the front line drugs such as chloroquine and antifolates registering widespread parasite resistance, the challenge of malaria treatment is a formidable aim. Now malarial drug therapy researchers are looking to discover new drugs which has become essential and very necessary to identify therapeutic strategies at the earliest. Among many approaches like finding a new drugs, look for some natural compounds or using drugs used for other diseases like microbial antibiotics, curcumin and garlic. Many groups are trying to treat malaria by using these drugs singly or in combination. Due to occurrence of resistant for all existing antimalarials according to WHO norms researchers are following the combination therapy for malaria specially with Artemisinin (ACT). Combination therapy has assumed considerable importance in the context of artemisinin derivatives being the sole, tested, efficacious antimalarials left in the basket. Now researchers are trying to use combination of antibiotics like tetracycline, rifampicin, triclosan, thiolactomycin, cotrimoxazole and many others for its antimalarial activity specially against drug resistant malaria. This review aims to show some of the microbial antibiotics used as an antimalarial and their targets with respect to malarial parasite life cycle.

Keywords: Malaria; Drug Resistant; Apicoplast; Antibiotics; Antimalarial Activity

Introduction

Malaria is a tropical mosquito-borne disease caused by a protozoan, *Plasmodium*. Studies in 2010 showed that mortality among children below 5 years of age in Sub-Saharan Africa (SSA) is at 50% comparative to previous estimation, with time trends since 2000 [1]. *Plasmodium* species related incidences are at 214 million and mortality at 4,29,000; most of them are children below 5 years of age and pregnant women being under risk, especially in African regions [2]. Due to widespread parasite drug resistance, effective malaria treatment is a daunting task. Statistical analysis of the Indian field samples of malaria parasites shows resistance, close to 90%, towards Chloroquine treatment [3-5]. Despite extensive efforts, the incidence and intensity of the disease is not decreasing at an expected rate in most disease endemic regions of the world. With the deficient control programmes and non-availability of antimalarial vaccines, it has become necessary to identify alternative therapeutic strategies in the short-term. There are several diverse approaches that are currently being pursued around the world to develop new antimalarials to combat drug resistance. One approach is to examine whether known drugs used for other diseases can be used to treat malaria. Second option is to screen known compounds of natural origin for antimalarial activity, and third is the use of existing microbial antibiotics as antimalarials. For more than a decade now studies with special focus on compounds of plant origin, such as curcumin and garlic oil, for malaria treatment has been carried out [6,7]. This short review would focus on the role played by antibiotics in effective control and treatment of malaria.

For many years chloroquine was the mainstay of malaria treatment and control. However, resistance to chloroquine by *Plasmodium falciparum*, the parasite that causes the most severe form of malaria, has spread and intensified in almost all malaria-endemic areas. Moreover, *P. falciparum* has also developed resistance to other antimalarial drugs in use. For example, in Southeast Asian region, resistance has been reported to chloroquine, sulfadoxine/pyrimethamine (S/P), mefloquine, halofantrine and quinine, thereby, leaving combination therapies that include artemisinin (artemisinin-based combination therapy) [8] and its derivatives as the only effective treatment. In addition, there are several reports that show that use of antibiotics to be effective against malaria parasites [9,10]. In 1989, a chloroquine-resistant strain of *Plasmodium vivax* was reported in Papua New Guinea, which was the first time a non-*P. falciparum* malaria species had exhibited resistance to any major antimalarial drug. These reports are critical for helping nations to establish appropriate malaria treatment policies and ensure the availability of drugs that will be effective against the disease. In 1973, chloroquine resistance had been reported from several countries in South America and Asia, but not until the late 1970s from Africa [11]. Resistance to chloroquine in Africa has spread from east to west. In the most affected countries, the first line of treatment was changed from chloroquine to S/P only to find resistance emerging to this drug as well [12-14]. The efficacy of quinine has diminished in some areas, and low level of resistance is now widespread in parts of Southeast Asia. Decreased sensitivity to quinine is present in more than 50% of *P. falciparum* infections in Southeast Asian regions [15].

