CHAPTER FIVE

Operational Research Needs Toward Malaria Elimination in China

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Abstract

Owing to the implementation of a national malaria elimination programme from 2010 to 2020, we performed a systematic review to assess research challenges in the People's Republic of China (P.R. China) and define research priorities in the next few years. A systematic search was conducted for articles published from January 2000 to December 2012 in international journals from PubMed and Chinese journals from the China National Knowledge Infrastructure (CNKI). In total, 2532 articles from CNKI and 308 articles from PubMed published between 2010 and 2012 related to malaria after unrelated references and review or comment were further excluded, and a set of research gaps have been identified that could hinder progress toward malaria elimination in P.R. China. For example, there is a lack of sensitive and specific tests for the diagnosis of malaria cases with low parasitemia, and there is a need for surveillance tools that can evaluate the epidemic status for guiding the elimination strategy. Hence, we argue that malaria elimination will be accelerated in P.R. China through the development of new tests, such as detection of parasite or drug resistance, monitoring glucose-6-phosphate dehydrogenase (G6PD) deficiency, active malaria screening methods, and understanding the effects of the environment and climate variation on vector distribution.

1. INTRODUCTION

With the completion of genome sequences and stage-specific transcriptomes of the intraerythrocytic developmental cycle of *Plasmodium falciparum* and *P. vivax*, a postgenomic era has begun and has opened the eyes of scientists to design new approaches for the prevention and control of malaria infections in the world (Gardner et al., 2002; Bozdech et al., 2003, 2008; Carlton et al., 2008; Neafsey et al., 2012). Additionally, much technical advancement was achieved in the discovery of diagnostic tools, vaccines and drug targets, as well as the biomarkers for antimalarial drug resistance (Chen et al., 2010a; Crompton et al., 2010; Hu et al., 2010; Fan et al., 2013; Miotto et al., 2013; Ariey et al., 2014). However, there were still an estimated 216 million episodes of malaria and 655,000 malaria deaths in 2010, which was less than 50% of morbidity and mortality levels in 2000 (Murray et al., 2012). Moreover, the efficacy of RTS,S/AS01E vaccine over the four-year period was only 16.8%, and the efficacy of the vaccine declined over time and with increasing malaria exposure (Olotu et al., 2013). Thus, the interruption of malaria transmission worldwide is one of the greatest challenges for global health and development communities (Alonso et al., 2011).

Much effort has been devoted to prevent and control malaria in the People's Republic of China (P.R. China), as well as basic and operational research, e.g.

the first discovery of artemisinin in the world and its use in the treatment of malaria – a medical advance that has saved millions of lives across the world, especially in the developing world. As a result, the 2011 Lasker DeBakey Clinical Research Award went to Youyou Tu, a Chinese Professor, for the discovery of artemisinin (Andersen et al., 2011; Miller et al., 2011). Despite the significant reduction of malaria over the last decade in P.R. China, considerable effort will be needed to prevent a resurgence, after launching the National Malaria Eradication Programme (NMEP). Control and eventual elimination of human parasitic disease in P.R. China requires novel, innovative approaches, particularly in areas of diagnostics, mathematical modelling, monitoring and evaluation, surveillance and public health response (Chen et al., 2012).

2. METHODS

2.1 Search strategy and selection criteria

A systematic search was conducted for articles published from January 2000 to December 2012 in international journals from PubMed and the Chinese journals from the China National Knowledge Infrastructure (CNKI), respectively. We used the following search criteria, *i.e.* (malaria or plasmodium[Title/Abstract]) AND China AND English[Language] AND ("2000/01/01"[Date - Create]: "2012/12/31"[Date - Create]), to search the MEDLINE database through PubMed. The references were exported from PubMed into Endnote X1 (Thompson Reuters, San Francisco, USA), duplicates were removed and the file was transferred to Excel 2007 (Microsoft Corp., Seattle, USA). Articles published in Chinese journals were searched using similar criteria (Figure 5.1).

Each article was assigned to at least one of the following subjects based on the keywords included in the reference: drug/drug resistance, immunology/vaccines, basic sciences, epidemiology/control, entomology/insecticides, diagnostics/detection and clinical. We excluded the following articles: review, education, software, comment, book, health promotion and commercial-related topics.

2.2 Statistical analysis

Data were processed using Excel (Microsoft, WA, USA) filters, and Excel was also used to compute polynomial regression for references published in CNKI and index analysis for references published in PubMed.

