Endemic Infectious Diseases in Pregnant Women in Central African Gabon
Epidemiology and Evaluation of new Interventions

Dissertation

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Abbreviations

WHO	World health organization
P. falciparum	*Plasmodium falciparum*
P. vivax	*Plasmodium vivax*
P. malariae	*Plasmodium malariae*
P. ovale	*Plasmodium ovale*
ACT	Artemisinin-based combination therapy
MIP	Malaria in pregnancy
IPTp	Intermittent preventive treatment in pregnancy
SP	Sulfadoxine/Pyrimethamine
$T_{1/2}$	The elimination half-time
MICs	Minimal inhibitory concentrations
LCV	*Lymphocryptovirus*
RDV	*Rhadinovirus*
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
EUCAST	the European committee on anti-microbial susceptibility testing
MQ	Mefloquine
HPLC	High performance liquid chromatographic
E. coli	*Eschrerichia coli*
E. faecalis	*Enterococcus faecalis*
N. gonorrhoeae	*Neisseria gonorrhoeae*
S. aureus	*Staphylococcus aureus*
S. agalactiae	*Streptococcus agalactiae*
S. pneumonia	*Streptococcus pneumonia*
NAD	Nicotinamide Adenine Dinucleotide
E. granulosus	*Echinococcus granulosus*
IHA	Indirect hemagglutination
IFA	Indirect fluorescent antibody
EIA	Enzyme immunoassay
IgG	Immunoglobulin G
CT	Computed tomography
MRI	Magnetic resonance imaging
GBS	Group B Streptococcus

DNA	Dioxyribonucleararacid
PCR	Polymerase Chain Reaction
LANA	Latent antigen
HHV8	Human Herpes Virus 8
FITC	Fluorescein isothiocyanate

Abstract

Malaria is one of the most important infectious diseases with high morbidity rates particularly in young children and in pregnant women in Sub-Saharan Africa. Intermittent preventive treatment in pregnancy (IPTp) is an effective way to reduce morbidity of malaria in pregnancy and to reduce neonatal morbidity. Sulphadoxine/pyrimethamine (SP) is the recommended drug for IPTp in sub-Saharan Africa. Because of increasing resistance against SP there is a need to develop alternative drugs to replace SP for IPTp. Among the most promising candidate drugs in clinical development are mefloquine and azithromycin. Besides their antimalarial activity, these antimalarial drugs show significant antibacterial activity when given as IPTp. This antibacterial activity may offer several additional public health benefits when used as IPTp in pregnant women. These drugs may– besides protecting from malaria in pregnancy - treat and prevent often undetected sexually transmitted diseases (STDs) and urinary tract infections (UTIs).

In this thesis, three studies are presented focusing on infectious diseases highly prevalent in sub-Saharan Africa. In the first study, the activity of mefloquine and azithromycin was assessed *in vitro* in comparison to sulphadoxine-pyrimethamine against bacterial pathogens with clinical importance in pregnancy. Results of this study demonstrated that SP was highly active against *Staphylococcus aureus* and *Streptococcus pneumoniae*. All tested Gram-positive bacteria, except *Enterococcus faecalis*, were sensitive to azithromycin. Additionally, azithromycin was active against *Neisseria gonorrhoeae*. Mefloquine showed good activity against pneumococci but lower *in vitro* activity against all other tested pathogens. These data indicate that the use of azithromycin may be associated with most collateral benefit in curing and preventing concomitant bacterial infections.

In the second study the impact of routine IPTp with mefloquine (MQ) and SP on *Streptococcus agalactiae* (GBS) was evaluated. GBS is an important cause of early neonatal infections leading to considerable morbidity and mortality. In this nested randomized controlled clinical trial GBS colonization rates in pregnant women were assessed. The overall prevalence of recto-vaginal GBS colonization was comparable to published findings in industrialized countries. Demographic characteristics showed significant association between GBS colonization and literacy. No difference of maternal GBS colonization was observed between SP- and MQ-IPTp regimes. This finding indicates that SP-IPTp was not superior to MQ-IPTp in preventing GBS colonization in pregnant women in Gabon.

In another analysis of the above described IPTp study, the prevalence of human herpes virus 8 (HHV-8) infections was assessed in Gabon. HHV-8 infection is associated with Karposi-sarcoma, Castleman disease and other malignant conditions and is known to be highly prevalent in Central Africa. In this sero-epidemiological study it was shown that 39% of pregnant women were seropositive for HHV-8 infections. Among 35% of seropositive women, real time PCR showed one pregnant women with detectable viraemia in peripheral blood. In analysis of cord blood samples of sero-positive women, no vertical transmission of HHV-8 was observed.

Finally, a pharmacokinetic analysis of albendazole for the treatment of extra-hepatic echinococcosis is presented. Echinococcosis is highly prevalent in sub-Saharan Africa and medical treatment is one option in resource limited settings. Here the ability of albendazole and its active metabolite albendazole sulphoxide to penetrate into *Echinococcus granulosus* cysts of patients with non-liver cysts was investigated. Analysis of two patients with lung and soft tissue cysts demonstrated a satisfactory penetration of albendazole sulphoxide into *Echinococcus* cysts. These data underline the usefulness of albendazole therapy in extra-hepatic cystic echinococcosis.

Zusammenfassung

Malaria ist global gesehen eine der wichtigsten Infektionserkrankungen, die zu hoher Morbidität und Mortalität besonders unter jungen Kindern und Schwangeren im Afrika südlich der Sahara führt. Die intermittierende präventive Therapie in der Schwangerschaft (intermittent preventive treatment in pregnancy, IPTp) ist ein wirksames Präventionsprogramm, um die Morbidität der Malaria in der Schwangerschaft zu reduzieren. Sulphadoxin/Pyrimethamin (SP) ist das empfohlene Medikament für IPTp in Afrika südlich der Sahara. Aufgrund zunehmender Resistenz gegen SP besteht der Bedarf für eine Alternative zu SP. Zu den vielversprechendsten Kandidaten, die sich derzeit in klinischer Entwicklung befinden, zählen Mefloquin und Azithromycin. Neben ihrer Wirkung gegen Malaria zeigen diese Wirkstoffe auch eine bedeutende antibakterielle Aktivität. Diese antibakterielle Wirkung könnte bei Anwendung als IPTp bei Schwangeren einen zusätzlichen Nutzen darstellen. So könnte mit diesen Medikamenten - neben einem Schutz vor Malaria in der Schwangerschaft - häufig unentdeckte sexuell übertragene und Harnwegsinfektionen vorgebeugt und behandelt werden.

In dieser Arbeit werden drei Studien mit einem Fokus auf Infektionserkrankungen im Afrika südlich der Sahara vorgestellt. In der ersten Studie wurde die Aktivität von Mefloquin und Azithromycin *in vitro* im Vergleich zu Sulphadoxin-Pyrimethamin gegen bakterielle Pathogene von klinischer Relevanz in der Schwangerschaft untersucht. Ergebnisse dieser Studie zeigen, dass SP hoch aktiv gegen *Staphylococcus aureus* und *Streptococcus pneumoniae* ist. Alle getesteten Gram-positiven Bakterien mit Ausnahme von *Enterococcus faecalis* zeigten ein gutes Ansprechen Reaktion auf Azithromycin. Zusätzlich zeigte Azithromycin Aktivität gegen *Neisseria gonorrhoeae*. Mefloquin zeigte gute Wirksamkeit gegen Pneumokokken, aber niedrigere *in vitro* Aktivität gegen alle anderen getesteten Pathogene. Diese Ergebnisse weisen darauf hin, dass die Verwendung von Azithromycin den umfassendsten Schutz gegen bakterielle Infektionen bieten könnte.

In der zweiten Studie wurde der Einfluss von IPTp mit Mefloquin (MQ) und SP auf *Streptococcus agalactiae* (GBS) untersucht. GBS ist eine bedeutende Ursache früher neonataler Infektionen , die zu signifikanter Morbidität und Mortalität führen. In dieser randomisiert-kontrollierten klinischen Studie wurden die GBS Kolonisationsraten schwangerer Frauen erhoben. Die Gesamt-Prävalenz rekto-vaginaler GBS-Kolonisation war vergleichbar mit publizierten Daten aus industrialisierten Ländern. Die demografischen Daten

zeigten eine signifikante Assoziation zwischen GBS-Kolonisation und Alphabetisierungsrate. Zwischen SP- und MQ-IPTp Gruppen fand sich kein Unterschied in der maternalen GBS-Kolonisation. Diese Ergebnisse zeigen, dass SP-IPTp bei der Prävention einer GBS-Kolonisation keine Vorteile unter schwangeren Frauen in Gabun zeigte.

In einer weiteren Analyse der oben beschriebenen Studie wurde die Prävalenz der Infektion mit dem humanen Herpesvirus 8 (HHV-8) in Gabun untersucht. HHV-8-Infektionen stehen in Verbindung mit Karposi-Sarkomen, Castleman Disease und anderen malignen Erkrankungen und ist in Zentralafrika bekanntermaßen hochprävalent. Unter 35% sero-positiven Frauen konnte mittels Real Time PCR bei einer schwangeren Frau HHV-8 mit nachweisbarer Virämie im peripheren Blutkreislauf nachgewiesen werden. Bei der Analyse von Nabelschnurblut-Proben von seropositiven Frauen konnte keine vertikale Übertragung von HV-8 beobachtet werden.

Die letzte Studie beschäftigte sich mit der medikamentösen Therapie der Echinokokkose. Die Echinokokkose ist in weiten Teilen Afrikas hochendemisch und Albendazol ist eine medikamentöse Therapieoption. Bisher gibt es aber nur beschränkt Daten zur Zystenpenetratio von Albendazol. In dieser Studie wurde die Fähigkeit von Albendazol und dessen aktivem Metaboliten Albendazol Sulphoxid untersucht, in Echinococcus granulosus Zysten von Patienten mit extrahepatischen-Zysten zu penetrieren. Die Analyse von zwei Patienten mit Lungen- und Weichgewebe-Zysten zeigte einen zufriedenstellenden Übertritt von Albendazol Sulphoxid in Echinococcus Zysten. Diese Ergebnisse unterstreichen die Nützlichkeit der Albendazol-Behandlung bei zystischer Echinokokkose.

Aims

The main objectives of the studies presented in the framework of this thesis were to gain better information regarding IPTp in sub-Saharan Africa, to investigate the epidemiology of other infectious diseases during pregnancy in the same region and to evaluate the impact of IPTp on infectious diseases other than malaria.

The aims were:

- To assess the anti-bacterial activity of sulphadoxine/pyrimetamine, mefloquine and azithromycin against common Gram-positive and Gram-negative bacteria in vitro.
- To evaluate the efficacy of IPTp drugs against GBS colonization and to describe the prevalence of GBS colonization in pregnant women adhering to IPTp in Gabon.
- To review the epidemiology and management of Group B Streptococcal colonization during pregnancy in African country Gabon
- To describe the prevalence of HHV-8 infection in pregnant women in Gabon

Another attempt was made to assess the ability of albendazole and its active metabolite albendazole sulphoxide to penetrate into cysts of two patients with extra-hepatic *E. granulosus* cysts – a zoonosis highly prevalent in some regions of Africa.

Publications

In this dissertation the following publications were summarized:

1. Mesküre Capan, Ghyslain Mombo-Ngoma, Athanasios Makristathis, Michael Ramharter. Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine. Malar J 2010 29;9:303.

2. Mesküre Capan, Ghyslain Mombo-Ngoma, Daisy Akerey-Diop, Arti Basra, Heike Würbel, Wenslance Lendamba, Lorenz Auer-Hackenberg, Rudolpho Mackanga, Joachim Melser, Sabine Belard, Michael Ramharter. Epidemiology and management of group B streptococcal colonization during pregnancy in Africa. Wiener klinische Wochenschrift 10/2012

3. Mesküre Capan, Sebastian Keltner, Florian Thalhammer, Stefan Winkler, Walter Jäger, Markus Zeitlinger, Michael Ramharter. Intra-cystic drug concentration of albendazole sulphoxide in patients with Echinococcus granulosus cysts. Am J Trop Med Hyg 2009 Oct;81(4):712-3

Manuscripts in Submission:

4. Prevention of Group B Streptococcus Colonization by Intermittent PreventiveTreatment of Malaria in Pregnant Women in Gabon: A Nested Analysis of aRandomized Controlled Clinical Trial of Sulfadoxine-Pyrimethamine versus Mefloquine

5. Epidemiology of Human Herpesvirus 8 Infection in Gabon: A Cross-Sectional Survey of Pregnant Women at Delivery

1. Introduction

1.1 Malaria

Malaria is an infectious disease caused by the parasite *Plasmodium*. Humans are intermediate hosts and mosquitoes the definitive hosts. In 1880, the French scientist "Alphonse Laveran" was the first to describe that the single-celled parasite from the genus *Plasmodium* as the causative agent of malaria. Malaria is a major public health problem in tropical and sub-tropical areas of the world, where it affects millions of people. According to the "World Health Organization (WHO)" an estimated 207 million cases of malaria occurring in 2012 led to 627.000 deaths. Ninety percent of these deaths occur in sub-Saharan Africa, mostly among children younger than five. Pregnant women, people with HIV/AIDS, and international travelers are additional risk groups [1].

There are numerous Plasmodium species that produce malaria in various animals' species. However, six species commonly infect humans [2-4].

1. *Plasmodium falciparum* is by far the deadliest of the human malarial species. *P. falciparum* infections may lead to severe diseases with progressive anemia, cerebral malaria, and other organ dysfunctions.
2. *Plasmodium vivax* is the geographically most widespread species and is traditionally thought to produce fewer complications. However *P. vivax* may lead to respiratory distress and relapses leading to debilitating symptoms.
3. *Plasmodium malariae* infections not only produce typical malaria symptoms but also can persist in the blood for very long periods, possibly decades, without ever producing symptoms.
4. *Plasmodium ovale* cause fewer cases and less severe forms of the disease. Recently the *P. ovale* species complex was further subdivided into *P. ovale wallikeri* and *P. ovale curtisi*.