Though microorganisms such as bacteria are not a causative agent for malaria, still antibiotics which kill bacteria would be of therapeutic use. Recent studies have suggested the effectiveness of antibiotics against the parasite and efforts are on to identify the mode of action. The role of antibiotics in treating malaria came in to spotlight after the discovery of a chloroplast-like organelle of apicomplexan parasites, the apicoplast [16]. This plastid-derived structure, which originates from a secondary endosymbiotic event (hence 3 to 4 membrane structure) [17], maintains certain parasite-specific functions [18] and involved in important metabolic as well as housekeeping activities, including fatty acid [19] and heme biosynthesis [20]. Like the mitochondrion, the apicoplast has a separate, prokaryote-like genome (35kb) which is completely mapped and the widespread occurrence of the genome is observed over a range of malaria species [21]; this probably explains the antimalarial effect of a number of antibacterial compounds that otherwise do not have any outcome on eukaryotes. Apicoplast synthesizes 23 proteins but is also estimated to import over 500 nuclear-encoded proteins, representing ∼ 10% of the proteins encoded by the nucleus [22]. Import into the apicoplast is by a specific mechanism involving two amino-terminal targeting sequences [23,24].

Apicoplast is an indispensible organelle in the parasite; hence it represents a promising target for antibiotic malaria therapy. Antibiotics such as tetracycline, doxycycline and azithromycin that act on prokaryotic organisms shown to have antimalarial activity by preventing parasite growth through the inhibition of 'prokaryote like' protein biosynthesis in the apicoplast – that is unique to apicomplexan parasites [25,26]. Here we describe the effect of different antibiotics against malarial parasite and also their effect on antibiotic target, the apicoplast (Figure 1).
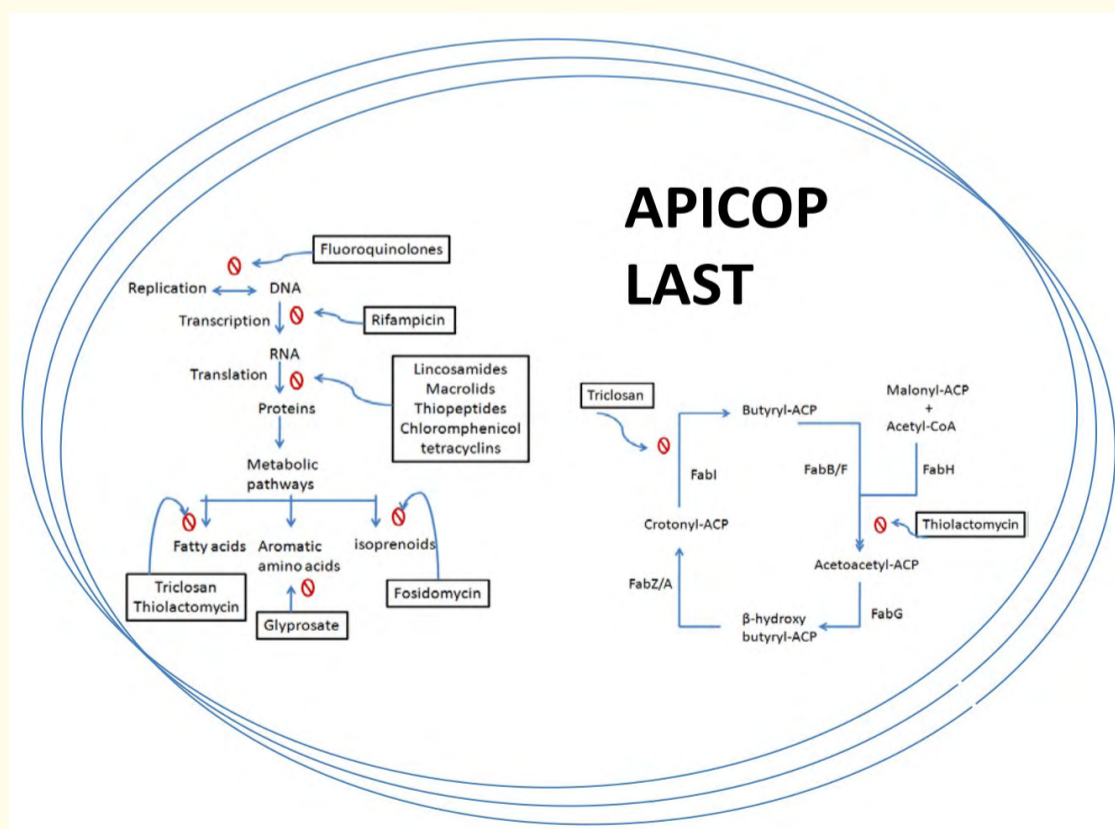

Figure 1: Antibiotics target in apicoplast of malaria parasite.