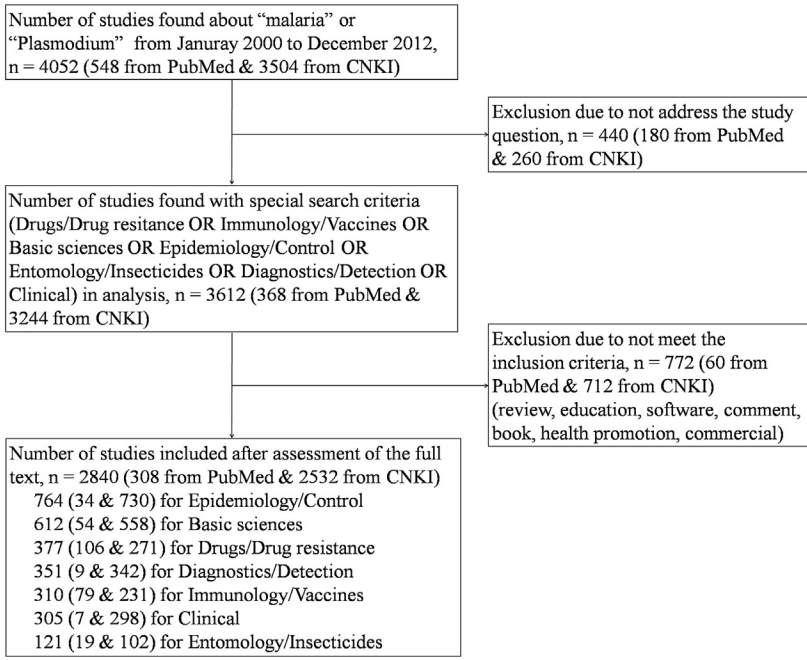

Figure 5.1 Flowchart visualizing the procedure for identifying relevant articles.

3. RESEARCH CHALLENGES IN THE STAGE OF MALARIA ELIMINATION

3.1 General information

There were far fewer references located from PubMed than from CNKI (Figure 5.2). In contrast to a binomial tendency of references published in CNKI ($R^2 = 0.8621$), an exponential increase of references published in PubMed ($R^2 = 0.8918$) was observed from 2000 to 2012.

In total, 308 references were found for malaria research in P.R. China from PubMed after unrelated references and reviews or comments were excluded from the search results. The remaining references were categorized according to subject, including drugs or drug resistance (34.4%), immunology or vaccines (25.6%), basic sciences (17.5%), epidemiology or control (11.0%), entomology or insecticides (6.2%), diagnostics or detection (2.9%) and clinical (2.3%) (Figure 5.3). The distribution of references in seven categories was much different from the distribution in a previous report about malaria references from the Asia Pacific Malaria Elimination Network (APMEN) (Andersen et al., 2011).

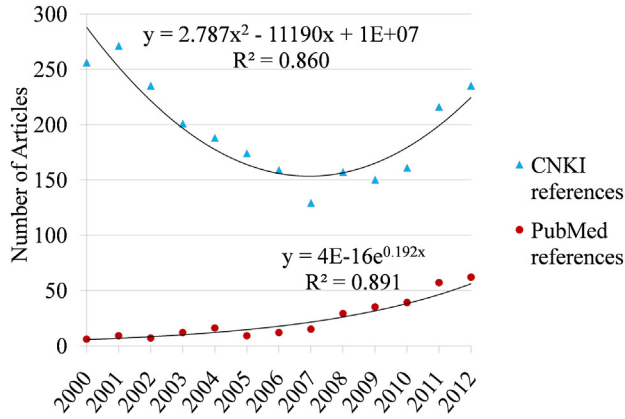

Figure 5.2 Distribution of references for malaria research in P.R. China searched from PubMed and CNKI.

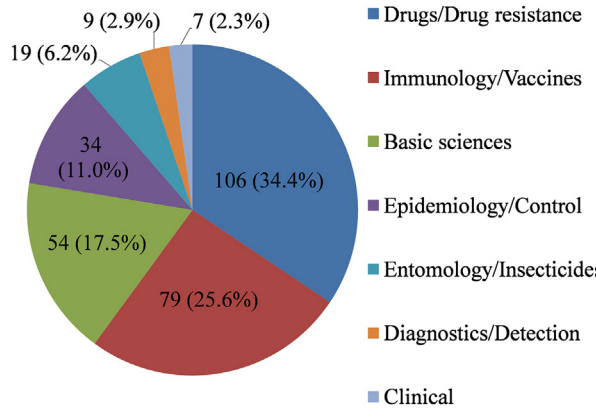

Figure 5.3 Percentage distribution of references for the malaria research in P.R. China searched from PubMed.

In total, 2532 references were found for malaria research in P.R. China from CNKI after unrelated references and reviews or comments were excluded from the search results. The remaining references were categorized according to subject, including epidemiology or control (28.8%), basic sciences (22.0%), diagnostics or detection (13.5%), clinical (11.8%), drugs or drug resistance (10.7%), immunology or vaccines (9.1%) and entomology or insecticides (4.0%) (Figure 5.4). The distribution of references in seven categories was much different from the distribution of references for malaria research in P.R. China from PubMed. In articles from the CNKI database, there was less research on

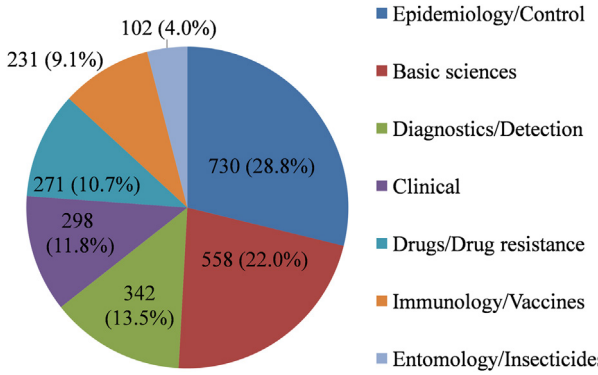

Figure 5.4 Percentage distribution of references for the malaria research in P.R. China searched from CNKI.

epidemiology/control, as well as on diagnostics/detection, and more research on immunology/vaccines.