In recent years, some human malaria cases have occurred with *Plasmodium knowlesi* – a species that causes malaria certain monkeys in South-East Asia [1].

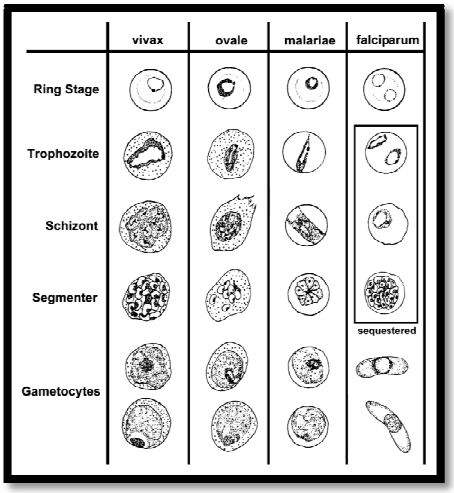

*Figure 1: Morphological differences between human **Plasmodium** species in blood smear.*

1.1.1 Transmission

In 1900s, Ronald Ross and Professor Grassi discovered independently that *Anopheles* mosquitoes are responsible for transmitting malaria [3, 4]. The intensity of transmission depends on factors associated with the parasites, the vector, the human host and the environment [1].

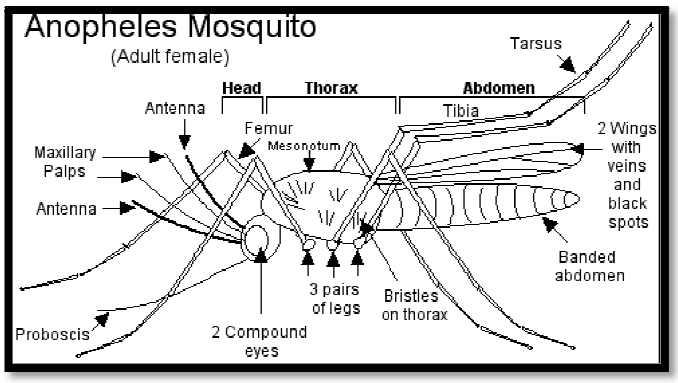

*Figure 2: **Anopheles** Mosquito*

The life cycle of all malaria types are similar. When an infected female *Anopheles* mosquito bites a human, the infectious form of the malaria parasite, so called sporozoites, are injected into the human bloodstream through the saliva of an *Anopheles* mosquito. They consecutively enter hepatocytes, where they transform into schizonts and start to multiply. If the hepatocyte ruptures, the blood stage parasites, known as merozoites, are released. Each merozoite invades a red blood cell and multiplies into more merozoites. A schizont red blood cell ruptures and releases more merozoites. This stage of life cycle causes the disease. Some

merozoites develop into the sexual form, the gametocytes, which do not cause disease but circulate in the bloodstream, awaiting the bite of a blood-seeking female *Anopheles* mosquito [2-4].

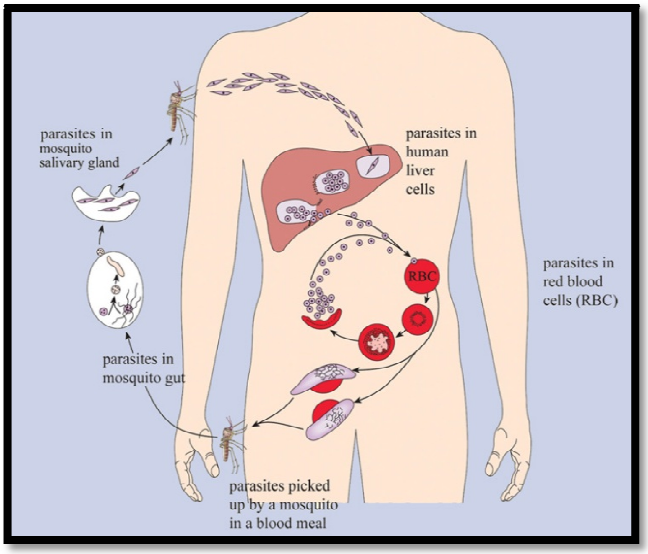

Figure 3: The Life Cycle of Malaria Parasites

In the mosquito's stomach a "male" gametocyte fertilizes a "female" to form an egg, or oocyst, which matures into thousands of sporozoites that move to the mosquito's salivary glands to be injected into a human host during the next bite [2-4].

1.1.2 Symptoms, Diagnosis and Treatment

The symptoms of malaria appear 10-16 days after the infectious mosquito bite. The symptoms are cyclical fever, shivering, headache, malaise, fatigue, muscular pains, occasionally nausea, vomiting, and diarrhea. If the patients are not treated within 24 hours, *P. falciparum* malaria can cause severe illness that may lead to death. The malaria attacks can repeat at regular time periods, like every 2 days for *P. vivax* and *P. ovale*, every 3 days for *P. malariae.* In case of infection by *P. falciparum*, fevers usually do not recur in a regular pattern.
The gold standard of malaria diagnosis is still the blood smear. A “thick smear” is made to establish the diagnosis and quantify the parasite burden, a “thin smear” allows the diagnosis of the species. Alternatively, rapid diagnostic tests can also be used for the qualitative diagnosis of malaria. Early treatment of malaria disease is essential and prevents deaths. The best available treatment for uncomplicated *P. falciparum* malaria is artemisinin-based combination therapy (ACT) including artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, or artesunate plus sulfadoxine-pyrimethamine [1].

The second line of recommended treatment is artesunate plus tetracycline, quinine plus tetracycline or doxycycline or clindamycin. Antimalarial treatment for uncomplicated P. vivax infection is chloroquine combined with primaquine [5].

1.2 Malaria in Pregnancy (MIP)

Malarial infection during pregnancy is a huge public health problems, associated with low birth-weight [6-8], pre-term delivery [9] and maternal anaemia [10] and leads therefore to increased maternal, perinatal, and neonatal morbidity and mortality in pregnancy and puerperium in sub-Saharan Africa [11, 12].
WHO recommends the use of IPTp with sulfadoxine-pyrimethamine (SP-IPTp) to reduce adverse health outcomes for pregnant women and their offsprings [13, 14]. However, due to an increasing rate of drug resistance of *Plasmodium falciparum* against SP, alternative antimalarial drugs have been evaluated for potential use as IPTp [15]. An ideal anti-malarial drug or drug combination for IPTp should be safe, well tolerated, and efficacious in the clearance of malaria parasites and provide a long period of chemoprotection. These drugs include amodiaquine, azithromycin, mefloquine, and combinations of these drugs with artemisinin derivatives or chloroquine [16, 17].

1.2.1 Sulphadoxine/Pyrimethamine (SP)

Sulphadoxine ($C_{12}H_{14}N_4O_4S$) is a sulfonamide with a molecular weight of 310.3*;* it is soluble in water. Sulphadoxine is rapidly absorbed from the gastrointestinal tract. The elimination half-time ($T_{1/2}$) is 4 to 9 days. Sulphadoxine is about 94% bound to plasma proteins. It is widely distributed to body tissues and fluids, passes into the foetal circulation and is detectable in breast milk. The side effects of sulphadoxine are similar to other sulfonamides. The most important adverse effect is allergic reaction to the compound; these hypersensivity reactions may affect different organ systems. The symptoms can be severe and include pruritus, photosensitivity reactions, exfoliative dermatitis, erythema nodosum, toxic epidermal necrolysis and Stevens-Johnson syndrome [18]. Other adverse effects include nausea, vomiting, anorexia, diarrhoea, fever, and interstitial nephritis, a syndrome resembling serum sickness, hepatitis, myocarditis, pulmonary eosinophilia, fibrosing alveolitis, peripheral neuropathy and systemic vasculitis.

Pyrimethamine ($C_{12}H_{13}ClN_4$) is a diaminopyrimidine with a molecular weight of 248.7, used in combination with a sulfonamide, usually sulphadoxine, or dapsone. Pyrimethamine is

well absorbed. The elimination half-time in healthy adults is between 2 and 6 days. It is mainly concentrated in the kidneys, lungs, liver and spleen, and about 80–90% is bound to plasma proteins. Pyrimethamine is generally well tolerated. However, high dosage can cause gastrointestinal symptoms such as atrophic glossitis, abdominal pain and vomiting, haematological effects including megaloblastic anaemia, leukopenia, thrombocytopenia and pancytopenia, and central nervous system effects such as headache and dizziness.

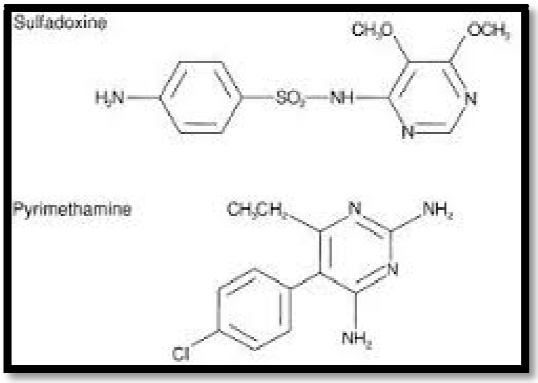

Figure 4: Sulfadoxine/pyrimethamine – antimalarial drug combination

A single treatment of the combination pyrimethamine-sulphadoxine is three tablets for an adult; equivalent to 1.25 mg/kg pyrimethamine and 25 mg/kg sulphadoxine. Following current recommendations of the WHO IPTp shall be provided in at least 2 treatment doses of SP given from the 2nd trimester onwards, given at least one month apart.[19]

1.2.2 Azithromycin

Azithromycin ($C_{37}H_{72}N_2O_{12}$) is a slow-acting anti-malarial macrolide [20]. The elimination half time is 68 hours; azithromycin accumulates in hepatic, renal, pulmonary and splenic tissue [21], and gradually leaches into the bloodstream over a period of one week [22].

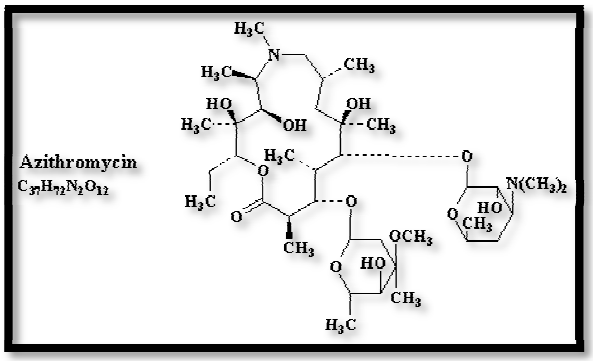

Figure 5: Azithromycin

In pregnant women, serum concentrations peak within six hours of oral administration and are sustained for 24 hours. As the drug disperses, peak concentrations are maintained three-times longer in the placenta, myometrial and adipose tissues [23]; only 2.6% of a maternal dose, however, perfuse the placenta [24]. Azithromycin is excreted in human milk with no adverse events observed as a consequence [25]. Azithromycin targets the 70S ribosomal subunit of the apical complex of susceptible micro-organisms including *P. falciparum* and *P. vivax* [22]. Azithromycin (500-2000mg) is used as an antibiotic during the pregnancy for upper and lower respiratory infections and skin diseases. Side effects of azithromycin in adults are: diarrhoea or loose stools (7%), nausea (5%), vomiting (2%), and vaginitis (2%); up to 1% of adults experience dizziness, headache, vertigo, and somnolence [26].

1.2.3 Mefloquine

Mefloquine is a 4-methanolquinoline and is related to quinine. It is slowly, but well absorbed. It has a terminal elimination half-life of approximately 2-4 weeks, and distributes into tissues, erythrocytes, and urine. The volume of distribution (Vd) ranges between 19L/kg in adults and 11,95L/kg in children 6-24 months of age. Mefloquine is excreted in small amounts in breast milk. It has a long elimination half-life of around 21 days, which is shortened in malaria to about 14 days [27-29].

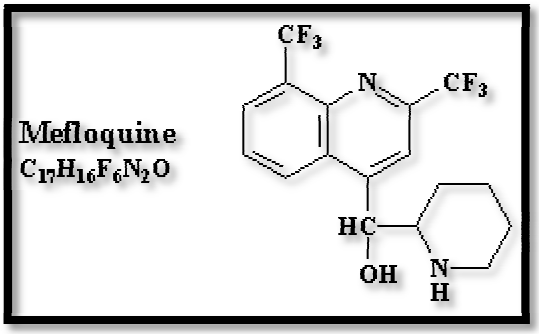

Figure 6: Mefloquine

Adverse effects are nausea, vomiting, abdominal pain, anorexia, diarrhoea, headache, dizziness, loss of balance, dysphoria, somnolence and sleep disorders, notably insomnia and abnormal dreams [30-33]. Other side effects reported rarely include skin rashes, pruritus and urticaria, hair loss, muscle weakness, liver function disturbances and very rarely thrombocytopenia and leucopenia.

1.2.4 Antibacterial activity of IPTp drugs

IPTp aims to reduce the burden of malaria by administering treatment doses of antimalarial drugs. Besides antiplasmodial activity, SP, mefloquine and azithromycin have proven antibacterial activity. The bactericidal activity of these drugs when used as IPTp in pregnant women may offer several additional public health benefits.

Sulphadoxine/Pyrimethamine: is an antifolate antibiotic, which has an antibacterial activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*, therefore it is an important treatment in urinary tract, skin and soft tissue infections [34, 35].

Azithromycin: was shown to be effective against bacteria causing sexually transmitted diseases like *Neisseria gonorrhea* [36] and *Chlamydia trachomatis* [37]. Since 1999, it has been used for chemoprophylactic (1,000 mg) and curative (2,000 mg) treatment against syphilis. Azithromycin is also sensitive against *Streptococcus pneumonia* [38].

Mefloquine: was demonstrated that it has an antibacterial activity against Gram-positive bacteria, including staphylococci, pneumococci and enterococci. However it exerted weaker activity against Gram negative bacteria and yeasts [39].

