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Mutation patterns in *P. falciparum* and *P. knowlesi* marker genes associated with drug resistance show discordance among mixed infection cases

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Human *Plasmodium knowlesi* infections have been reported from several Southeast Asian countries. The drug susceptibility profile of these two parasites in mixed infection cases need to be investigated. Here, we conducted PCR amplification on blood samples of malaria patients living in Andaman and Nicobar islands of India for the presence of *P. knowlesi* small subunit ribosomal RNA. Besides 18S rRNA, merozoite surface protein1 (MSP1), chloroquine resistance transporter (CRT) and dihydrofolate reductase (DHFR) genes of *P. knowlesi* were also PCR amplified from the positive samples and sequenced. The CRT and DHFR genes of other *Plasmodium* species present in these samples were also sequenced. Only 53 of 445 samples showed *P. knowlesi* specific 18S rRNA gene amplification. While three of 53 cases (5.66%) had *P. knowlesi* mono-infection, rest were co-infected with *P. falciparum* (86.79%, n=46) or *P. vivax* (7.55%, n=4), but none with *P. malariae*, or *P. ovale*. There was discordance in the drug resistance associated mutations among co-infecting *Plasmodium* species as the *P. knowlesi* isolates contained the wild type sequences of CRT and DHFR but the respective *P. falciparum* genes had mutations at the key amino acid positions associated with higher level of chloroquine and antifolate drug resistance. The mutation pattern indicates that the same patient, having mixed infection, may be harboring the drug sensitive *P. knowlesi* and a highly drug resistant *P. falciparum* parasite. We conclude that a larger human population in Southeast Asia may be at the risk of *P. knowlesi* infections than reported so far. The different drug susceptibility genotypes of *P. knowlesi* than its co-infecting *Plasmodium* species in mixed infections adds a new dimension to the malaria control program requiring formulation of the appropriate drug policy.

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