Presently, use of antibiotics as prophylaxis drug for malaria is the focus of many research groups. Two families of antibiotics that have been studied extensively are tetracyclines and macrolides and their derivatives [9,10]. Other drugs such as co-trimoxazole, quinolones, tigecycline, mirincamycin, ketolides, fusidic acid and thiopeptides are also studied. Antibiotics exhibiting antimalarial activity are listed in table 1.

No.	Existing antibiotics	Target molecule	Pathway/Mechanism
1	Tetracycline [27]	Apicoplast ribosome	Protein synthesis
2	Clindamycin [28]	DNA gyrase	DNA synthesis
3	Rifampicin [29]	RNA polymerase	RNA polymerase
4	Thiolactomycin [24]	Fab H	TypeII FAB
5	Triclosan [19]	Fab I/PfENR	Isoprenoid synthesis
6	Fosmidomycin [30]	DOXP reducto- isomerase	Translation
7	Ciprofloxacin [31]	DNA topoisomerase II	DNA replication
8	Doxycycline [32]	16S rRNA of apicoplast	Translation
9	Amythiamicin [33]	Elongation factor Tu	Translation
10	Azithromycin [34]	Not characterized	Translation
11	Thiostrepton [35]	Not characterized	Translation
12	Micrococcin [36]	Not characterized	Translation
13	Chloramphenicol [37]	Not characterized	Translation

***Table 1:** List of antibiotics exhibiting antimalarial activity.*

Tetracycline is a broad-spectrum antimicrobial drug that has potent but slow action against the asexual blood stages of all *Plasmodium* species. It is also active against the primary intra hepatic stages of *P. falciparum*. The combination of quinine plus tetracycline given over 5 - 7 days is still highly effective for treatment in areas of multidrug resistance in Thailand if adherence with the treatment regimen can be assured [38]. However, it should not be used in monotherapy because of its slow action. Tetracycline can be used in combination with quinine for treatment of falciparum malaria to decrease the risk of recrudescence. Doxycycline, like tetracyclines, can be used for therapy in combination with quinine in areas where resistance to quinine has been reported. Doxycycline in combination with mefloquine or artesunate has been used successfully in Thailand to treat multiresistant uncomplicated falciparum malaria. In contrast to tetracycline, doxycycline can also be used for chemoprophylaxis. Doxycycline prophylaxis is recommended in areas of mefloquine-resistant falciparum malaria [39] and has been used successfully in Cambodia and Somalia [40]. Triclosan is a chemical often referred to as a "biocide" instead of an "antibiotic". However, its mode of action seems to suggest that it is an antibiotic. Triclosan binds to bacterial enoyl-acyl carrier protein reductase (ENR) enzyme, which is encoded by the gene FabI. Co-trimoxazole is an antibacterial, antifungal [41] drug and reported to be effective as an antimalarial in combination with trimethoprim and sulfamethoxazole [42,43] but some reports have reported cross-resistance [44,45]. This drug has been studied for both prophylaxis and treatment of malaria [46,47]. Bactericidal quinolones are synthetic antibiotics and was discovered as a byproduct during the synthesis of chloroquine [48]. The drug shows significant anti-malarial activity and is reported to target gyrase enzyme of the parasite [49,50]. Mild antimalarial activity has been shown by norfloxacin, ofloxacin, pefloxacin, trovafloxacin and ciprofloxacin [41]. Ciprofloxacin works in synergy with artemisinin [51]. Acridinones, like quinolones, targeted to inhibit heme polymerization, as chloroquine does [52] and also blocks the transmission to mosquitoes by preventing oocysts development [53]. Tigecycline is a new class of antimicrobials, the glycylcyclines, which belong to the tetracycline class and its anti-malarial activity was first tested *ex vivo* [54]. The complete cure of malaria in *P. berghei*-infected mice with tigecycline in combination with a subcurative dose of chloroquine has been reported [55]. This drug has been used in ACT group with artesunate in Southeast Asia region [56] for treatment of complicated malaria. Mirincamycin is a lincosamide antibiotic similar to clindamycin that is synthetically produced. This older molecule was studied in 2009 on *P. falciparum* isolates from Gabon [57]. *In vitro* study on ketolides showed anti-plasmodial activity against chloroquine-susceptible and chloroquine resistant strains *of P. falciparum* [58]. Due to prokaryote-like gene expression in the apicoplast, several antibiotics which target DNA replication, transcription and translation in bacteria also kill the parasite [59]. Several malaria research groups have been working on microbial antibiotics which have shown a way for the development of antibiotic-derived drug resistant malaria therapy.