1.3 Other infectious diseases in Sub-Saharan Africa

1.3.1 Group B streptococcus – Streptococcus agalactiae

Group B *Streptococcus* (GBS), also known as *Streptococcus agalactiae*, is best known as a cause of postpartum infection and as the most common cause of neonatal sepsis. *S. agalactiae* is a Gram-positive bacterium with a narrow zone of beta haemolysis. The virulence of *S. agalactiae* is related to the polysaccharide toxin it produces. Immunity is mediated by antibodies to the capsular polysaccharide and is serotype-specific. Several serotypes are known: Ia, Ib, Ic, II, III, IV, V, VI, VII, and VIII.

S. agalactiae infection is extremely rare in healthy individuals and is associated with following underlying diseases: diabetes mellitus, malignancy [40], cardiovascular and genitourinary abnormalities, neurologic deficits, cirrhosis, steroid use, AIDS, renal dysfunction, and peripheral vascular disease [41]. Group B streptococci are found commonly in the vaginal and gastrointestinal tract and have been found to colonize the urethra in both men and women without causing infection. Group B streptococci can also colonize the upper respiratory tract. Urinary tract infections are a common manifestation of group B streptococcal disease and are observed in both pregnant and non-pregnant adults. Other

presentations of group B streptococcal infection include pneumonia, skin and soft-tissue infections, septic arthritis, osteomyelitis, meningitis, peritonitis, and endo-ophthalmitis [42]. Neonates can be infected by the organism vertically in utero or during delivery from the maternal genital tract. Although the transmission rate from mothers colonized with *S. agalactiae* to neonates delivered vaginally is approximately 50%, only 1-2% of colonized neonates go on to develop invasive group B streptococcal disease [43]. Neonatal group B streptococcal disease is divided into early and late disease. Early onset disease presents within the first 7 days of life. Late onset disease is defined as infection that presents between one week postpartum and age 3 months. Early onset and late onset diseases commonly include sepsis, pneumonia and meningitis. The prevention of group B streptococcal infection in pregnancy requires intra-partum antimicrobial prophylaxis in term women with culture evidence of recent vaginal or rectal group B streptococcal infection [44]. Penicillin or ampicillin is the initial approach. However, some isolates have shown minimum inhibitory concentrations (MICs) approaching the upper limits of susceptibility for some of the beta-lactam agents [45]. Clindamycin and erythromycin are standard in individuals with penicillin allergy.

1.3.2 Human Herpes Virus 8

The *Herpesvirinea* family is subdivided into three subfamilies: the *Alpha-*, *Beta-*, and *Gammaherpesvirinea*. Human Herpes Virus 8 (HHV-8) is a member of *Gammahepesvirinea*. The *Gammaherpesvirinea* subfamily contains two genera (a classification of closely related viruses) that include both the gamma-1 or *Lymphocryptovirus* (LCV) and the gamma-2 or *Rhadinovirus* (RDV) virus genera. HHV-8 is the only RDV discovered in humans and shows typical herpesvirus morphology with a central icosahedral capsid surrounded by a lipid bilayer [46, 47]. The capsid is composed of four structural proteins and three of them have significant homologies to alpha- and beta-herpes viruses. The analysis of the open reading frames K1 and K15 led to the identification of six viral subtypes (A, B, C, D, E and N) with marked clustering to geographic regions [48, 49] . HHV-8 infects several cell types, usually as a latent form. Lytic replication can take place in blood and tissues from Kaposi's sarcoma and multicentric Castleman's disease. The virus can be latently maintained in B lymphocytes and monocytes which may serve as a reservoir [50].

Human herpes virus 8 (HHV-8) – also known as Kaposi's sarcoma associated herpes virus (KSHV) – is the infectious agent causing Kaposi's sarcoma [51-56], primary effusion

lymphoma [57], and multicentric Castleman's disease [58]. HHV-8 transmission is thought to occur primarily by sexual contact [52, 59, 60]. However, the high seroprevalence rates in children living in Africa and the Western and Eastern Mediterranean countries [61-64] suggest other epidemiologically relevant routes of transmission.

The serologic prevalence of HHV-8 infection has been explored in most continents worldwide and in different populations at different levels of risk for HHV-8 infection. The prevalence rates varied depending on the geographic origin of the sera tested and the specific tests used to determine these prevalences; in particular, there was also a remarkable difference between the prevalence of antibodies against latent form antigens or lytic antigens.

1.4 Echinococcosis

Echinococcosis is a parasitic disease caused by the larval stage of tapeworms of the genus *Echinococcus*. It is a zoonosis highly prevalent in many parts of Africa.[65] There are two important sorts of diseases, which differ in presentation, behaviour and clinical management.

1. Alveolar echinococcosis is caused by *Echinococcus multilocularis*, in which a multiversiculated tumour forms, mainly in the liver. The asymptomatic incubation period is 5 to 15 years. Serological tests are helpful for diagnosis. However, the diagnosis is challenging and experience of clinicians, radiologist, and microbiologist are important for the first diagnosis. Lack of awareness of the disease often leads to misinterpretation of findings.
2. Cystic echinococcosis is caused by *Echinococcus granulosus,* in which fluid filled cysts develop mainly in the liver and lung. The incubation period may take just as long as in the case of alveolar echinococcosis. The spectrum of symptoms depends on the following:

- involved organs
- size of cysts and their sites within the affected organ or organs
- interaction between the expanding cysts and adjacent organ structures, particularly bile ducts and the vascular system of the liver
- complications caused by rupture of cysts
- bacterial infection of cysts and spread of protoscolices and larval material into bile ducts or blood vessels
- immunologic reactions such as asthma, anaphylaxis, or membranous nephropathy secondary to release of antigenic material

1.4.1 Echinococcus granulosus – Biology

The adult *E. granulosus* is 3 to 6 mm long and lives in the small intestine of canines.

Figure 7: Echinococcus granulosus adult worm

Adult worms live in the bowels of the definitive hosts and are passed in faeces. The eggs are ingested by humans or other intermediated host such as sheep, horses, goats, rodents and others. When ingested, the eggs hatch and penetrate the intestinal wall. Via the blood stream, they are carried to various organs; liver (75%), lung (25%) and other organs (6%) like the brain, heart, kidney and spleen. There, so-called hydatic cysts are formed by the larval stage of the tape worm. This cyst enlarges gradually, producing protoscolices. Protoscolices ingested by definitive hosts, evaginate and attach to small intestine, where they grow into the adult worm within 32-80 days.

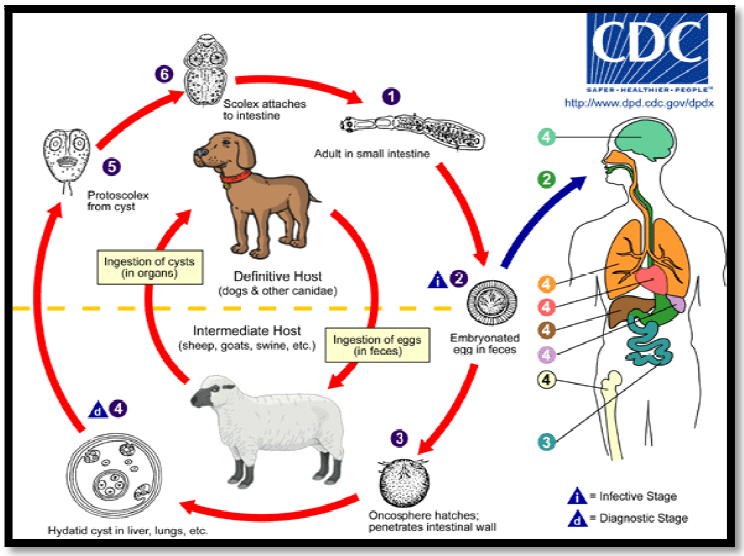

Figure 8: E. granulosus Life Cycle

1.4.2 Echinococcus granulosus – Diagnosis and Treatment

Diagnosis: There are several different serological methods for detecting antibodies. Among these, indirect haemagglutination (IHA), indirect fluorescent antibody (IFA) tests and enzyme immunoassay (EIA) are the most sensitive methods. Sensitivity rates of these methods range from 60% to 90%. IHA test and enzyme-linked immunoassay are the most widely used methods for detection of anti-*Echinococcus IgG* antibodies. Positive reactions in these methods usually have to be confirmed by a more specific method, such as a Western Blot (WB) for example.

Besides serological methods, medical imaging is crucial in the diagnosis of human echinococcosis. Ultrasonography is a safe, non-invasive and cheap method for detecting and monitoring cysts in solid organs, especially the liver [66].

In 2003, the World Health Organization proposed a standardized ultrasound classification based on the active-transitional-inactive status of the cyst as suggested by its sonographic appearance [67]. Computed tomography and magnetic resonance imaging are useful for other sites, such as rare cardiac lesions for example [68]. Simple X-ray can be useful for cysts in the lungs, bone, and muscle and for detecting calcified cysts.

- *Treatment*: Mebendazole and albendazole are effective drugs against cystic echinococcosis. Both drugs are well tolerated but have different efficacy. Albendazole is more effective than mebendazole in the treatment of liver cysts. The cure rate of *E. granulosus* infection treatment with albendazole varies from 40% to 50%. Duration of therapy and doses are important. Before the introduction of antihelminthic drugs the first choice of treatment for echinococcosis was surgery. However, surgery is also associated with mortality (up to 2%), morbidity [69] and recurrence rates (2-25%) [70].

2. Material and Methods

2.1 Chemicals and Agars

Mueller Hinton broth, Merck KGaA, Darmstadt, Germany

New York City agar, HiMedia™ Laboratories Pvt Ltd, India

Chocolate agar, Becton Dickinson GmbH, Heidelberg, Germany

Columbia broth base, Becton Dickinson GmbH, Heidelberg, Germany

Neopeptone, Becton Dickinson GmbH, Heidelberg, Germany

Agarose, Sigma-Aldrich, Seelze, Germany

Haematin solution, Sigma-Aldrich, Seelze, Germany

Tween, Sigma-Aldrich, Seelze, Germany

Pyridoxal solution, Sigma-Aldrich, Seelze, Germany

Amies transport medium, eSwab, Copan Diagnostics, Brescia, It.

Lim Broth, Becton Dickinson, Germany

Granada agar, Becton Dickinson, Germany

SYBR Green I, Roche Diagnostic GmbH, Mannheim, Germany

2.2 Pathogens and Drugs

Escherichia coli, Clinical Isolate and American Type Culture Collection (ATCC) strain

Enterococcus faecalis, Clinical Isolate and American Type Culture Collection (ATCC) strain

Neisseria gonorrhoeae, Clinical Isolate and American Type Culture Collection (ATCC) strain

Stahphylococcus aureus, Clinical Isolate and American Type Culture Collection (ATCC) strain

Streptococcus agalactiae, Clinical Isolate and American Type Culture Collection (ATCC) strain

Mefloquine, Sigma-Aldrich, Seelze, Germany

Sulfadoxine/pyrimethamine, Sigma-Aldrich, Seelze, Germany

Azithromycin, Sigma-Aldrich, Seelze, Germany

Albendazole and Albendazole/Sulfoxide, Sigma-Aldrich, Seelze, Germany

2.3 Instruments

"UltiMate 3000" system, Dionex, Sunnyvale, CA

"Hypersil BDS-C18 column", Thermo Fisher Scientific, Waltham, MA

"Anaerobic chamber", BugBox, LedTechno, Heusden-Zolder, B.

"QIAamp tissue kit", Qiagen, Bechman Instruments Inc, US

"KSHV/HHV-8 IgG IFA Kit", Advanced Biotechnologies Inc, Columbia, US

2.4 Techniques

2.4.1 Minimal Inhibitory Concentration

- The minimal inhibitory concentrations (MIC) of mefloquine, azithromycin, and SP were examined against the following bacterial pathogens
    a. *Escherichia coli*,
    b. *Enterococcus faecalis*,
    c. *Neisseria gonorrhoeae*,
    d. *Staphylococcus aureus*,
    e. Group B beta-hemolytic streptococci; i.e. *Streptococcus agalactiae*.
- Bacteria were grown overnight at 37°C in Mueller Hinton broth with or without 2-5% horseblood.
- *Neisseria gonorrhoeae* was incubated overnight at 35°C in an atmosphere containing 5% CO2 on New York City agar and chocolate agar.
- Drugs were obtained from Sigma-Aldrich (Seelze, Germany) and were dissolved and diluted (1:2) to stock solutions.
- Fastidious broth medium was prepared and consisted:
    - 35 g Columbia broth base,
    - 5 g glucose,
    - 5 g yeast extract,
    - 2 g neopeptone,
    - 0.75 g agarose dissolved in 960 ml of distilled water [71].
    - A total of 30 ml haematin solution (0.05% [wt/vol] in 0.1 M NaOH) and 5 ml Tween 80 (10% [wt/vol]) was then added.
- The resultant broth was sterilized by autoclaving and 6 ml of pyridoxal solution (0.1% [wt/vol]) and 1.5 ml of NAD solution (1% wt/vol) were added.

2.4.2 Determination of MICs

- MICs were determined by using a standard microdilution assay with a bacterial turbidity of 0.5 McFarland (Clinical and Laboratory Standards Institute (CLSI) guidelines).
- The bacterial density was approximately 10^5 CFU/ml.
- The 96-well plates were incubated for 24 h at 35°C in a moist atmosphere containing 5% CO2.
- Positive control wells contained microorganisms without antibiotics.
- All tests were performed in duplicate and MICs were reported as arithmetic means.