Research references in the basic sciences and clinical sciences of malaria were excluded in the following analysis because they were not in close connection with the prevention and control of malaria.

3.2 Diagnostics and detection

Diagnostics and detection accounts for nine references from PubMed and 342 from CNKI, respectively (Figures 5.3 and 5.4). When the composition of research topics was analysed for diagnostics and detection, it was shown that most references focused on diagnostic tools, such as polymerase chain reaction (PCR), rapid diagnostic tests (RDTs), microscopic examination (eight from PubMed and 245 from CNKI), and imported and border malaria (one from PubMed and 97 from CNKI), while no reference concentrated on detection of low parasitemia and case tracing (Figure 5.5).

In the last few years, innovative tools to detect *Plasmodium* infection were developed and improved (Chen et al., 2012). The loop-mediated isothermal amplification (LAMP) test is a high-performance method for detecting DNA, which holds promise for use in the first-line battle against malaria. LAMP uses a set of primers that initiate large-scale nucleic acid synthesis by *Bst* DNA polymerase at isothermal conditions. It has been claimed that the LAMP method can detect as few as 100 copies of DNA template in blood samples (equal to roughly five parasites/μl of blood). This sensitivity is notably higher than any currently known immunochromatography-based malaria rapid diagnostic test (RDT) as recommended by the World Health Organization (WHO) as

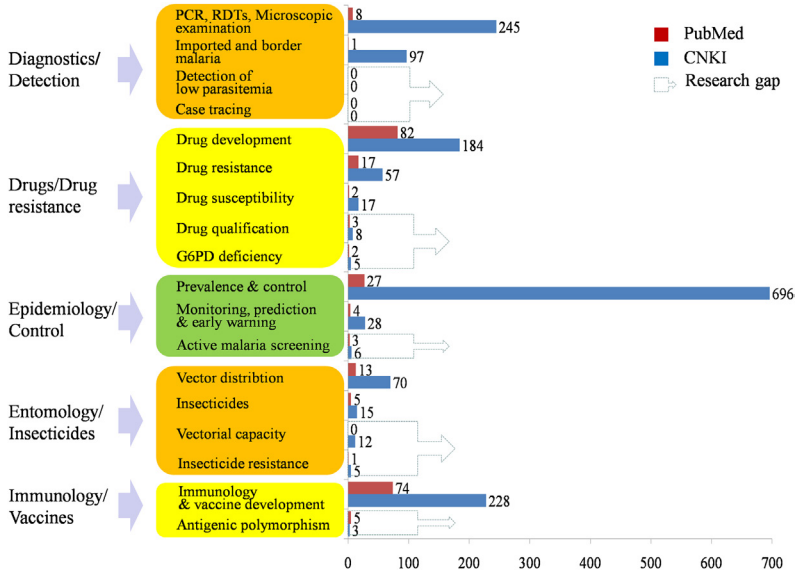

Figure 5.5 Distribution of malaria-related articles in P.R. China by topic searched from PubMed and CNKI.

part of the global malaria control strategy (Moody, 2002). A visualized LAMP method was established by the addition of a microcrystalline wax dye capsule containing the highly sensitive DNA fluorescence dye SYBR Green I to a normal LAMP reaction prior to the initiation of the reaction (Tao et al., 2011). Although further validation is needed and indeed ongoing, we can conclude that this novel, cheap and quick visualized LAMP method is feasible for malaria diagnosis under resource-constrained field settings in rural parts of P.R. China.

3.3 Drugs and drug resistance

In total, 106 references from PubMed and 271 references from CNKI were identified as associated with drug development and drug resistance (Figures 5.3 and 5.4). The composition of papers on drug and drug resistance are shown in Figure 5.5. Most articles were associated with drug development (82 from PubMed and 184 from CNKI) and drug resistance (17 from PubMed and 57 from CNKI), followed by drug susceptibility (two from PubMed and 17 from CNKI), drug quantification (three from PubMed and eight from CNKI) and Glucose-6-phosphate dehydrogenase (G6PD) deficiency (two from PubMed and five from CNKI).

The research on antimalarial drugs has a long history in P.R. China, with notable achievements. Due to the first discovery of artemisinin in the world, Chinese

scientists have paid close attention to the antimalarial studies using artemisinin and artemisinin derivatives (Li et al., 2000, 2010; Wang et al., 2010; Liu et al., 2011; Zheng et al., 2013). However, *P. falciparum* parasites with reduced in vivo susceptibility to artemisinin derivatives have emerged in western Cambodia (Noedl et al., 2008; Dondorp et al., 2009, 2010; Amaratunga et al., 2012; Phyo et al., 2012).