Conclusion

As reviewed in this article, serious attempts are underway all over the world to identify new drug targets in the malarial parasite and develop new antimalarials. With the recognition of deficiencies in control programmes based on insecticides and non-availability of vaccines, antimalarial drugs have acquired importance as a frontline control measure against malaria. While this effort is very essential, there is also a need to pursue alternative approaches that could, perhaps, lead to cheaper therapeutic options such as antibiotics. All these approaches have become very essential; since resistance is wide spread against all existing antimalarials and cheaper alternatives need to be looked at. Further studies should be promoted to assess the effects of antibacterials as antimalarials especially in artemisinin combination therapy, as the most apt partner drug to treat malaria.

Bibliography

1. L Liu., *et al.* "Global, regional, and national causes of child mortality: an updated systematicanalysis for 2010 with time trends since 2000". *Lancet* 379.9832 (2012): 2151-2161.

2. World health organization. "World malaria report-2016". WHO (2016).

3. Vinayak S., *et al.* "Prevalence of K76T mutation in the Pfcrt of Plasmodium falciparum among chloroquine responders in India". *Acta Tropica* 87.2 (2003): 287-293.

4. Vathsala PG., *et al.* "Widespread Occurrence of The Plasmodium falciparum Chloroquine Resistance Transporter (PFCRT) Gene Haplotype SVMNT In P. falciparum Malaria In India". *The American Journal of Tropical Medicine and Hygiene* 70.3 (2004): 256-259.

5. Pooja M., *et al.* "Progressive Increase in Point Mutations Associated with Chloroquine Resistance in Plasmodium falciparum isolates from India". *The Journal of Infectious Diseases* 193.9 (2006): 1304-1312.

6. Reddy RC., *et al.* "Curcumin for malaria therapy". *Biochemical and Biophysical Research Communications* 326.2 (2005): 472-474.

7. Vathsala PG., *et al.* "Assessment of in vivo antimalarial activity of arteether and garlic oil combination therapy". *Biochemistry and Biophysics Reports* 5 (2016): 359-364.

8. Tilley L., *et al.* "Artemisinin action and resistance in Plasmodium falciparum". *Trends in Parasitology* 32.9 (2016): 682-696.

9. Gaillard T., *et al.* "Tetracyclines in malaria". *Malaria Journal* 14 (2015): 445.

10. Gaillard T., *et al.* "Macrolides and associated antibiotics based on similar mechanism of action like lincosamides in malaria". *Malaria Journal* 15 (2016): 85.

11. D'Alessandro U and Buttiens H. "History and importance of antimalarial drug resistance". *Tropical Medicine and International Health* 16.11 (2001): 845-848.

12. Plowe CV., *et al.* "Sustained clinical efficacy of sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Malawi after 10 years as first line treatment: five year prospective study". *British Medical Journal* 328.7439 (2004): 545-549.

13. Talisuma AO., *et al.* "Efficacy of sulphadoxine-pyrimethamine alone or combined with amodiaquine or chloroquine for the treatment of uncomplicated falciparum malaria in Ugandan children". *Tropical Medicine and International Health* 9.2 (2004): 222-229.

14. Terlouw DJ., *et al.* "Sulfadoxine-pyrimethamine in treatment of malaria in Western Kenya: increasing resistance and underdosing". *Antimicrobial Agents and Chemotherapy* 47.9 (2003): 2929-2932.

15. Giboda M and Denis MB. "Response of Kampuchean strains of Plasmodium falciparum to antimalarials: In vivo assessment of quinine and quinine plus tetracycline multiple drug resistance in vitro". *The Journal of Tropical Medicine and Hygiene* 91.4 (1988): 205-211.

16. Kohler S., *et al*. "A plastid of probable green algal origin in apicomplexan parasites". *Science* 275.5305 (1997): 1485-1489.

17. Wilson RJM., *et al*. "Complete gene map of the plastid-like DNA of the malaria parasite Plasmodium falciparum". *Journal of Molecular Biology* 261.2 (1996): 155-172.