2.4.3 Classifications of anti-bacterial activity

- CLSI consensus cut off levels were used for the categorization of anti-bacterial activity. CLSI recommendation :
    - Cut off levels for azithromycin; classification as sensitive, intermediate and resistant:
        - *S. aureus* ≤2 µg/ml, >2 - <8 µg/ml, ≥8 µg/ml;
        - *S. pneumonia* and *S.agalactiae* ≤0.5 µg/ml, >0.5 - <2 µg/ml, ≥ 2 µg/ml.
        - *N. gonorrhoeae* ; due to CLSI recommendations and the European committee on anti-microbial susceptibility testing (EUCAST);
          ≤ 0.25 µg/ml; > 0.25 µg/ml - ≤ 0.5 µg/ml; > 0.5 µg/ml
        - *E. coli* and *Enterococcus* no cut off levels by CLSI and EUCAST, therefore the cut off levels of *S. aureus* were used: ≤2 µg/ml, >2 - <8 µg/ml, ≥ 8 µg/ml for susceptible, intermediate and resistant, respectively.
    - Sulfadoxine/Pyrimethamine: No recommendations are available for SP by CLSI or EUCAST. Therefore, CLSI and EUCAST cut off levels for trimethoprim- sulfamethoxazole - due to comparable anti-bacterial pharmacodynamics in the class of antifolate antibiotics and similar molecular weights for trimethoprim and pyrimethamine (290 and 249, respectively) - were used. The CLSI cut off levels for classification as sensitive, intermediate and resistant are the following:
        - *S. aureus* and *E.coli*: ≤ 2 µg/ml; > 2 - < 4 µg/ml; ≥ 4 µg/ml,
        - *S. pneumonia*: ≤ 0.5 µg/ml; > 0.5 - < 4 µg/ml; ≥ 4 µg/ml.

- The EUCAST cut off levels for SP (trimethoprim/pyrimethamine):
    - *S. agalactiae*: ≤ 1 µg/ml; > 1 - ≤ 2 µg/ml; > 2 µg/ml,
    - *E. faecalis*: < 0.03 µg/ ml; ≥ 0.03 µg/ml - ≤ 1 µg/ml, > 1 µg/ml

- For mefloquine: No recommendations are available by CLSI or EUCAST, therefore the threshold of drug resistance was set considering available pharmacokinetic data in human patients and published in vitro inhibitory concentrations against Plasmodium falciparum [72-78]. Thus, the threshold for drug resistance was 0.265 µg/ml for mefloquine.

2.4.4 Detection of GBS colonization

- ***Study participants:*** Pregnant women participating in a multicenter study evaluating alternative drugs for intermittent preventive treatment of malaria in pregnancy (MIPPAD: "Evaluation of the safety and efficacy of mefloquine as intermittent preventive treatment of malaria in pregnancy (IPTp) (NCT 00811421).") were invited to participate in this study.
- ***Research ethic:*** The study was approved by the institutional ethics review board, and written informed consent was obtained from HIV-negative pregnant women permanently residing in the study area with a gestational age at the first visit of ≤ 28 weeks. Exclusion criteria included known allergy to the study drugs, known clinical history of severe renal, hepatic, psychiatric or neurological diseases or antimalarial treatment in the preceding 4 weeks. After the study details had been explained and informed consent had been signed, participants were given a study number and randomized to SP or mefloquine IPTp.
- ***Detection of group B streptococcus colonization***:
    - The swabs were taken from the cervix and rectally from 557 women at delivery.
    - All samples were collected using nylon flocked swabs that were submerged into 1 ml of liquid Amies transport medium.
    - A total of 200µl of the transport medium of each of the cervical/rectal swabs was inoculated into 5 ml of Lim Broth (Todd-Hewitt broth, 1% yeast extract, 15 µg nalidixic acid/ml and 10 µg colistin/ml) [79], which is selective for Gram positive bacteria.

- This culture was incubated under aerobic conditions at 37 °C for 24 hours and then sub-cultured for 24h on Granada agar [80] at 37 °C in an anaerobic chamber.
- Granada agar was examined for yellow-orange pigment colonies that confirm the presence of GBS.
- The statistical analysis was performed with a commercial software package (JMP 5.0, SAS Institute Inc., NC)

2.4.5 Detection of HHV8 infection

- ***Study Participants:*** Pregnant women participating in a multicentre study for the preventive treatment of malaria in pregnancy (MIPPAD: "Evaluation of the safety and efficacy of Mefloquine as Intermittent preventive treatment of malaria in pregnancy (IPTp) (NCT 00811421).") were invited to participate in this study.
- ***Research ethic:*** The study was granted ethical approval by the Institutional Review Board of the Centre de Recherches Médicales de Lambaréné at the Albert Schweitzer Hospital in Gabon.
- ***Sample collection:***
  - Blood samples from mother-child pairs were obtained at delivery after provision of informed consent.
  - Two millilitre of maternal peripheral blood was obtained together with a biopsy from the maternal side of the placenta.
  - After delivery cord blood and a biopsy from the foetal side were collected. Whole blood and placental biopsies were used for the detection of virus specific DNA by PCR.
- ***Serological detection of HHV8:***
  - Blood samples were centrifuged at 2500 rpm for 10 minutes to remove plasma for the detection of specific antibody directed against latent nuclear antigen (LANA) of HHV-8.
  - Plasma samples were tested at a 1/20 dilution for the HHV-8-specific IgG by an indirect immunofluorescence assay [81] following instructions by the manufacturer.

- Specific HHV-8 antibodies binding to the antigen present in infected cells were visualized after incubation with Fluorescein Isothiocyanate (FITC) under a fluorescence microscope.

- ***Detection of HHV8 by PCR:***
  - DNA was extracted from maternal and cord blood and placental biopsies using QIAamp tissue kit.
  - DNA extracts were further assays by PCR using the LightCycler® arousel-based system.
  - Amplification was carried out in a 15µl PCR master mix containing:
    - 10pmol/µl of each primers specific for ORF25 region [82],
    - 3mM of MgCl2, 1x Lightcycler® LightCycler® FastStart DNA Master SYBR Green I.
    - and 1,5µl of DNA.
  - After an initial 10-min denaturation at 95°C, 50 cycles were run for 10 sec at 95°C; for 5 sec at 65°C and for 22 sec at 72°C. These cycles were followed by a 15 sec melting at 65°C and 30 sec cooling at 40°C.
  - Each PCR sample (15µl) was analyzed by electrophoresis on a 2 % Agarose gel, and the bands were visualized by ethidium bromide staining.
  - Data were recorded on paper record forms. An available software package was used for statistical analysis (JMP 7.0, SAS Institute Inc., NC).

2.4.6 Concentration and Separation of albendazole and albendazole sulphoxide

- Venous blood samples for drug analysis were taken from two patients before albendazole intake and during the intervention.
- Concentrations of albendazole and albendazole sulphoxide in plasma and cyst fluid were measured by a validated high performance liquid chromatographic (HPLC) method using an “UltiMate 3000” system (Dionex, Sunnyvale, CA).
- Separation of albendazole sulphoxide was carried out using a Hypersil BDS-C18 column (Thermo Fisher Scientific, Waltham, MA) with an acetic acid/methanol mobile phase.
- The limit of quantification for albendazole and albendazole sulphoxide was 0.063 and 0.072 µg/ml, respectively.
- Coefficients of accuracy and precision for both compounds were < 8

3. Results

Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin

The bacterial isolates were sub-cultured after thawing prior to susceptibility assays (n = 34).

Median MIC values of SP against Gram-positive bacteria were as follows:

- S. aureus 16 µg/ml,
- S. agalactiae 24 µg/ml,
- S. pneumoniae 4 µg/ml, and E. faecalis 12 µg/ml.

However, the MIC values of SP against Gram negative bacteria were considerably higher:

- N. gonorrhoeae 256µg/ml
- E. coli 128 µg/ml

SP showed high or intermediate activity against all tested Gram-positive bacteria, whereas E. coli and *N. gonorrhoeae* were classified as resistant to SP (Table 1 and Table 2).

Table 1: Median minimal inhibitory concentrations (MIC) of anti-malarial drugs against Gram-positive and Gram-negative bacteria.

Microorganisms	**Median Minimal Inhibitory Concentration (µg/ml)**		
	Sulphadoxine/Pyrimethamine	**Mefloquine**	**Azithromycin**
S. aureus (n = 5)	16	16	0.5
S. agalactiae (n = 4)	24	16	0.06
S. pneumoniae (n = 5)	4	0.06	0.01
E. faecalis (n = 5)	12	16	4
N. gonorrhoeae (n = 10)	256	8	0.01
E. coli (n = 5)	128	128	4

Thresholds for drug resistance:

Mefloquine: sensitive: ≤ 0.265 µg/ml;

Sulphadoxine/Pyrimethamine (threshold based on fractional pyrimethamine concentration in 1:20 combination): sensitive, intermediate, resistant,

- *S. aureus* and *E. coli*: ≤ 2 µg/ml; > 2 - < 4 µg/ml; ≥ 4 µg/ml,
- *S. pneumonia*: ≤ 0.5 µg/ml; > 0.5 - < 4 µg/ml; ≥ 4 µg/ml,
- *S. agalactiae*: ≤ 1 µg/ml; > 1 - ≤ 2 µg/ml; > 2 µg/ml,
- *E. faecalis*: < 0.03 µg/ml; ≥ 0.03 - ≤ 1 µg/ml; > 1 µg/ml.

Azithromycin: sensitive, intermediate, resistant

- *S. aureus*; *E. coli* and *E. faecalis* ≤ 2 µg/ml, > 2 - < 8 µg/ml, ≥ 8 µg/ml;
- *S. pneumonia* and *S. agalactiae* ≤ 0.5 µg/ml, > 0.5 - < 2 µg/ml, ≥ 2 µg/ml;
- *N. gonorrhoeae* ≤ 0.25 µg/ml; > 0.25 µg/ml - ≤ 0.5 µg/ml; > 0.5 µg/ml;

All bacterial isolates were assessed for their in vitro drug susceptibility against mefloquine. The MIC values of mefloquine against Gram positive and Gram negative bacteria were as followed:

- *S. aureus* 16 µg/ml,
- *S. agalactiae* 16 µg/ml,
- *S. pneumoniae* 0.06 µg/ml,
- *E. faecalis* 16 µg/ml,
- *N. gonorrhoeae* 8 µg/ml
- *E. coli* 128 µg/ml

Based on these data *S. pneumonia* was classified as sensitive, whereas *S. aureus*, *S. agalactiae*, *E. faecalis*, *N. gonorrhoeae*, and *E. coli* as resistant to the in vitro activity of mefloquine (Table 1 and Table 2).

In vitro anti-bacterial activity of azithromycin against bacterial isolates was as followed:

- *S. aureus* 0.5 µg/ml,
- *S. agalactiae* 0.06 µg/ml,
- *S. pneumoniae* 0.01 µg/ml,
- *E. faecalis* 4 µg/ml,
- *N. gonorrhoeae* 0.01 µg/ml
- *E. coli* 4 µg/ml4

Median MIC values of azithromycin showed that *N. gonorrhoeae*, *S. aureus*, *S. agalactiae* and *S. pneumonia* were sensitive to Azithromycin. The activity of Azithromycin against *E. faecalis* was weak. *E. coli* showed intermediate drug susceptibility against azithromycin (Table 1 and Table 2).

Table 2: *In vitro anti-bacterial activity of sulphadoxine-pyrimethamine, mefloquine, and azithromycin.*

	Sulphadoxine/Pyrimethamine	**Mefloquine**	**Azithromycin**
S. aureus	**+**	**-**	**+**
S. agalactiae	**~**	**-**	**+**
S. pneumoniae	**+**	**+**	**+**
E. faecalis	**~**	**-**	**~/-**
N. gonorrhoeae	**-**	**-**	**+**
E. coli	**-**	**-**	**~**

Insufficient *in vitro* activity; ~ intermediate *in vitro* activity; + good *in vitro* activity

Prevention of Group B Streptococcus Colonization by Intermittent Preventive Treatment of Malaria in Pregnant Women in Gabon: A Nested Analysis of a randomized Controlled Clinical Trial of Sulphadoxine-Pyrimethamine versus Mefloquine

During the study period, 557 pregnant women participate in this sub-study and recto-vaginal swabs were taken. 346 participants had previously received mefloquine as IPTp and 186 were in the SP group. Baseline characteristics of participants including age, gravidity, anaemia, and literacy as well as delivery outcomes and birth weight of infants were collected and analysed.

Table 1: Baseline Characteristic of Participants

	Total N	Percent of Group B Streptococcal Colonization		Relative Risk (95% Confidence Interval)
		Positive (%)	Negative (%)	
Age, years				
14-17	77	18	82	1
18-20	138	15	85	0.80 (0.38-1.69)
21-24	116	22	78	1.3 (0.63-2.68)
25-30	112	21	79	1.22 (0.59-2.56)
31-49	114	22	78	1.26 (0.61-2.62)
Literacy				
yes	441	17	83	1
no	116	31	69	2.23 (1.40-3.56)
Gravidity				
primigravida	131	13	87	1
sekundigravida	198	18	82	1.43 (0.77-2.70)
multigravida	228	25	75	2.28 (1.26-4.12)
Anaemia				
no	155	21	79	-
moderate	393	19	81	-
severe	5	20	80	-
Rapid plasma regain (RPR)				
positive	7	14	86	-
negative	535	19	81	-
Study drugs (IPTp)				
MQ	346	19	81	1
SP	186	22	78	1.28 (0.83-1.98)
Delivery- outcome				
Live Birth	536	19	81	1
Stillbirth	14	14	86	0.69 (0.15-3.14)
Spontenous abortion	5	40	60	6.23 (1.02-37.7)
Birth weight- infants				
Normal birth weight	452	20	80	1
Low birth weight	90	14	86	0.66 (0.35-1.25)

The prevalence of GBS colonization at delivery was 19.8% (110 of 557 participants). Risk factor analysis showed significant associations between GBS colonization and literacy of the pregnant women with prevalence rates of 31% (95% CI = 1.40-3.56); and 17% (95% CI= 1) in illiterate and literate women, respectively. Importantly, 5 out of 557 pregnancies ended as spontaneous abortion, with 3 in GBS colonized women (95% CI = 1.02-37.7) and 2 in non-colonized women (95% CI = 0.15-3.14). The occurrences rate of spontaneous abortion for GBS positive and GBS negative patients were 60% to 40%, respectively.

Analysis of GBS colonization rates in the two IPTp groups showed prevalence of 18% (MQ group) and 22% (SP group), respectively, indicating no significant difference between IPTp regimens. Similarly there was no difference in GBS prevalence at delivery in MQ full dose and split dose groups.