3.4 Epidemiology and disease control

Epidemiology and disease control accounts for 34 references from PubMed and 730 references from CNKI, respectively (Figures 5.3 and 5.4). Of these papers (Figure 5.5), most references report disease prevention and control (27 from PubMed and 696 from CNKI). The remaining references were found to be related to monitoring, prediction and early warning (four from PubMed and 28 from CNKI) and active malaria screening (three from PubMed and six from CNKI).

In P.R. China, continued effort has restrained the transmission and re-emergence of malaria (Zhou et al., 2013b). The NMEP needs to establish effective surveillance response systems tailored to local transmission patterns. Currently, the malaria transmission in P.R. China can be divided into three major strata in terms of intensity. The first stratum focuses on the southern and southwestern regions. The second stratum focuses on the central part of the country. The remaining areas, where malaria transmission is very low or might have been interrupted, belong to the third stratum (Yang et al., 2012).

Malaria transmission is influenced by various factors including climatic and nonclimatic factors. The spatial and seasonal distribution of malaria is largely determined by climate, and climatic factors including rainfall, temperature and humidity have been widely used and recognized in the malaria early warning system (MEWS). However, climatic factors are not enough for MEWS, which requires comprehensive and integrated indicators. To predict the timing and severity of malaria epidemics in MEWS, epidemiological surveillance indicators (e.g. slide positivity rates) should be considered (Bi et al., 2012).

3.5 Entomology and insecticides

In all references, 19 papers from PubMed and 102 papers from CNKI were identified as associated with entomology and insecticides (Figures 5.3 and 5.4). When the composition of research topics was analysed for entomology and insecticides (Figure 5.5), it was shown that the highest number of reported literatures was on the topic of distribution of vectors (13 from PubMed and

70 from CNKI), followed by insecticides (five from PubMed and 15 from CNKI) and vectorial capacity (zero from PubMed and 12 from CNKI).

In the NMEP of P.R. China vector control is one of the important components in rapid response to the malaria transmission in outbreak foci as well as to improve the efficiency of case management, which has been promoted by both the WHO and Roll Back Malaria Partnership (RBM) for reduction of malaria transmission (Pan et al., 2012). One of the most difficult issues in the elimination process is to have real-time surveillance and response systems to monitor the changes of transmission patterns in order to guide the elimination efforts in the high risk areas (Yang et al., 2010). In order to better understand the role of the vector in the transmission of malaria during outbreaks, the vector capacity of *Anopheles sinensis* in the Huanghuai valley of central P.R. China was investigated. The study suggested that *P. vivax* malaria outbreaks in Huanhuai valley is highly related to the enhancement in vector capacity of *An. sinensis* for *P. vivax*, which was attributed to the local residents' habits and the remarkable drop in the number of large livestock leading to the disappearance of traditional biological barriers (Pan et al., 2012).

3.6 Immunology and vaccines

Immunology and vaccines account for 79 references from PubMed and 231 references from CNKI, respectively (Figures 5.3 and 5.4). The composition of papers reported on immunology and vaccines are shown in Figure 5.5. It was shown that most references focused on immunology and vaccine development (74 from PubMed and 228 from CNKI) rather than antigenic polymorphism (five from PubMed and three from CNKI).

Malaria vaccine and related immunological studies are essential and important in the global malaria communities. Until now, protective efficacy of the RTS,S/AS01 *P. falciparum* malaria vaccine was 55%, and the overall reduction in severe malaria was 35% (Agnandji et al., 2011, 2012; White, 2011; Agnandji et al., 2012; Daily, 2012). The most effective malaria vaccination, up to date, is on the immunization of human volunteers by means of repeated exposure to live *P. falciparum* sporozoites through bites of sporozoite-infected *An. stephensi* mosquitoes; parasitemia developed in none of the 10 immunized volunteers but did develop in all five nonimmunized volunteers (Campbell, 2009; Roestenberg et al., 2009). Recently, *P. falciparum* sporozoites (PfSPZ) vaccine, composed of attenuated, aseptic, purified, cryopreserved PfSPZ, was administered four to six times intravenously to adults. Zero of six subjects who received five doses of 1.35 x

10^5 PfSPZ vaccine developed into malaria episode after controlled human malaria infection, a standard process in malaria vaccine trials that the vaccinee was immunized by the bites of mosquitoes carrying the PfSPZ Vaccine. These data indicate that high-level protection against malaria can be achieved with intravenously administration of the vaccine that is safe and meets regulatory standards (Good, 2013; Seder et al., 2013). Malaria scientists at Second Military Medical University and Tongji University School of Medicine, Shanghai, P.R. China have developed PfCP2.9, a fusion protein containing domain III of AMA1 strain 3D7 and the 19 kDa c-terminal portion of MSP1 strain K1/FVO, expressed in *Pichia pastoris* and adjuvanted with Montanide ISA 720. PfCP2.9 was the only vaccine candidate from P.R. China listed on the WHO malaria vaccine rainbow table (Qian et al., 2002; Pan et al., 2004; Zhang et al., 2005; Hu et al., 2008; Xue et al., 2010; Schwartz et al., 2012).