18. Roos DS., *et al*. "Mining the Plasmodium genome database to define organellar function: What does the apicoplast do?" *Philosophical Transactions of the Royal Society of London* 357.1417 (2002): 35-46.

19. N Surolia and A Surolia. "Triclosan offers protection against blood stages of malaria by inhibiting enoyl-ACP reductase of Plasmodium falciparum". *Nature Medicine* 7.2 (2001) 167-173.

20. N Suroliaand and G Padmanaban. "De novo biosynthesis of heme offers a new chemotherapeutic target in the human malarial parasite" *Biochemical and Biophysical Research Communications* 187.2 (1992) 744 -750.

21. Roberts F., *et al*. "Evidence for the shikimate pathway in apicomplexan parasites". *Nature* 393 (1998): 801-805.

22. Ralph SA., *et al*. "Metabolic maps and functions of the Plasmodium falciparum apicoplast". *Nature Reviews Microbiology* 2.3 (2004): 203-216.

23. Foth BJ and McFadden GI. "The apicoplast: A plastid in Plasmodium falciparum and other apicomplexan parasites". *International Review of Cytology* 224.57 (2003): 57-110.

24. Waller RF., *et al*. "Nuclear - encoded proteins target to the plastid in Toxoplasma gondii and Plasmodium falciparum". *Proceedings of the National Academy of Sciences of the United States of America* 95.21 (1998): 12352-12357.

25. Fichera ME and Roos DS. "A plastid organelle as a drug target in apicomplexan parasites". *Nature* 390.6658 (1997): 407-409.

26. Clough B and Wilson RJM. "Antibiotics and the plasmodial plastid organelle". In Antimalarial Chemotherapy: Mechanisms of Action, Resistance, and New Directions in Drug Discovery (ed. PJ. Rosenthal), Totawa, NJ: Humana Press (2001): 265-286.

27. EL Dahl., *et al*. "Tetracyclines specifically target the apicoplast of the malaria parasite Plasmodium falciparum". *Antimicrobial Agents and Chemotherapy* 50.9 (2006): 3124-3131.

28. EL Dahl and PJ Rosenthal. "Apicoplast translation, transcription and genome replication: targets for antimalarial antibiotics". *Trends in Parasitology* 24.6 (2008): 279-284.

29. M Strath., *et al*. "Antimalarial activity of rifampicin in vitro and in rodent models". *Transactions of the Royal Society of Tropical Medicine and Hygiene* 87.2 (1993): 211-216.

30. H Jomaa., *et al*. "Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs". *Science* 285.5433 (1999): 1573-1576.

31. R Gozalbes., *et al*. "Anti-Toxoplasma activities of 24 quinolones and fluoroquinolones in vitro: prediction of activity by molecular topology and virtual computational techniques". *Antimicrobial Agents and Chemotherapy* 44.10 (2000): 2771-2776.

32. DG Lalloo., *et al*. "UK malaria treatment guidelines". *Journal of Infection* 54.2 (2007): 111-121.

33. B Clough., *et al*. "Antibiotic inhibitors of organellar protein synthesis in Plasmodium falciparum". *Protist* 150.2 (1999): 189-195.

34. ABS Sidhu., *et al*. "In vitro efficacy, resistance selection, and structural modeling studies implicate the malarial parasite apicoplast as the target of azithromycin". *The Journal of Biological Chemistry* 282.4 (2007) 2494-2504.

35. GA McConkey., *et al.* "Inhibition of Plasmodium falciparum protein synthesis targeting the plastid-like organelle with thiostrepton". *The Journal of Biological Chemistry* 272.4 (1997): 2046-2049.

36. MJ Rogers., *et al.* "The antibiotic micrococcin is a potent inhibitor of growth and protein synthesis in the malaria parasite". *Antimicrobial Agents and Chemotherapy* 42.3 (1998): 715-716.

37. S Chaubey*., et al.* "The apicoplast of Plasmodium falciparum is translationally active". *Molecular Microbiology* 56.1 (2005): 81-89.

38. Looareesuwan S., *et al.* "Randomised trial of mefloquine-tetracycline and quinine-tetracycline for acute uncomplicated falciparum malaria". *ActaTropica* 57.1 (1994): 47-53.

39. Schwartz E and Regev-Yochay G. "Primaquine as prophylaxis for malaria in non-immune travellers: a comparison with mefloquine and doxycycline". *Clinical Infectious Diseases* 29.6 (1999): 1502-1506.