Table 2: GBS colonization and IPTp groups

	Total	Group B Streptococcal Colonization			
		Positive		Negative	
	N	N	%	N	%
Mefloquine (MQ)					
MQ Full dose	192/557	34	19	158	34
MQ Split dose	179/557	34	18	145	32

Epidemiology of Human Herpesvirus 8 Infection in Gabon: A Cross-Sectional Survey of Pregnant Women at Delivery

From May 2010 to October 2011, 366 pregnant women were serologically tested for HHV-8 infection. Ninety-eight cord blood samples from seropositive women were collected for serological testing and 76 mother-child pairs were available for PCR analysis.

Baseline characteristics of all study participants were age (median 24 years), literacy, gravidity (median 2), and birth weight of infants (median 3000g). Forty-five patients were below 18 years old (36%), 299 patients were above 18 years old (35%), and data of 22 were missing. Thirty-three percent of study participants have the first gravidity. 143 pregnant women (39%) tested positive in the serologic assay. There were no significant differences between illiterate (29%) participants and literate participants (36%). There was also no difference in HHV-8 sero-positivity between patients pregnant for the second time or those for the third or more times (38% vs. 35%). Sixty-two infants were born with low birth weight; however, low birth weight was not correlated with HHV-8 infection (Table 1).

The cord bloods of infants of HHV8-positive mothers were tested. Thirty percent (30/98) were HHV-8 seropositive. No cord bloods of infants of seronegative mothers were tested. Seventy-six mother-child pairs were available for PCR analysis. In only one mother blood sample was HHV-8 specific DNA sequences detected. All other samples were found to be negative.

Table 1: Baseline Characteristics of Study Participants

	Total	Percent of HHV8 Infection	
	N	Positive (%)	Negative (%)
Age, years			
14-17	45	36	64
18-20	92	37	63
21-24	72	31	69
25-30	67	39	61
31-49	68	32	68
Literacy			
yes	282	36	64
no	62	29	71
Gravidity			
primigravida	86	33	67
sekundigravida	128	37	63
multigravida	130	35	65
Birth weight- infants			
Normal birth weight	276	35	65
Low birth weight	64	32	67

Intra-Cystic Drug Concentration of Albendazole Sulphoxide in Patients with Echinococcus granulosus Cysts

A 60-year-old woman of Turkish origin (Patient A) and a 30-year old Turkish citizen (Patient B) were participated in this study. Patient A had a cystic lesion in the proximal *Musculus satorius* of 7.5 cm diameter and patient B had a distinctive pleural effusion on the right side of the lung with transgression of the diaphragm. Both patients were treated with 400 mg albendazole to be taken twice daily with a fatty meal for 5 and 4 days before surgical removal, respectively.

Albendazol sulphoxide concentrations were measured in plasma and in cystic fluid. HPLC measurement showed that the concentration of albendazol sulphoxide in pre-operative and intra-operative plasma samples were in the range of 0.10 and 1.3µg/ml. Cystic fluid samples were analysed and the albendazol sulphoxide concentration in cystic fluid samples was 0.16 and 0.59µg/ml (Table 1).

The relative intra cystic drug concentration was as following: patient A 48% and patient B 156%. These data demonstrate that albendazole sulphoxide has acceptable tissue penetration into echinococcal cysts.

Table 1: *Albendazole sulphoxide (µg/ml) and relative intra-cystic drug concentration*

	Pre-operative plasma	Intra-operative plasma	Cyst fluid	Relative intra-cystic drug concentration
Patient A	1.26	1.24	0.59	48%
Patient B	0.18	0.10	0.16	156%

4. Discussion

Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin

This *in vitro* drug susceptibility study showed a broad spectrum of antibacterial activity of SP against all tested Gram positive bacteria and intermediate activity against *E. coli*. Among the two currently proposed alternative drugs for IPTp – mefloquine and azithromycin – the latter shows a similarly broad antibacterial spectrum of activity as SP but additionally also good activity against *N. gonorrhoeae*. Mefloquine activity was classified as high against pneumococci and intermediate against all other bacteria except *E. coli*.

The evaluation of the antimicrobial activity of antimalarials *in vitro* provides the basis for further clinical evaluation. Based on our data a clinically important effect on certain bacterial co-infections in pregnant women may be anticipated for SP and azithromycin and to a lesser extent for mefloquine. Previous data show that bacterial infections including sexually transmitted diseases, pneumococcal infections, and *S. agalactiae* colonisation contribute significantly to adverse pregnancy outcome in sub-Saharan Africa [83]. Similarly, there is evidence for significant improvement of maternal and child health by routine administration of appropriate antimicrobial drugs during pregnancy [84]. Given that urinary and genital tract infections are an important cause for premature delivery, it may be speculated that the routine administration of SP and azithromycin as IPTp may confer a reduction in the rate of prematurity. Similarly, the potential for eradication of vaginal *S. agalactiae* colonization by IPTp with SP or azithromycin might prevent cases of neonatal sepsis. However, caution must be used when extrapolating data on *in vitro* activity of drugs to anticipated *in vivo* efficacy. Clinical efficacy will ultimately depend – besides the intrinsic antibacterial activity as assessed in this study – on drug absorption, drug concentrations at the target sites, and half-lives of drugs. Additionally, a limitation of our study lies in the absence of validated thresholds for the *in vitro* activity of SP and mefloquine. The proposed levels are based on comparative thresholds of *in vitro* activity against *P. falciparum* and known pharmacokinetic characteristics of the drugs and need further clinical validation [71]. Based on the hypothesis of a collateral health benefit by the administration of antimalarials with antibiotic activity against relevant pathogens, it may seem desirable to choose the next IPTp drug based on both its antimalarial and antibacterial activity. Whether such an approach is justified or not is however at least to date unknown. Although it may look attractive to simultaneously treat

concomitant and potentially deleterious bacterial infections by routine administration of antimalarials, this strategy may also prove hazardous. Large scale use of drugs with antibacterial activity may speed up the process of selection of drug resistant bacterial isolates. Interestingly, there is evidence for the development of antibiotic drug resistance by cross resistance with antimalarial drugs [85]. Epidemiologic evidence linking the development of quinolone resistant Gram negative bacteria with large scale use of chloroquine and the recent development and spread of quinolone resistant *N. gonorrhoea* strains is an illustrative example for this phenomenon [86]. The threat of promoting drug resistance against commonly used antibiotics is particularly worrying for sub-Saharan Africa where microbiologic analysis of infections is rarely performed and alternative antibiotics for drug resistant pathogens are often not affordable. Therefore, future studies on alternative IPTp drugs should include evaluations of the clinical efficacy against concomitant bacterial infections and the impact of large scale use of IPTp on the development and spread of antibiotic drug resistance.

Epidemiology and Management of Group B Streptococcal Colonization during Pregnancy in Africa

Epidemiology of GBS colonization

Streptococcus agalactiae – often referred to as Group B streptococcus (GBS) – is a Gram-positive bacterium that has been identified as a human pathogen since the early 1900s [87]. GBS is of particular medical importance during pregnancy and the postpartum period because it may lead to invasive disease in both mother and infant. Maternal colonization with GBS in the genitourinary and gastrointestinal tract and transmission to the infant during labour and delivery is an important cause of early neonatal morbidity and mortality [88, 89].
Numerous studies report the prevalence of GBS in pregnant women living in high income regions. Maternal colonization rates range from 10-30% in the USA [90], 11-19.5% in Canada [91], 20% in New Zealand [92], and 6.5-36% in Europe [93]. In these studies prevalence of GBS colonization was associated with ethnicity, certain sub-regional differences, age, gravidity, socioeconomic factors, and sexual behaviour. Methodological differences in diagnosing GBS including sampling strategies and culture conditions may have influenced prevalence rates and direct comparability of study results is therefore problematic. To overcome methodological differences, standard culture conditions were published by the Centres for Disease Control and Prevention (CDC) to enhance GBS detection and improve comparability of individual studies [94-96].

GBS infections in pregnant women and newborns

In pregnant women, GBS may cause urinary tract infection, which may progress to maternal sepsis. Similarly, GBS may be a cause of chorioamnitis, endometritis and puerperal wound infection [97, 98]. On a population level, mortality from GBS-related diseases in pregnant women is however comparably low. Controversially, pregnancy-associated GBS infection may lead to adverse pregnancy outcome and early infections in newborns within the first few days of life [99]. Two forms of GBS infections are distinguished in newborns. Early-onset disease is defined as isolation of GBS from normally sterile sites including blood and cerebrospinal fluid. Early-onset disease occurs within the first 24 hours of birth and until 7 days of life. Early-onset disease follows vertical transmission of GBS in the birth canal or by direct transmission from maternal GBS infection. Approximately 80% of infants with early-onset disease reportedly become ill within 24 hours In the United States [100]. The most important early-onset diseases in infants are bacteraemia, sepsis with or without pneumonia and meningitis resulting in high mortality. A clinical study in the USA demonstrated that

early-onset disease caused by GBS was characterized by bloodstream infection, pneumonia, and meningitis in 40%, 7%, and 6% of patients, respectively. The overall case fatality rate associated with early-onset disease was 4% in this report [99]. Established risk factors for early-onset disease include preterm labour (< 37 weeks gestation), prolonged rupture of membranes (≥ 18 hours), and maternal fever (≥ 38.0° C) [97, 101, 102].
Late-onset disease occurs in infants between 7 days and 3 months of age. Infection follows vertical transmission from mother to their infants [103] or horizontal transmission [104]. Again bloodstream infection and meningitis are the most common clinical features but other sites of infection occur. The overall case fatality rate of late-onset disease is reportedly approximately 2.5% [99]. Risk factors for the occurrence of late-onset disease are not yet well understood.

Prevention of GBS infections in the newborn

Over the past three decades several clinical trials demonstrated that the use of intra-partum antibiotic prophylaxis decreased the risk of vertical GBS transmission by a reduction of GBS-related disease in the first week of life. Therefore, many national guidelines in high income settings recommend universal GBS screening and antimicrobial treatment of all pregnant women between 35 to 37 weeks of gestation [90, 102, 105]. Oral penicillins are the first line treatment based on their comparably narrow antimicrobial spectrum and high activity against GBS. Erythromycin and clindamycin are suggested as alternatives, particularly in the case of suspected allergy to penicillin [90]. Intra-partum treatment (IP) is effective in reducing the risk of early-onset disease in newborns to colonized women and neonatal colonization. A clinical trial in the USA demonstrated that the use of antimicrobial intra-partum chemoprophylaxis reduced the incidence rate of early-onset disease caused by GBS from 1.7 per 1000 live births in 1993 to 0.6 per 1000 in 1998 [99]. Since GBS is a non-invasive colonising agent, local disinfection has attracted considerable attention as a potential tool to eliminate GBS colonisation from the female genital tract. Chlorhexidine was evaluated in several clinical trials in Europe, Africa and the USA for this purpose. In a European trial, chlorhexidine application reduced the rate of hospital admission due to GBS in neonates. However, the effectiveness of vaginal disinfectant against GBS-related neonatal sepsis and meningitis was not shown [106]. Importantly, a study in Malawi reported that the disinfection of the birth-canal with chlorhexidine solution reduced GBS related neonatal mortality from 17.9 to 7.8 per 1000 live births. In addition this intervention also had a positive impact on maternal postpartum GBS infection [107]. Although local disinfection proved less effective

than antimicrobial drug prophylaxis, one of its potential advantages is the feature not to promote the development and spread of drug resistance. Vaginal disinfection could therefore constitute a potential alternative prevention strategy for resource limited regions due to the ease of use and low cost.

The development of effective vaccines against GBS would without any doubt constitute a major advantage for the prevention of GBS infections. The underlying mode of action for such vaccines is the induction of specific antibodies to capsular GBS polysaccharides that protect against invasive GBS disease in the mother and the newborn. To date, GBS polysaccharide antigens are tested as conjugate vaccines and promising results have been reported for rodent models [108]. Besides the uncertainty of the translation of these results to humans, more detailed knowledge about the prevalence of GBS subtypes in high and low income regions and potential cross-protection of strain specific epitopes are mandatory for further development of future vaccine candidates.

Current evidence for GBS related diseases and prevention strategies for sub-Saharan Africa

Several studies conducted in developing countries investigated the prevalence of maternal GBS colonization and some reported the distribution of GBS serotypes. Prevalence rates in various geographic regions ranged from 12 – 22% (India/Pakistan: 12%, Asia/Pacific 19%, sub-Saharan Africa 19%, Middle East/North Africa 22% , America 14%) [98]. Similarly, when focusing on sub-Saharan African countries, considerable variability of GBS prevalence was observed, probably due to differences in sampling strategies and microbiologic processing of samples. Reported prevalence rates in these countries varied between 1% and 32% (Nigeria 20%, Ivory Cost 19%, Togo 4%, Gambia 22%, Mozambique 1% [96], Malawi 16.5% [109], Zimbabwe 20-32% [110]). Stoll and Schuchat summarized 34 reports from 23 developing countries and indicated that the overall prevalence of maternal GBS colonization was approximately 18%, which is within a similar range as reported for women in the United States and Europe [96]. Despite the absence of important differences in antimicrobial susceptibility between GBS serotypes, their relative distribution is of great importance for ongoing efforts to identify vaccine candidates for clinical development. Clinical studies demonstrated that strains Ia, Ib, II and III were the most commonly encountered serotypes in high income regions and interestingly, these serotypes were associated with early-onset disease [100, 111-113].

Serotype III caused more than half of neonatal group B streptococcal disease and mortality [114]. To date, available data indicate a similar serotype distribution in high income regions and sub-Saharan Africa. Reporting from African regions indicates that serotypes Ia, II, III, and V were most frequently encountered [96, 115, 116]. Based on these studies further molecular characterisation of GBS isolates from sub-Saharan Africa is important to evaluate most suitable vaccine targets for potential future vaccines.

