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Small subunit ribosomal RNA sequence analysis of E. histolyticastrains from rural South Africa

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Background: *Entamoeba histolytica* is a protozoan parasite and the causative agent of amoebic liver abscess and amoebic dysentery in humans. It is most common in developing countries such as Mexico, India, East and South Africa and regions of Central and South America. The estimations of the worldwide burden of amoebiasis indicated that approximately 500 million people were infected by the parasite and 10% of these individuals had invasive amoebiasis. Genetic variation among *E. histolytica* isolates collected from a wide geographical range has already been demonstrated in numerous studies. However, the level of intra-species genetic variation in *E. histolytica* populations in South Africa remains unknown.

Methods & Materials: Stool samples were collected from patients attending different health centres in the Limpopo Province and Pretoria. Genomic DNA was isolated from these samples and a nested PCR was used to amplify the 16S-like ribosomal RNA genes. The PCR amplicons were sequenced and the genetic variation as well as the phylogenetic relationships among the detected *E. histolytica* isolates was investigated.

Results: Sequence analysis revealed 13 unique genotypes grouped into 5 different clades from among 61 *E. histolytica* stool isolates. Interestingly, two strains were exactly identical, in that A was substituted by G in the same position, indicating that they are possibly from the same source. The other genotypes showed varying degree of polymorphism in the 439 bp PCR product of the 16S-like rRNA gene revealing the existence of genetic variations within the species of *E. histolytica* infecting humans.

Conclusion: In this study the presence of new *E. histolytica* genotypes in two area of South Africa exploring a small region of the 16S-like rRNA gene may be a unique finding yet genotyping the whole gene would probably reveal more genetic diversity and clustering which could point towards the existence of intra-species variation in *E. histolytica* infecting humans and shed light on clinical outcome factors. Moreover, molecular phylogenetic results have demonstrated that the 16S-like rRNA gene of all *E. histolytica* isolates tested are closely clustered together and are possibly restricted to the study region and have shown how valuable the 16S-like rRNA gene is in epidemiological studies.

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Asymptomatic falciparum malaria and the prevalence of Pfcrt K76T mutation: Challenges for malaria control in Nigeria

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Background: Malaria remains a public health challenge especially in sub-Sahara Africa where asymptomatic malaria in adults and pregnant women is not uncommon. In the present study, the prevalence of asymptomatic malaria was investigated in a cohort of Nigerian children (*almajiris*) and the genetic polymorphisms of CQ resistance biomarkers were assessed.

Methods & Materials: Four hundred and forty (440) apparently healthy *almajiris* aged 3-12 years were randomly enrolled in Maiduguri, Nigeria between July and December 2010. Falciparum malaria was diagnosed by microscopy and nested polymerase chain reaction (PCR). Using PCR, the genetic polymorphisms of *Plasmodium falciparum* chloroquine transporter codon 76 (*Pfcrt* K76T) and *Plasmodium falciparum* multidrug resistant locus 1 codon 86 (*Pfmdr1* N86Y) were determined in the *Plasmodium falciparum* isolated from the subjects. The ethical approval was granted by Ethics Committee, Ministry of Health, Borno State.

Results: The mean age of the subjects was 8.3 + 4.5years; with subjects < 5years accounted for 10.7% (47/440). The male subjects accounted for higher proportion – 74.5% *vs* 15.5% for male and female, respectively ($\chi^2 = 7.5$; df = 1; p = 0.00001). The prevalence of asexual parasitaemia was 12.7% (56/440) with geometric mean parasite density of 240 (160–630) parasites/μl blood. Gametocyte carriage was 8.6% (38/440). *Pfcrt* T76 mutation was detected in 5.4% (3/56) of the isolates while none of the isolates harbored *Pfmdr1* Y86 mutation.

Conclusion: Asymptomatic malaria is uncommon in children and the discovery in a cohort of Nigerian children could pose a remarkable challenge to malaria control as they could serve as reservoir of malaria infection.

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