Vaccines based on polymorphic malaria proteins may not elicit responses against all variants of the target antigen circulating in the parasite population, thus it is important to understand the natural variation in the frequency of polymorphisms in a malaria vaccine antigen (Takala et al., 2007). Genetic diversity was only analysed in several vaccine candidates in P.R. China, such as MSP1 and GLURP of *P. falciparum* (Zhu et al., 2002; Pan et al., 2010) and AMA1, MSP1, Pvs25 and Pvs28 of *P. vivax* (Figtree et al., 2000; Feng et al., 2011).

Currently, the most promising vaccine, RTS,S, is in phase III clinical trials in various African countries; however, both the efficacy (30–50%) and the duration of protection (a few months) are limited (Good, 2013), and the vaccines are far from the usage in the prevention and control of malaria, thus it is excluded in the further analysis.

3.7 Research gap analysis

A series of research gaps have been identified that will hinder progress toward malaria elimination in P.R. China (Figures 5.5 and 5.6). First, in the control and pre-elimination stage, PCR, RDTs and the microscopic examination of a slide were commonly used in the field, but there is a lack of sensitive and specific tests for the diagnosis of malaria cases with low parasitemia and tracing the original source of *Plasmodium* parasites. Besides that, most references focused on detection of imported malaria, while only one reference discussed transmission patterns of malaria in the border areas where more mobile population crossed the border frequently.

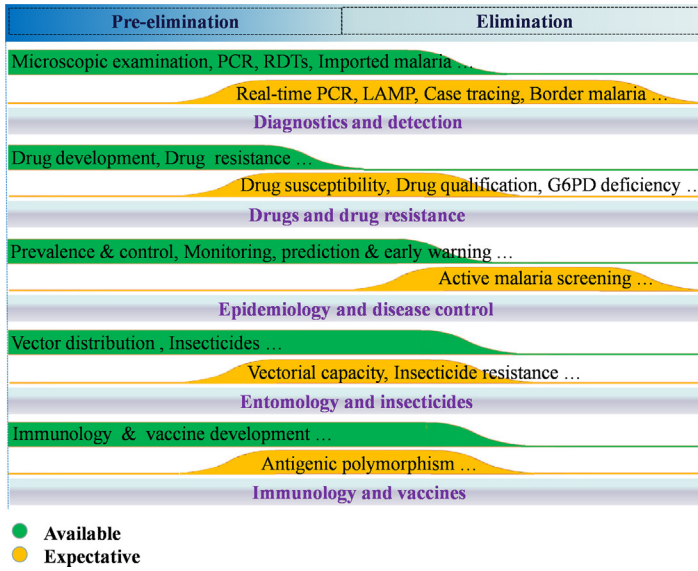

Figure 5.6 Gap analysis of research references toward malaria elimination in P.R. China, searched from CNKI and PubMed.

Second, the research output of drug development and drug susceptibility accounted for the highest number of publications in the field of drugs and drug resistance. However, it is short of innovative and efficient techniques for screening G6PD deficiency and monitoring the drug qualification. In the past few years, researchers have paid great attention to drug resistance, and it is still the research focus during the pre-elimination/elimination stage in China.

Third, a great number of articles focused on prevalence and control, monitoring, prediction and early warning of malaria, which suggests that monitoring and controlling the transmission of malaria is a key priority during the pre-elimination stage in P.R. China. Furthermore, there is also an urgent need for surveillance tools that can evaluate the epidemic status for guiding the elimination strategy.

Fourth, further study is warranted to investigate the effect of the environment and climate variation on vector distribution during the pre-elimination/elimination stage despite efforts by researchers concentrated simply on vector distribution in the past. Meanwhile, it is an essential to pay more attention to the assessment of insecticidal impact on the malaria mosquito's vectorial capacity and keep a close eye on insecticide resistance in the future investigation.

Fifth, although a lot of articles have been focused on immunology and vaccines development rather than antigenic polymorphism in malaria, *Plasmodium* parasite infections do not readily evoke an effective protective immunity against reinfection. Possible reasons for this include the ability of the parasites to interfere with the host's immune response and to evade the response in an immune host by, for example, exploiting antigenic polymorphism. Hence, there is a strong need to investigate antigenic polymorphism in malaria and its implication for immune evasion.