To date, the incidence of invasive GBS infections is incompletely understood in many low income regions. One study from Malawi indicated an incidence of early onset sepsis caused by GBS of 0.92 per 1000 live births [117]. However, a significant proportion of deliveries occur outside hospitals in many parts of sub-Saharan Africa and infants developing early onset sepsis may succumb before attending a health care centre. Importantly, routine identification of GBS is lacking in most routine institutions in Africa. The risk for overall mortality and rates of prematurity in GBS colonization remains therefore incompletely understood. [115]. However, some studies indicate that maternal GBS colonization may constitute an important risk factor for unfavourable birth outcome including spontaneous abortion, preterm delivery, and low birth weight in Africa [118] [96]. Importantly these conditions may outweigh the direct effect of invasive GBS infections on a population level. For a better understanding of the importance of GBS on a population level, further detailed epidemiologic studies are therefore needed.

Contrary to high income regions, prevention strategies for GBS colonised pregnant women are hampered by a lack of facilities for appropriate diagnosis in most countries in sub-Saharan Africa. Since routine screening is not performed in pregnancy, targeted interventions are not feasible. Since overall incidence of neonatal sepsis due to GBS carriage is low, routine administration of antimicrobial prophylaxis to all pregnant women is most probably not cost-effective. In addition this approach may increase the selective pressure for specific drug resistance, which may prove particularly worrisome in regions with limited availability of second line antimicrobials. Whether routine vaginal disinfection may prove safe, effective, and cost-effective, needs to be further investigated.

Prevention of Group B Streptococcus Colonization by Intermittent Preventive Treatment of Malaria in Pregnant Women in Gabon: A Nested Analysis of a randomized Controlled Clinical Trial of Sulphadoxine-Pyrimethamine versus Mefloquine

This study investigates the epidemiology of pregnancy-associated GBS colonization in Central Africa. The data show that the prevalence rate of GBS colonization among pregnant women reached 20%, which was comparable with the rate of GBS colonization of pregnant women in industrialized countries. No association of GBS colonization was observed with risk factors such as gravidity and maternal age. However, colonization was positively associated with literacy. This finding indicates the connection between the maternal GBS colonization and the socioeconomic status of study population. Moreover, maternal GBS colonization has been demonstrated to play an important role in spontaneous abortion [119]. This study shows that the occurrence of spontaneous abortion is also related with maternal GBS colonization. According to literature, the prenatal screening for GBS in pregnant women between 35 to 37 weeks of gestation and intra-partum antibiotic prophylaxis reduces the incidence of neonatal diseases. The use of penicillin, which has a narrow spectrum of antimicrobial activity, was encouraged by CDC for treatment. Erythromycin and clindamycin are the alternatives to penicillin [120]. Few clinical trials from Sub-Saharan Africa exist to describe the GBS - related neonatal morbidity and mortality. The existing data indicate that maternal GBS colonization rates of pregnant women and incidence of invasive disease in their offspring are comparable to industrialized countries. However, in resource poor settings, infrastructure for diagnostics, peripartal chemoprophylaxis and adequate case management is mostly lacking [115, 121]. Therefore, most infections will remain undetected and untreated. Intermittent preventive treatment of malaria in pregnancy (IPTp) could effect on concurrent infectious diseases in pregnancy. SP - recommended by WHO as IPTp drug of choice - has a broad spectrum of antibacterial activity against Gram positive bacteria and intermediate activity against Gram negative bacteria. Among the alternative IPTp drugs, mefloquine is one of the most promising treatments against malaria in pregnancy; however, this in vitro drug susceptibility study showed a low antibacterial activity against Gram positive and Gram negative bacteria [122]. Because of routine IPTp treatment in many African countries and their effects as prophylaxis may solve the lack of detection and treatment of GBS colonization during pregnancy. However, in this study no specific activity of SP and mefloquine on GBS colonization was demonstrated.

In conclusion, these data indicate that maternal colonization with GBS during pregnancy is an underestimated health problem of pregnant women and their newborns in Sub-Saharan Africa. Active screening and the recommended treatment must be implemented in these countries for preventing maternal GBS colonization and for protecting newborns from invasive diseases. Nevertheless, the vaccine development for maternal immunization against group B streptococcus will maximize potential benefits in all low income countries.

Epidemiology of Human Herpesvirus 8 Infection in Gabon: A Cross-Sectional Survey of Pregnant Women at Delivery

This study aimed to better understand the epidemiology of HHV8 infection during pregnancy in Sub-Saharan Africa. In this study, HHV8 infections were widespread among pregnant women reaching 39%. However, infection was not associated with socioeconomic status. Also age was not associated with HHV8 infection in pregnant women. According to literature, herpes virus infections during pregnancy can lead to fatal results including spontaneous abortion, premature delivery, and miscarriage [123-125]. In this study, HHV8 infection had no influence on birth outcomes. In the US and Europe, the major transmission route of HHV-8 infection is anal intercourse [52, 126]. However, the increased level of HHV-8 prevalence during childhood in endemic areas like sub-Saharan Africa provides evidence of nonsexual horizontal transmission. During our study the cord blood samples from seropositive patients were tested for latent nuclear antigen of HHV8. This finding shows the possible passive transmission of maternal IgG. However, no transplacental transmission was determined in spite of using highly sensitive real-time PCR analysis. In summary, the data indicate that the prevalence of HHV8 infection in pregnant women is high in Sub-Saharan Africa. In future studies nonsexual horizontal transmission routes of HHV-8 infection in sub-Saharan Africa need to be investigated.

Intra-Cystic Drug Concentration of Albendazole Sulphoxide in Patients with Echinococcus granulosus Cysts

These data reveal a comparatively good tissue penetration of albendazole sulphoxide into echinococcal cysts in human patients with approximately 50% variation compared to plasma drug concentrations. A previous report indicated maximum plasma levels for albendazole-sulphoxide in the range of 0.45 to 2.96 µg/ml [127] and intra-cystic drug concentrations between 0.06 and 0.42 µg /ml [128]. Morris et al. reported approximately 20% intra-cystic concentration of albendazole sulphoxide in patients treated with 10 mg/kg albendazole for one month prior to operation [129]. Although these data are in line with our results for plasma drug concentrations, the relative intra-cystic drug concentration of albendazole sulphoxide was significantly higher in our patients. Interestingly, praziquantel, another antihelminthic drug with activity against tapeworms, led to a considerable increase in albendazole plasma levels. The activity against protoscolices was significantly higher in patients treated with a combination of albendazole and praziquantel [130]. So far, most pharmacokinetic data on target site drug concentrations derive from echinococcal cysts of the liver and information on intra-cystic drug penetration for other organs are scarce. Despite the limited number of patients in this study, our data indicate that drug penetration in cysts located in the pleural cavity and the skeletal muscle is comparable or potentially higher compared to previous data on liver cysts. Albendazole is used for treatment of human echinococcosis since the 1980s and – depending on the stage of the disease – continuous long-term albendazole therapy shows cure rates between 30% and 80%. Although the oral bioavailability of albendazole is characterized by significant variation, intra-cystic drug penetration seems less variable and comparable to plasma drug levels. Although many questions regarding the optimal medical treatment of echinococcosis remain, the use of albendazole has been shown to allow safer manipulation of liver cysts during surgery, thus minimizing the chance of recurrence. Based on our data this may similarly be true for cysts localized in other organs.[131, 132]. Further data on anthelminthic combinations therapy and the development of novel anthelminthic drugs with improved oral bioavailability are desirable to further improve the management of echinococcosis.

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6. Publications

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Short Report: Intra-Cystic Drug Concentration of Albendazole Sulphoxide in Patients with *Echinococcus granulosus* Cysts

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Abstract. Albendazole therapy—alone or in combination with surgery—remains the standard of care for human echinococcosis depending on the stage of disease. However, only limited data are available on target site concentrations in liver cysts and data for non-liver cysts are lacking. We report on intra-cystic concentrations of the biologically active metabolite albendazole sulphoxide in non-liver cysts indicating a relative intra-cystic drug concentration of 48–156% compared with plasma levels. These data are evidence for a satisfactory penetration of albendazole sulphoxide into *Echinococcus* cysts localized in other organs than the liver and underline the usefulness of albendazole therapy for this indication.

Echinococcosis is caused by the ingestion of eggs of the dog tapeworm or fox tapeworm belonging to the genus *Echinococcus*. *E. granulosus* causes considerable human morbidity and mortality, with the highest prevalence in rural areas because of close co-habitation of humans, animal intermediate hosts, and final hosts. Oncospheres migrate after ingestion to different organs and form cysts most commonly located in the liver (60–70%) and lungs (20%). Other localizations of cysts, including the kidney, spleen, muscles, bone, pancreas, central nervous system, and soft tissue, have been described but are less common.[1–3]

Treatment recommendations for *E. granulosus* cysts include long-term therapy with albendazole alone or in combination with praziquantel, percutaneous drainage, and surgical removal of the lesions.[4–6] Albendazole is a benzimidazole derivative and is highly active against echinococcal protoscolices. However, the *in vivo* therapeutic response is limited by its low and erratic bioavailability.[7–10] Nevertheless albendazole is the most commonly used drug for pre- and post-operative anthelminthic treatment and continued suppressive therapy of inoperable hydatid cysts.[11] On the background of its variable absorption, the penetration of albendazole and its active metabolite albendazole sulphoxide into cysts is of major importance for adequate *in vivo* activity. Whereas limited data are available for *Echinococcus* cysts of the liver, little is known about the intra-cystic concentrations of albendazole in patients treated for echinococcal cysts located in other organs than the liver. In this study, we aimed to assess the ability of albendazole and its active metabolite albendazole sulphoxide to penetrate into *E. granulosus* cysts of two patients with non-liver cysts.

A 60-year-old women of Turkish origin (Patient A) residing in Austria for > 5 years was diagnosed with a painless tumor on the left thigh, which became noticeable after voluntary weight loss of 25 kg. The second patient (Patient B), a 30-year-old Turkish citizen, presented at the hospital with progressive cough, night sweats, and dyspnea. Inflammation markers were increased in both patients, with C-reactive protein at 8.7 and 2.4 mg/dL for Patients A and B, respectively (normal range, < 1 mg/dL). Marked eosinophilia was recorded (550 and 3,020/μL blood for Patients A and B, respectively) in differential blood count, and serologic investigation by enzyme-linked immunosorbent assay and Western blot confirmed the diagnosis of *E. granulosus* infection. Computer tomography and magnetic resonance imaging showed a cystic lesion in the proximal *Musculus satorius* of 7.5 cm diameter in Patient A. Patient B had a distinctive pleural effusion on the right side of the lung with transgression of the diaphragm. Following current treatment recommendations, patients were prescribed 400 mg albendazole to be taken twice daily with a fatty meal for 5 and 4 days before surgical removal, respectively.[12] Histologic examination of cyst tissues confirmed diagnosis by visualization of typical protoscolices. Patients continued anthelminthic treatment for 1 month after surgery and had no signs of recurrence of *Echinococcus* cysts.

Venous blood samples for drug analysis were taken in the morning of surgery before albendazole intake and during the intervention. Undiluted cyst fluid was obtained during operation and was subsequently stored at −80°C until further analysis. Concentrations of albendazole and albendazole sulphoxide in plasma and cyst fluid were measured by a validated high-performance liquid chromatographic (HPLC) method using an "UltiMate 3000" system (Dionex, Sunnyvale, CA). Separation of albendazole sulphoxide was carried out using a Hypersil BDS-C18 column (Thermo Fisher Scientific, Waltham, MA) with an acetic acid/methanol mobile phase. The limit of quantification for albendazole and albendazole sulphoxide was 0.063 and 0.072 μg/mL, respectively. Coefficients of accuracy and precision for both compounds were < 8%.

HPLC measurement showed that, in all clinical samples, albendazole was below the limit of detection. However, albendazole sulphoxide—the active metabolite—was quantified in pre-operative and intra-operative samples in the range of 0.10 and 1.3 μg/mL in plasma. Furthermore, the analysis of cystic fluid samples also showed albendazole sulphoxide concentrations of 0.16 and 0.6 μg/mL, indicating a relative intra-cystic drug concentration of 48% and 156% compared with plasma, respectively (Table 1).

These data show—besides the known variability of plasma concentrations—good tissue penetration of albendazole sul-

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TABLE 1
Albendazole sulphoxide (μg/mL) and relative intra-cystic drug concentration

	Pre-operative plasma	Intra-operative plasma	Cyst fluid	Relative intra-cystic drug concentration
Patient A	1.26	1.24	0.59	48%
Patient B	0.18	0.10	0.16	156%

phoxide into echinococcal cysts, with ~50% variation compared with plasma drug concentrations. In general, plasma concentrations of albendazole A previous report indicated maximum plasma levels for albendazole sulphoxide in the range of 0.45–2.96 μg/mL and intra-cystic drug concentrations between 0.06 and 0.42 μg/mL.[12,13] Morris and others[14] reported an ~20% intra-cystic concentration of albendazole sulphoxide in patients treated with 10 mg/kg albendazole for 1 month before surgery. Although these data are in line with our results for plasma drug concentrations, the relative intra-cystic drug concentration of albendazole sulphoxide was significantly higher in our patients. Interestingly, in a previous study, praziquantel, another anthelminthic drug with activity against tapeworms, led to a considerable increase in albendazole plasma levels.[15] In that report, the activity against protoscolices was significantly higher in patients treated with a combination of albendazole and praziquantel.

Thus far, most data on target site drug concentrations derive from echinococcal cysts of the liver and information on intracystic drug penetration for other organs are scarce. Despite the limited number of patients in this study, our data indicate that drug penetration in cysts located in the pleural cavity and the skeletal muscle is comparable or potentially higher compared with previous data on liver cysts.

Albendazole has been used for the treatment of human echinococcosis since the 1980s and—depending on the stage of the disease—continuous long-term albendazole therapy shows cure rates between 30% and 80%. Although the oral bioavailability of albendazole is characterized by significant variation, intra-cystic drug penetration seems less variable and comparable to plasma drug levels. Although many questions about the optimal medical treatment of echinococcosis remain, the use of albendazole has been shown to allow safer manipulation of liver cysts during surgery, thus minimizing the chance of secondary recurrence. Based on our data, this may similarly be true for cysts localized in other organs.[16,17] Further data on anthelminthic combination therapy and the development of novel anthelminthic drugs with improved oral bioavailability are desirable to further improve the management of echinococcosis.