40. Shanks GD., *et al.* "Doxycycline for malaria prophylaxis in Australian soldiers deployed to United Nations missions in Somalia and Cambodia". *Military Medicine* 160.9 (1995): 443-445.

41. Fehintola FA. "Cotrimoxazole, clinical uses and malaria chemotherapy". *African Journal of Medicine and Medical Sciences* 39.1 (2010): 63-68.

42. Sowunmi A., *et al.* "Effects of antifolates-co-trimoxazole and pyrimethamine- sulfadoxine on gametocytes in children with acute, symptomatic, uncomplicated, Plasmodium falciparum malaria". *Memórias do Instituto Oswaldo Cruz* 100.4(2005): 451-455.

43. Omar SA., *et al.* "Co-trimoxazole compared with sulfadoxine-pyrimethamine in the treatment of uncomplicated malaria in Kenyan children". *Transactions of the Royal Society of Tropical Medicine and Hygiene* 95.6 (2001): 657-60.

44. Iyer JK., *et al.* "Plasmodium falciparum cross-resistance between trimethoprim and pyrimethamine". *Lancet* 358.9287 (2001): 1066-1067.

45. Triglia T., *et al.* "Mutations in dihydropteroate synthase are responsible for sulfone and sulfonamide resistance in Plasmodium falciparum". *Proceedings of the National Academy of Sciences USA* 94.25 (1997): 13944-13949.

46. Manyando C., *et al.* "Safety and efficacy of co-trimoxazole for treatment and prevention of Plasmodium falciparum malaria: a systematic review". *PLoS ONE* 8.2 (2013): 56916.

47. Suthar AB., *et al.* "Co-trimoxazole prophylaxis in adults, including pregnant women, with HIV: a systematic review and meta-analysis". *Lancet HIV* 2.4 (2015): e137-50.

48. Lesher GY., *et al.* "1,8-naphthyridine derivatives. A new class of chemotherapeutic agents". Journal of Medicinal Chemistry 5.5 (1962): 1063-5.

49. Dubar F., *et al.* "Ester prodrugs of ciprofloxacin as DNA-gyrase inhibitors: synthesis, antiparasitic evaluation and docking studies". *MedChemCommun* 2 (2011): 430-435.

50. Beteck RM., *et al.* "Recent progress in the development of anti-malarial quinolones". *Malaria Journal* 13 (2014): 339.

51. Goodman CD., *et al.* "The effects of anti-bacterials on the malaria parasite Plasmodium falciparum". *Molecular and Biochemical Parasitology* 152.2 (2007): 181-191.

52. Agarwal D., *et al.* "In vitro synergistic effect of fluoroquinolone analogues in combination with artemisinin against Plasmodium falciparum their antiplasmodial action in rodent malaria model". *Malaria Journal* 14 (2015): 48

53. Fernández-Calienes A., *et al.* "Antimalarial activity of new acridinone derivatives". *Biomedicine and Pharmacotherapy* 65.3 (2011): 210-214.

54. Saenz FE., *et al.* "4-(1H)-quinolones and 1,2,3,4-tetrahydroacridin-9(10H)-ones prevent the transmission of Plasmodium falciparum to Anopheles freeborni". *Antimicrobial Agents and Chemotherapy* 57.12 (2013): 6187-6195.

55. Starzengruber P., *et al.* "Antimalarial activity of tigecycline, a novel glycylcycline antibiotic". *Antimicrobial Agents and Chemotherapy* 53.9 (2009): 4040-4042.

56. Sahu R., *et al.* "In vitro and in vivo anti-malarial activity of tigecycline, a glycylcycline antibiotic, in combination with chloroquine". *Malaria Journal* 13 (2014): 414.

57. Ashley EA., *et al.* "Spread of artemisinin resistance in Plasmodium falciparum malaria". *The New England Journal of Medicine* 371.5 (2015): 411-423.

58. Held J., *et al.* "In vitro activity of mirincamycin (U24729A) against Plasmodium falciparum isolates from Gabon". *Antimicrobial Agents and Chemotherapy* 54.1 (2010): 540-542.

59. Makgatho M., *et al.* "Ketolide agents HMR 3004 and HMR 3647 (telithromycin) inhibit the growth of Plasmodium falciparum in vitro". *African Health Sciences.* 15.4 (2015): 1271-1276.