4. RESEARCH PRIORITY TOWARD MALARIA ELIMINATION IN CHINA

4.1 Development of detection techniques for *Plasmodium* with low parasitemia

Early diagnosis followed by treatment is an essential component to the national malaria elimination programme. Microscopy is regarded as the gold standard for malaria diagnosis (WHO, 1999) and is used as the principal means for diagnosis and surveillance of malaria in P.R. China as well. However, the lack of skilled technologists in medical facilities in endemic areas often leads to poor interpretation of data. Furthermore, microscopy is time-consuming and labour-intensive, cannot detect sequestered *P. falciparum* parasites (Leke et al., 1999) and is less reliable at low-density parasitaemia, such as parasitaemia < 50 parasites $(\mu l \text{ blood})^{-1}$ (Kilian et al., 2000; Bell et al., 2005). Most rapid diagnostic tests (RDTs) are made based on antigen capture, and do not work with low parasitemias (Zhou et al., 2013a). By now, there are several *Plasmodium* antigens for diagnosis targets, such as histidine-rich protein-2 (HRP-2) and parasite-specific lactate dehydrogenase (pLDH). Of these, HRP-2 is able to distinguish from other *Plasmodium* parasites because of its specific expression in *P. falciparum*. Hence, recombinant antigen of the China strain is recommended as coating antigen for detection of *P. falciparum* and *P. vivax* by colloidal gold immunochromatographic assay (GICA) strips which has been used in the elimination stage of the NMEP. Moreover, real-time PCR has been developed and applied for the diagnosis of malaria. Microscopic examination, GICA and real-time PCR were employed to identify parasitemia patients and people without parasitemia (Khairnar et al., 2009). There were no statistical differences in specificity among three methods, but sensitivity of real-time PCR was better than others, especially for low parasitemias.

The recently developed LAMP assay is a relatively simple and field-applicable technique to detect parasite infections that can overcome the shortage of the time-consuming and expensive purification of DNA

prior to amplification (Han et al., 2007; Chen et al., 2010b; Han, 2013). LAMP tests have recently been evaluated in detecting the infections of *P. falciparum* (Yi et al., 2010 and *P. vivax* (Zhu et al., 2010; Lu et al., 2012; Wang et al., 2012) in a China reference laboratory and a rural clinic in field, with promising results. LAMP test was evaluated for samples, from 272 outpatients at a rural Ugandan clinic. For samples, with a *P. falciparum* qPCR titer of ≥2 parasites/μL, LAMP sensitivity was 97.8%, similar to that of single well-nested PCR in a United Kingdom reference laboratory. LAMP dramatically lowers the detection threshold achievable in malaria-endemic settings, providing a new tool for diagnosis in elimination strategies (Hopkins et al., 2013).

4.2 Tracing the original source of *Plasmodium* parasites

In P.R. China, as a result of active implementation of malaria control measures for more than 40 years, considerable success to control and eliminate malaria transmission has achieved. No local infection cases within three years is one of important indexes for malaria elimination, so the tracing origin technique based on molecular biology is necessary for malaria elimination. In P.R. China, MAD20-type MSP1 allele and 3D7-type MSP2 allele were dominant in *P. falciparum* population in the Hainan Province. The mixed infection rate of different types of MSP1 or MSP2 alleles was low (Jiang et al., 2003). Moreover, MSP2 gene polymorphisms of different geographical strains of *P. falciparum* in Yunnan is evident through random amplified DNA polymorphism- PCR techniques (Yang et al., 2004).

As genetic makers, microsatellites are abundant and distributed throughout the eukaryotic genomes. Their advantages include high polymorphism and abundance, codominance, selective neutrality and high reliability. Microsatellite has been applied for the investigation of source tracing and genetic diversity (Koepfli et al., 2011; Iwagami et al., 2012, 2013). In the 1990s, microsatellites (MS) were introduced to map the chloroquine (CQ)-resistant gene (Su et al., 1996, 1997). Analysis of the population structure with microsatellites demonstrated that *P. vivax* isolates from different areas of China showed high genetic diversity and regionally centered difference (Guo et al., 2012).

The completion of many malaria parasite genomes provides great opportunities for genome-wide characterization of gene expression and high-throughput genotyping (Carlton et al., 2008; Winzeler, 2008). Substantial progress in malaria genomics and genotyping has been made recently, particularly the development of various microarray platforms for large-scale characterization of the *P. falciparum* genome. Microarray has been used for gene

expression analysis, detection of single nucleotide polymorphism (SNP) and copy number variation (CNV), characterization of chromatin modifications and other applications. In the 2000s, SNP is becoming the marker of choice because of the development of high-throughput SNP genotyping methods (Su et al., 2007; Maresso et al., 2008). SNP genotyping is actually based on the same polymorphism as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP); the difference is in the method for detecting the polymorphism. Large numbers of MS and SNP have been developed for *P. falciparum* (Su et al., 1999; Jeffares et al., 2007; Mu et al., 2007; Volkman et al., 2007) and genetic markers for other parasites such as *P. vivax* and rodent malaria parasites are also available or being developed (Grech et al., 2002; Feng et al., 2003; Martinelli et al., 2004; Li et al., 2007; Karunaweera et al., 2008). High-throughput genotyping methods are now available for typing DNA from *P. falciparum* and for mapping parasite traits, and many more typing methods are under development, including those for other malaria species. Unfortunately, the malaria parasite is a single-cell organism, and it is thus challenging to detect or measure reproducible phenotypic variation between individual parasites. The future direction for mapping malaria traits should focus on developing methods to accurately characterize and measure phenotypic variation among individual parasites.

4.3 Border malaria in P.R. China

There are more than 35 minorities distributed in Yunnan Province, and most of them are living in remote areas, including the border with other countries, such as Myanmar, Vietnam, Lao PDR, etc. Malaria prevalence is at highest level among ethnic minorities, migrants and forest workers, with the most vulnerable population being pregnant women, the very poor and the malnourished (ADB, 1998; Xia et al., 2013). The previous study investigated knowledge of malaria prevention and use of personal protection measures among ethnic minority populations in the Yunnan Province in Southwest China. Most Chinese are Han-Chinese, but the populations interviewed during this study are recognized as ethnic nationalities by the state: having culture, language and lifestyle unique to their groups (Xu et al., 2003).