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Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative *in vitro* study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin

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Abstract

Background: Intermittent preventive treatment of malaria with sulphadoxine-pyrimethamine (SP) is recommended for the prevention of malaria in pregnancy in sub-Saharan Africa. Increasing drug resistance necessitates the urgent evaluation of alternative drugs. Currently, the most promising candidates in clinical development are mefloquine and azithromycin. Besides the anti-malarial activity, SP is also a potent antibiotic and incurs significant anti-microbial activity when given as IPTp - though systematic clinical evaluation of this action is still lacking.

Methods: In this study, the intrinsic anti-bacterial activity of mefloquine and azithromycin was assessed in comparison to sulphadoxine-pyrimethamine against bacterial pathogens with clinical importance in pregnancy in a standard microdilution assay.

Results: SP was highly active against *Staphylococcus aureus* and *Streptococcus pneumoniae*. All tested Gram-positive bacteria, except *Enterococcus faecalis*, were sensitive to azithromycin. Additionally, azithromycin was active against *Neisseria gonorrhoeae*. Mefloquine showed good activity against pneumococci but lower *in vitro* action against all other tested pathogens.

Conclusion: These data indicate important differences in the spectrum of anti-bacterial activity for the evaluated anti-malarial drugs. Given the large scale use of IPTp in Africa, the need for prospective clinical trials evaluating the impact of antibiotic activity of anti-malarials on maternal and foetal health and on the risk of promoting specific drug resistance of bacterial pathogens is discussed.

Background

Malaria in pregnancy is associated with low birth-weight [1-3], pre-term delivery [4] and maternal anaemia [5] and is therefore an important cause of maternal, perinatal, and neonatal morbidity and mortality in pregnancy and the puerperium in sub-Saharan Africa [6,7]. The World Health Organization recommends intermittent preventive treatment of malaria in pregnancy with sulphadoxine-pyrimethamine (SP-IPTp) in order to reduce adverse health outcomes for pregnant women and their offspring [8,9]. Curative doses of SP are administered during routine antenatal visits at least twice after the first trimester in HIV negative and at least three times in HIV positive women. Due to rising drug resistance of *Plasmodium falciparum* against SP, potential alternative anti-malarial drugs have been proposed for future use as IPTp [10]. These compounds include amodiaquine, azithromycin, mefloquine, and combinations of these drugs with artemisinin derivatives or chloroquine [11,12].

Bacterial infections including sexually transmitted diseases, urinary tract infections, and group B streptococcal carriage are causes for considerable morbidity and mortality in pregnant women and the unborn child. In sub-Saharan Africa adequate diagnosis and treatment of these infections are often lacking. SP belongs to the class of anti-folates exerting considerable anti-microbial activity besides its anti-malarial activity. Anti-folate antibiotics

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show clinically important activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and other pathogenic bacteria and are, therefore, used on a large scale for the treatment of urinary tract infections, skin and soft tissue infections, and in other indications [13,14]. Whereas mefloquine use is currently restricted to treating falciparum malaria, azithromycin is in use for the treatment of a variety of bacterial infections including respiratory tract infections and sexually transmitted diseases and it is under investigation for combination therapy of falciparum malaria [15,16].

IPTp with a drug exerting anti-bacterial activity may, therefore, offer a significant additional public health benefit by providing treatment for undetected or previously untreated bacterial infections in pregnant women. Conversely, widespread use of antibiotics may increase the risk for the development of drug resistance leading to future difficulties in the clinical management of bacterial infections.

This study aimed to assess the anti-bacterial activity of SP, mefloquine and azithromycin - the most promising candidate drugs for the replacement of SP for IPTp [15] - against common Gram-positive and Gram-negative bacteria *in vitro*.

Methods

The minimal inhibitory concentrations (MIC) of mefloquine, azithromycin, and SP was assessed against bacterial pathogens with medical importance during pregnancy. For this purpose, common bacterial pathogens causing urinary tract infections (*Escherichia coli, Enterococcus faecalis)*, sexually transmitted diseases (*Neisseria gonorrhoeae*), skin and soft tissue infections (*Staphylococcus aureus*) and neonatal sepsis (Group B beta-haemolytic *streptococci; i.e. Streptococcus agalactiae*) were studied. Tested microorganisms consisted of clinical isolates and American Type Culture Collection (ATCC) strains as external controls. Bacteria were grown overnight at 37°C in Mueller Hinton broth with or without 2-5% horseblood. *Neisseria gonorrhoeae* was incubated overnight at 35°C in an atmosphere containing 5% CO_2 on New York City agar and chocolate agar.

All drugs were obtained from Sigma-Aldrich (Seelze, Germany) and were first dissolved and diluted to stock solutions. Further 1:2 dilutions of stock solutions were done with culture medium in order to achieve respective drug concentrations. Mueller Hinton broth medium was commercially prepared (Merck KGaA, Darmstadt, Germany). Fastidious broth medium was used for cultivation of *N. gonorrhoeae* and was prepared as described previously consisting of 35 g Columbia broth base, 5 g glucose, 5 g yeast extract, 2 g neopeptone, and 0.75 g agarose dissolved in 960 ml of distilled water [17]. A total of 30 ml haematin solution (0.05% [wt/vol] in 0.1 M NaOH) and 5 ml Tween 80 (10% [vol/vol]) was then added. The resultant broth was sterilized by autoclaving and 6 ml of pyridoxal solution (0.1% [wt/vol]) and 1.5 ml of NAD solution (1% wt/vol) were added.

Determination of MICs

MICs were determined by employing a standard microdilution assay following Clinical and Laboratory Standards Institute (CLSI) guidelines with fastidious broth medium and Mueller Hinton broth and a bacterial turbidity of 0.5 McFarland. The final bacterial density was approximately 10^5 CFU/ml. The 96-well plates were incubated for 24 h at 35°C in a moist atmosphere containing 5% CO_2. Positive control wells contained microorganisms without antibiotics. All tests were performed in duplicate and MICs were reported as arithmetic means.

Classifiction of anti-bacterial activity

CLSI consensus cut off levels were used for the categorization of anti-bacterial activity. Azithromycin susceptibility was assessed using the following cut off levels for classification as sensitive, intermediate and resistant: *S. aureus* ≤2 μg/ml, >2 - <8 μg/ml, ≥8 μg/ml; *S. pneumonia* and *S. agalactiae* ≤0.5 μg/ml, >0.5 - <2 μg/ml, ≥ 2 μg/ml. In case of *N. gonorrhoeae*, due to a lack of CLSI recommendations, the European committee on anti-microbial susceptibility testing (EUCAST) definitions for azithromycin cut-off values (≤ 0.25 μg/ml; > 0.25 μg/ml - ≤ 0.5 μg/ml; > 0.5 μg/ml as sensitive, intermediate, resistant) were employed.

No break points are defined for the activity of azithromycin against *E. coli* and *Enterococcus* by CLSI and EUCAST. Cut off levels as proposed for *S. aureus* (≤2 μg/ml, >2 - <8 μg/ml, ≥ 8 μg/ml for susceptible, intermediate and resistant, respectively) were therefore used.

No recommendations are available for mefloquine and SP by CLSI or EUCAST and no previous publications on the interpretation of *in vitro* anti-bacterial activity of the two anti-malarial drugs were found. For the classification of SP activity - due to comparable anti-bacterial pharmacodynamics in the class of antifolate antibiotics and similar molecular weights for trimethoprim and pyrimethamine (290 and 249, respectively) - CLSI and EUCAST threshold levels of anti-bacterial activity were used as defined for trimethoprim-sulphamethoxazole. These definitions are based on trimethoprim drug concentrations and were employed for pyrimethamine to classify SP activity. The CLSI cut off levels for classification as sensitive, intermediate and resistant are the following: *S. aureus* and *E.coli*: ≤ 2 μg/ml; > 2 - < 4 μg/ml; ≥ 4 μg/ml, *S. pneumonia*: ≤ 0.5 μg/ml; > 0.5 - < 4 μg/ml; ≥ 4 μg/ml. The EUCAST cut off levels: *S. agalactiae*: ≤ 1 μg/

ml; > 1 - ≤ 2 μg/ml; > 2 μg/ml, *E. faecalis*: < 0.03 μg/ml; ≥ 0.03 μg/ml - ≤ 1 μg/ml, > 1 μg/ml. For mefloquine the threshold of drug resistance was set considering available pharmacokinetic data in human patients and published *in vitro* inhibitory concentrations against *Plasmodium falciparum* [18-24]. Thus, the threshold for drug resistance was 0.265 μg/ml for mefloquine.

Results

All bacterial isolates were sub-cultured after thawing prior to susceptibility assays (n = 34). Median MIC values of SP against Gram-positive bacteria were as follows: *S. aureus* 16 μg/ml, *S. agalactiae* 24 μg/ml, *S. pneumoniae* 4 μg/ml, and *E. faecalis* 12 μg/ml (Table 1, Table 2 and Additional File 1). Median MICs were considerably higher for *N. gonorrhoeae* and *E. coli* (256 μg/ml and 128 μg/ml, respectively). SP showed high or intermediate activity against all tested Gram-positive bacteria, whereas *E. coli* and *N. gonorrhoeae* were classified as resistant to SP.

A total of 34 bacterial isolates were evaluated for their *in vitro* drug susceptibility against mefloquine (Table 1, Table 2 and Additional File 1). The observed MIC of mefloquine was 16 μg/ml in all tested *S. aureus* and *E. faecalis* isolates. Similarly growth of *S. agalactiae* was completely inhibited at 16 μg/ml except for one isolate with a MIC of 32 μg/ml. The MIC of mefloquine against pneumococci and *N. gonorrhoeae* varied between 0.03 - 0.06 μg/ml and 4 - 16 μg/ml, respectively. Based on the observed MIC values *S. pneumonia* was classified as sensitive, *S. aureus*, *S. agalactiae*, *E. faecalis*, and *N. gonorrhoeae*, and *E. coli* (Median MIC 128 μg/ml) as resistant to the *in vitro* activity of mefloquine.

Table 2 Summary of *in vitro* anti-bacterial activity of sulphadoxine-pyrimethamine, mefloquine, and azithromycin

	Sulphadoxine/ Pyrimethamine	Mefloquine	Azithromycin
S. aureus	+	-	+
S. agalactiae	~	-	+
S. pneumoniae	+	+	+
E. faecalis	~	-	~/-
N. gonorrhoeae	-	-	+
E. coli	-	-	~

- Insufficient *in vitro* activity; ~ intermediate *in vitro* activity; + good *in vitro* activity.

Median MIC values of azithromycin were 0.5 μg/ml, 0.06 μg/ml, 0.01 μg/ml and 0.01 μg/ml against *S. aureus*, *S. agalactiae*, *S. pneumonia*, and *N. gonorrhoeae*, respectively (Table 1, Table 2 and Additional File 1). Activity against *E. coli* (median MIC 4 μg/ml) and *E. faecalis* (median MIC 4 mg/ml) was considerably weaker. *Neisseria gonorrhoeae* and all Gram-positive bacteria - except for *E. faecalis* -were classified as being sensitive to azithromycin. *Escherichia coli* strains showed intermediate drug susceptibility against azithromycin *in vitro*.

Discussion

This *in vitro* drug susceptibility study showed a broad spectrum of anti-bacterial activity of SP against Gram-positive and low activity against Gram-negative bacteria. Among the two currently proposed alternative drugs for IPTp - mefloquine and azithromycin - the latter shows

Table 1 Median minimal inhibitory concentrations (MIC) of anti-malarials against selected Gram-positive and Gram-negative bacteria

Microorganisms	Median Minimal Inhibitory Concentration (μg/ml)		
	Sulphadoxine/Pyrimethamine	Mefloquine	Azithromycin
S. aureus (n = 5)	16	16	0.5
S. agalactiae (n = 4)	24	16	0.06
S. pneumoniae (n = 5)	4	0.06	0.01
E. faecalis (n = 5)	12	16	4
N. gonorrhoeae (n = 10)	256	8	0.01
E. coli (n = 5)	128	128	4

Thresholds for drug resistance:

Mefloquine: sensitive: ≤ 0.265 μg/ml

Sulphadoxine/Pyrimethamine (threshold based on fractional pyrimethamine concentration in 1:20 combination): sensitive, intermediate, resistant

S. aureus and *E. coli*: ≤2 μg/ml; >2 - <4 μg/ml; ≥4 μg/ml,

S. pneumonia: ≤0.5 μg/ml; >0.5 - <4 μg/ml; ≥4 μg/ml,

S. agalactiae: ≤1 μg/ml; >1 - ≤2 μg/ml; >2 μg/ml,

E. faecalis: ⊠0.03 μg/ml; ≥ 0.03 - ≤ 1 μg/ml; > 1 μg/ml.

Azithromycin: sensitive, intermediate, resistant

S. aureus; *E. coli* and *E. faecalis* ≤2 μg/ml, >2 - <8 μg/ml, ≥8 μg/ml;

S. pneumonia and *S. agalactiae* ≤0.5 μg/ml, >0.5 - <2 μg/ml, ≥2 μg/ml;

N. gonorrhoeae ≤0.25 μg/ml; >0.25 μg/ml - ≤0.5 μg/ml; >0.5 μg/ml;

an even broader anti-bacterial spectrum of activity as SP with good activity against *N. gonorrhoeae*. Interestingly, previous reports indicate *in vitro* activity of mefloquine against *E. coli* [25,26]. The present study, however, demonstrated high activity of mefloquine against pneumococci and low activity against all other bacteria. Whether these findings similarly translate into clinically relevant in vivo activity of mefloquine needs further investigation since no validated resistance thresholds are available for mefloquine.