Along the border areas, it is very common that people from one ethnic group may reside in several countries. They frequently cross international borders to visit family members, make cross-border marriages, and conduct trades (Xu et al., 2003), where they have exposed to mosquito biting while travelling, and finally import the disease when they go back to their villages (Xu et al., 1997). The Yunnan Province is one of the two remaining epidemic areas

of China with high annual transmission of both *P. vivax* and *P. falciparum*; the other being Hainan Island before 2010. In 2004, the annual reported malaria incidence was 3.09/10,000 (Zhou et al., 2006), although the estimated number of actual cases is at least 18 times greater (Sheng et al., 2003). Malaria is a particularly severe social and health problem along the border with Myanmar, where mobile workers move back and forth across the border and malaria control is weak. One-third of malaria cases in P.R. China came from the Yunnan Province in 2005, and about a quarter of these were actually infected in Myanmar during trips to visit relatives and conduct business (Fund, 2007).

The use of personal protection must be increased, particularly among outdoor workers that have higher risk of malaria infection. However, personal protection is widely used and widely accepted to prevent nuisance-biting mosquitoes, with the major barrier to use being affordability. Therefore, social marketing campaigns aimed at women and those that work outdoors that provide highly subsidized products, especially insecticide impregnation kits for bed nets and hammock nets are most likely to succeed in lowering malaria morbidity among non-Han-Chinese groups in rural China (Moore et al., 2008).

4.4 Drug resistance monitoring

The discovery in the 1940s that the synthetic drug CQ could effectively treat individuals safely and cheaply helped spur malaria eradication efforts in the 1950s. However, the emergence of CQ resistance diminished its therapeutic efficacy and doomed initial efforts to eradicate the disease. This is of particular importance because in recent years artemisinin class drugs, the current recommended first-line treatment for uncomplicated and severe malaria (WHO, 2006), may lose their effectiveness. However, a delayed-clearance phenotype has already been reported both in western Cambodia (Dondorp et al., 2010) and Thailand (Phyo et al., 2012). This delayed-clearance phenotype, whilst not of clinical significance yet (Phyo et al., 2012), is the first indication that resistance to artemisinin may emerge soon. This has important implications for global eradication efforts, as it will likely be at least a decade before a new compound is capable of replacing the artemisinins (Olliaro et al., 2009). The sensitivity surveillance of *Plasmodium* parasites to drug contains in vivo and in vitro. Culture system in vitro of *P. falciparum* has been well developed. However culture method in vitro of *P. vivax* is not mature yet. Two monoclonal strains of dihydroarthemisinin-resistant *P. falciparum* were obtained from chloroquine-resistant *P. falciparum* originating in Yunnan, China (Yang et al., 2013). *P. falciparum* isolates in China–Myanmar showed resistance to chloroquine and pyronaridine, and most isolates were still sensitive to piperaquine (Zhang et al., 2012).

4.5 Detection of G6PD deficiency

Primaquine is an essential tool for malaria control and elimination since it is the only available drug preventing multiple clinical attacks by relapses of *P. vivax*. It is also the only antimalarial agent against the sexual stages of *P. falciparum* infectious to mosquitoes, and is thus useful in preventing malaria transmission (Baird et al., 2011; Bousema et al., 2011). However, the difficulties of identifying glucose-6-phosphate dehydrogenase deficiency (G6PDd) greatly hinder primaquine's widespread use, as this common genetic disorder makes patients susceptible to potentially severe and fatal primaquine-induced haemolysis. The risk of such an outcome varies widely among G6PD gene variants (Howes et al., 2013b).

G6PD is a potentially pathogenic inherited enzyme abnormality and, similar to other human red blood cell polymorphisms, is particularly prevalent in historically malaria-endemic countries (Cappellini et al., 2008; Howes et al., 2012). The spatial extent of *P. vivax* malaria overlaps widely with that of G6PD deficiency; unfortunately, the only drug licensed for the radical cure and relapse prevention of *P. vivax*, primaquine, can trigger severe haemolytic anaemia in G6PD deficient individuals. According to the past and current data on this unique pharmacogenetic association, G6PDd is becoming increasingly important as several nations now consider strategies to eliminate malaria transmission rather than control its clinical burden (Wells et al., 2010). G6PD deficiency is a highly variable disorder, in terms of spatial heterogeneity in prevalence and molecular variants, as well as its interactions with *P. vivax* and primaquine. Consideration of factors including aspects of basic physiology, diagnosis and clinical triggers of primaquine-induced haemolysis is required to assess the risks and benefits of applying primaquine in various geographic and demographic settings. Given that haemolytically toxic antirelapse drugs will likely be the only therapeutic options for the coming decade, it is clear that we need to understand G6PD deficiency and primaquine-induced haemolysis in depth to determine safe and effective therapeutic strategies to overcome this hurdle and achieve malaria elimination (Howes et al., 2013a).