This evaluation of the anti-microbial activity of anti-malarials *in vitro* may provide the basis for further clinical evaluation. Based on our data a clinically important effect on concurrent infectious diseases in pregnant women may be anticipated for SP and azithromycin and to a lesser extent for mefloquine. Previous data show that bacterial infections including sexually transmitted diseases, pneumococcal infections, and *S. agalactiae* colonisation contribute significantly to adverse pregnancy outcome in sub-Saharan Africa [27]. Similarly, there is evidence for significant improvement of maternal and child health by routine administration of appropriate anti-microbial drugs during pregnancy [28]. Given that urinary and genital tract infections are an important cause for premature delivery, it may be speculated that the routine administration of SP and azithromycin for IPTp may confer a reduction in the rate of prematurity due to the antibiotic effect. Similarly the potential for eradication of vaginal *S. agalactiae* colonization by IPTp with SP or azithromycin might prevent cases of neonatal sepsis. However, caution must be employed by the extrapolation of data on *in vitro* activity of drugs to anticipated *in vivo* efficacy. Clinical efficacy will ultimately depend - besides the intrinsic anti-bacterial activity as assessed in this study - on drug absorption, drug concentrations at the target sites, half-lives of drugs, and the local pattern of drug resistant pathogens. Additionally a limitation of our study lies in the absence of validated thresholds for the *in vitro* activity of SP and mefloquine. The proposed levels are extrapolated from antifolate antibiotics or based on thresholds of *in vitro* activity against *P. falciparum* and need further clinical validation [17].

The next generation of IPTp drugs will be chosen based on pharmacodynamic properties and its safety, tolerability, simplicity of administration, and cost. Based on the hypothesis of a collateral health benefit by the administration of anti-malarials with activity against relevant bacterial pathogens, it may seem desirable to choose the next IPTp drug based on both its anti-malarial and anti-bacterial pharmacodynamic properties. Whether such an approach is justified or not is however to date unknown. Whereas it may look attractive to simultaneously treat concomitant and potentially deleterious bacterial infections by routine administration of anti-malarials, this strategy may also prove hazardous. Large-scale use of drugs with anti-bacterial activity may speed up the process of selection of drug resistant bacterial isolates. Interestingly, there is evidence for the development of antibiotic drug resistance by cross-resistance with anti-malarial drugs [29]. Epidemiologic evidence linking the development of quinolone resistant Gram-negative bacteria with large-scale use of chloroquine and the recent development and spread of quinolone resistant *N. gonorrhoeae* strains are illustrative examples for this phenomenon [30]. In this context the potential selection of drug resistance against anti-folate and macrolide antibiotics by the use of SP and azithromycin as IPTp is of particular concern. The threat of promoting drug resistance against commonly used antibiotics is particularly worrying for sub-Saharan Africa where microbiologic analysis of infections is rarely performed and alternative antibiotics for drug resistant pathogens are often not affordable.

Conclusion

These data indicate that sulphadoxine-pyrimethamine and azithromycin are active against a broad spectrum of bacterial pathogens whereas mefloquine's activity is restricted to pneumococci. Whether the choice of a second generation IPTp drug with broad or narrow anti-bacterial spectrum is favourable for maternal and foetal health, is currently unknown. Further clinical trials evaluating the efficacy of IPTp against concomitant bacterial infections and the impact of their large scale use on the development and spread of antibiotic drug resistance are therefore necessary to allow an informed decision on the next IPTp drug for Africa.

Additional material

Additional file 1: Minimal inhibitory concentrations (MIC) of anti-malarials against selected Gram positive and Gram negative bacteria. Listing of minimal inhibitory concentrations of all isolates.

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Authors' contributions
MR and GMN developed the concept and design of the study, were responsible for data analysis, and drafting of the manuscript. MC participated in study design, was responsible for the collection of data, and contributed

to data analysis, interpretation, and drafting of the manuscript. AM participated in study design, data analysis, and critical review of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Epidemiology and management of group B streptococcal colonization during pregnancy in Africa

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Epidemiologie und Prävention der Gruppe B Streptokokken Besiedlung während der Schwangerschaft in Afrika

Zusammenfassung Gruppe B Streptokokken stellen eine bedeutende Ursache der frühen Neugeborenen-Sepsis dar. Das in Europa und den USA eingeführte mikrobiologische Screening schwangerer Frauen auf urogenitale Gruppe B Streptokokken Kolonisation sowie die antimikrobielle Therapie bei Gruppe B Streptokokken Besiedlung reduzierte die Inzidenz der invasiven Neugeboreneninfektion durch Gruppe B Streptokokken deutlich. Für einkommensschwache Länder gibt es jedoch nur unzureichende Daten zur Prävalenz mütterlicher Gruppe B Streptokokken Kolonisation und Inzidenz invasiver Neugeboreneninfektion durch Gruppe B Streptokokken. Vereinzelte Berichte aus dem südlichen Afrika lassen jedoch annehmen, dass die Raten mütterlicher Gruppe B Streptokokken Besiedlung und assoziierter neonataler Morbidität und Mortalität ähnlich hoch sind wie in Industrieländern. Zur Entwicklung geeigneter Präventionsstrategien für das tropische Afrika sind dringend Studien zur lokalen Epidemiologie der Gruppe B Streptokokken und Kosteneffizienz potentieller Präventionsmaßnahmen notwendig.

Schlüsselwörter: Gruppe B Streptokokken, Streptococcus agalactiae, Neugeboreneninfektion, Afrika

Summary Group B streptococcal infections are a leading cause of neonatal morbidity and mortality. Maternal microbiological screening during pregnancy and intrapartum antimicrobial treatment of maternal group B streptococcus (GBS) colonization constitutes an effective prevention strategy to reduce early neonatal invasive disease due to GBS in the European and North American setting. Data on the prevalence of GBS colonization in pregnancy and incidence of neonatal invasive GBS disease are very limited for low-income regions. However, the first reports from sub-Saharan Africa indicate that GBS colonization rates may be comparable to industrialized countries and that related neonatal morbidity and mortality is of significance. Prior to the development of suitable prevention strategies, which are undoubtedly needed in resource poor settings, more evidence on GBS epidemiology in sub-Saharan Africa and assessment of cost effectiveness of different prevention strategies are essential.

Keywords: Group B Streptococcus, Streptococcus agalactiae, Neonatal sepsis, Africa

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Epidemiology and management of group B streptococcus in industrialized countries

Streptococcus agalactiae—often referred to as group B streptococcus (GBS)—is a Gram-positive bacterium that has been identified as a human pathogen since the early 1900s. GBS is of particular medical importance during

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pregnancy and the postpartum period since it may lead to invasive disease in both mother and newborn. Maternal colonization with GBS in the genitourinary and gastrointestinal tract, leading to intrapartum transmission, is a primary risk factor for early neonatal morbidity and mortality [1].

Numerous studies report the prevalence of GBS colonization in pregnant women living in high-income regions. Approximately 10–30% of pregnant women are colonized with GBS in industrialized countries [1]. Because of the considerably varying colonization rates reported in individual studies, standard specimen and culture conditions were published by the Centers for Disease Control and Prevention to enhance GBS detection and improve the comparability of individual studies. Classification of GBS is based on ten different capsular polysaccharides and a clear predominance of several serotypes has been shown with respect to the prevalence and the virulence in industrialized countries [2].

Pregnancy-associated GBS colonization may lead to adverse pregnancy outcome and invasive infection in the newborn. Neonatal invasive GBS disease is distinguished into early-onset disease (symptoms within the first 7 days of life) and late-onset disease (symptoms from day 8 to 90), both including sepsis, pneumonia and meningitis. Improved case management in industrialized countries reduced case fatality rates from 50 to 5 % [3, 4]; nevertheless, surviving neonates often recover with long-term neurological sequelae.

The introduction of prenatal screening for GBS colonization in pregnant women and intrapartum antibiotic prophylaxis in 1996 reduced the incidence of early-onset disease by 80 % to 0.28 cases per 1,000 live births in the United States [5–7]. Therefore, many national guidelines in high-income countries recommend universal GBS screening for all pregnant women between 35 and 37 weeks of gestation and intrapartum antibiotic prophylaxis in the case of GBS colonization. Oral penicillin is the antibiotic of choice based on its high activity against GBS and its comparably narrow antimicrobial spectrum. Erythromycin and clindamycin are suggested as alternatives, particularly in the case of suspected allergy to penicillin. Unfortunately, current evidence suggests that intrapartum antibiotic prophylaxis does not reduce the incidence of late-onset disease.

Epidemiology of group B streptococcus in sub-Saharan Africa and the prospect for tailored prevention strategies

For Africa, a considerable discrepancy of GBS colonization rates in pregnant women is reported, probably due to the differences in sampling strategies and microbiologic processing of samples. Reported prevalence rates vary between 1 and 32 % [8–11]. The average maternal GBS colonization rate is around 20 %, when considering only reports with adequate microbiologic methodology, appears to be comparable to those in industrialized regions [8]. Neonatal invasive GBS disease in Africa is sporadically reported with incidences up to 3 per 1,000 live births (corresponding to the preintrapartum antibiotic prophylaxis area of the high-income regions) and high case fatality rates [12]. First reports on GBS serotype distribution indicate the predominance of several serotypes, largely overlapping with the predominant serotypes from industrialized countries [3, 11, 13]. Still, for most parts of Africa, knowledge on current GBS epidemiology is still lacking [3, 4, 7, 12–14]. In addition, GBS disease burden in Africa is likely to be underestimated since cases occurring outside the healthcare facilities are not reported, infrastructure for microbial diagnosis is not available and antibiotics are used prior to the microbiological specimen collection.

For sub-Saharan Africa, no prevention strategies to reduce neonatal invasive GBS disease are available. In most African settings, maternal screening and intrapartum antibiotic prophylaxis are not implementable due to the lacking infrastructure and resources. Even in a country with high-quality medical services such as Germany, where intrapartum antibiotic prophylaxis is recommended, adherence to intrapartum antibiotic prophylaxis was remarkably low [2].

With the aim to circumvent the necessity for microbiologic screening, intrapartum vaginal disinfection attracted considerable attention as a potential tool to eliminate GBS colonization from the female genital tract. Chlorhexidine was evaluated in several clinical trials in Europe, Africa, and the United States of America for this indication, but no randomized clinical trial could demonstrate protection against early-onset disease [4]. Therefore, intrapartum disinfection with chlorhexidine is currently not seen as a viable alternative to active screening and treatment. Hence, development of a prevention strategy based on vaccines is of paramount importance, since vaccination can easily be integrated in existing healthcare structures such as antenatal care (ANC) programs [15]. Maternal immunization is a highly efficacious and cost-effective tool in preventing the infectious diseases in the neonate as previously demonstrated by the example of neonatal tetanus [16]. Maternal antibodies against type-specific polysaccharide capsular antigens were shown to be protective against invasive disease in the newborn [17]. Today, several GBS vaccine candidates have entered phase I to III clinical trials; however, it is yet unclear whether an efficacious vaccine against GBS may become available in the near future [18–21]. Even after a potentially successful development of an efficacious vaccine, the main challenge will lie in its affordability and availability for populations in resource-poor regions. Finally, more research on the microbiological and molecular epidemiology of African GBS and prospective population-based GBS surveillance is necessary to accompany vaccine development in order to inform the ongoing initiative for clinical development of vaccine candidates and to project and analyze the potential impact of such a prevention strategy.

Conclusion

Despite the fact that GBS colonization of pregnant women is a common health risk in high- and low-income regions and appropriate strategies for active screening and treatment are implemented in high-income regions, no viable preventive strategies have been defined for low-income regions. Current efforts to develop a vaccine for maternal immunization against GBS and protecting newborns from invasive GBS disease are propitious. However, further molecular epidemiologic studies on the serotype distribution of GBS in low-income regions are needed to adapt the ongoing vaccine development to maximize the potential benefits for low-income regions.

Conflict of interest
The authors declare that there is no conflict of interest.

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PERSONAL PROFILE

Self-motivated, creative and open-minded clinical research associate

Experienced in clinical and laboratory research

Excellent in working with others

Teaching experience with technicians and students

EDUCATION

2009-2015	Eberhard Karls University Tübingen, Germany PhD Degree in Biology
2004-2008	University of Vienna, Austria MSc. Degree in Biology
1998-2002	University of Mersin, Turkey BSc. Degree in Biology
1992-1998	Humanistic High School, Adana/Turkey

RESEARCH EXPERIENCE

2010-2012 Medical Research Unit, Albert Schweitzer Hospital Lambaréné/Gabon
Research Investigator

Clinical Skills:

- Developing and writing trial protocols
- Monitoring the clinical trial throughout its duration
- Archiving study documentation and correspondence
- Source data/document verification
- Preparing manuscripts for publication

Teaching Skills:
- Supervising the clinical trial site staff
- Organizing Trainings and Seminars

Laboratory Skills:
- Leading of Laboratory Examinations in Hematology, Biochemistry, Serology, Parasitology
- Conducting experiments including Polymerase-chain reaction (PCR), Real time-PCR, Southern/Western-Blot, Flow Cytometry techniques, Bacterial culture techniques, cell culture techniques

2009-2010 General Hospital – Infectious Diseases and Tropical Medicine Vienna/Austria
Project assistant
Conducting following studies:

- "Antimicrobial activities of Malaria drugs on Gram +/- bacteria"
- "*Echinococcus granulosus* Cysts and Albendazole Sulphadoxine Concentration"

Developing and maintaining projects
Preparing study reports and manuscripts
Interpreting of study results

EDUCATIONAL TRAININGS

E-learning courses
- Introduction of Good Clinical Practice (GCP) - Vienna School Clinical Research (VSCR), 2010
- Introduction to Clinical Research - Global Health Trials, 2012
- The Research Questions - Global Health Trials, 2012
- The Study Protocol – Global Health Trials, 2012
- Data safety and monitoring boards for African clinical trials, 2012

Trainings
- Introduction of Good Clinical Laboratory Practice (GCLP) - Vienna School of Clinical Research (VSCR), 2010
- Malaria "Standard operation procedure" (SOP) workshop, 2010
- GSK, Determination of Malaria Parasites, 2011

SKILLS

Languages: Fluent in English and German, Basic in French
Computer: Proficient with Microsoft Excel, PowerPoint and Word; Endnote

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