4.6 Active malaria screening methods

Many of malaria's signs and symptoms are indistinguishable from those of other febrile diseases. Detection of the presence of *Plasmodium* parasites is essential, therefore, to guide case management in NMEP (Bojang et al., 2000). Improved diagnostic tools are required to enable targeted treatment of infected individuals. In addition, field-ready diagnostic tools for mass screening and surveillance that can detect asymptomatic infections of very low parasite densities are needed to monitor transmission reduction and

ensure elimination. Antibody-based tests for infection and novel methods based on biomarkers need further development and validation, as do methods for the detection and treatment of *P. vivax*. Current rapid diagnostic tests (RDTs) targeting *P. vivax* are generally less effective than those targeting *P. falciparum* (malERA Consultative Group on Diagnoses and Diagnostics, 2011). Identification of parasitemia in febrile patients is essential in all of the programmatic phases of the continuum, from malaria control to elimination, although the challenges for health systems in maintaining this activity in areas where malaria has become rare will be more prominent, as will the importance of detecting asymptomatic infections of low parasite density. Analyses of past experiences and operations research are required to guide decisions on when these changes in emphasis should take place as control progresses (malERA Consultative Group on Health Systems and Operational Research, 2011; malERA Consultative Group on Modeling, 2011).

Current antigen-detecting RDTs are likely to miss a significant proportion of asymptomatic cases in low-transmission settings (Roper et al., 1996; Kidson et al., 1998; Collins et al., 1999). Thus, although the current generation of RDTs can indicate the presence of malaria in a community, they cannot determine the true prevalence of parasite carriage. Research aimed towards increasing the sensitivity of existing RDTs may not change this situation because of the limitations of the currently available technology. Some antigen or antibody detecting enzyme-linked immunosorbent assay (ELISA) are more sensitive than RDTs (Chen et al., 2011). Furthermore, because ELISAs can also be used to quantify antigen, they have been used to monitor drug efficacy, and may also facilitate high-throughput testing. However, their use is currently limited by laboratory and training requirements. Detection of antisporozoite antibodies (so-called anti-CSP antibodies) alone or in combination with antibodies to blood-stage parasites has also been suggested as a surrogate for detecting individuals with a high likelihood of carrying *P. vivax* hypnozoites to provide evidence of infection (Cho et al., 2001; Kim et al., 2003; Lee et al., 2003; Park et al., 2003; Suh et al., 2004).

4.7 Effect of the environment and climate variation on vector distribution

Malaria is transmitted to humans by mosquitoes of the genus *Anopheles*. Improved vector control is essential for the elimination or eradication of malaria in P.R. China. In regions where transmission rates are at low or moderate level, existing tools may be sufficient to achieve elimination, but in many malaria-endemic regions, new vector control interventions, including new insecticides and formulations, are needed. Better understanding of

vector biology is an essential prerequisite for the development of new control interventions (malERA Consultative Group on Vector Control, 2011). The overarching goal of malaria vector control is to reduce the vectorial capacity of local vector populations below the critical threshold needed to achieve a malaria reproduction rate (R0, the expected number of human cases that arise from each human case in a population) of less than one. Because of the long extrinsic incubation time of *Plasmodium* in its *Anopheles* vectors, the most effective vector control strategies in use today rely on insecticide interventions like indoor residual insecticide sprays (IRSs) and long-lasting insecticide-treated nets (LLINs) that reduce vector daily survival rates (Enayati et al., 2010). For many malaria-endemic regions, these tools can make substantial contributions to malaria control and may be sufficient for local malaria elimination.

5. CONCLUSIONS AND RECOMMENDATIONS

A systematic search was conducted for literatures published from January 2000 to December 2012 in the international journals from PubMed and the Chinese journals from CNKI. The number of studies found from PubMed were far fewer than those searched from CNKI. In contrast to a binomial tendency of references published in CNKI, an exponential increasing number of references published in PubMed was observed from 2000 to 2012.

In total, 308 and 2532 references were found on the malaria research carried out in P.R. China from PubMed and CNKI, respectively. Articles on drugs or drug resistance were the largest proportion of publications located from PubMed; in contrast, articles on epidemiology or control were the largest proportion of publications from CNKI.

A series of research gaps have been identified that will hinder progress toward malaria elimination in P.R. China. For example, there is a lack of sensitive and specific tests for the diagnosis of malaria cases with low parasitemia, and there is a need for surveillance tools that can evaluate the epidemic status for guiding the elimination strategy. Hence, we expected that malaria elimination could be accelerated in China through development of new tests both used in the detection of parasite or drug resistance and monitoring G6PD deficiency, active malaria screening methods, and understanding the effect of the environment and climate variation on vector distribution.

Moreover, combined with improved tools for diagnosis and surveillance and connected to effective response packages will lead to integrated, multipronged strategies for the control and elimination of malaria in P.R. China. Experiences reviewed here will be important for other middle and low income countries that are moving from morbidity control towards transmission interruption and eventual elimination of malaria.

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