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Thesis Title: Detection of common enteric parasites in Kolkata and characterization of the pathogenic factors of local isolates of *Giardia lamblia*

Abstract:

Background: Enteric parasitic infections remain a significant public health concern, particularly in regions with favorable environmental conditions for pathogen transmission. This study aimed to investigate the prevalence, genetic diversity, and clinical implications of enteric protozoan parasites, with a focus on *Giardia lamblia*, among diarrheal patients and asymptomatic individuals in eastern India. A cross-sectional study was conducted in the eastern region of India. Human fecal samples were collected from diarrheal patients and asymptomatic individuals from different community. Microscopic and molecular assays were performed to identify common enteric protozoan parasites. *Giardia lamblia* isolates were subjected to multilocus sequence typing (MLST) analysis using recommended genetic markers. A novel multiplex PCR-RFLP approach was developed for simultaneous detection and differentiation of G. lamblia assemblages and sub-assemblages. Additionally, genetic variability of virulence genes and genes involved in Metronidazole (MTZ) metabolic pathways was investigated. The overall prevalence of intestinal parasitic infections among diarrheal patients was 18.56%. *Giardia lamblia* (6.67%) emerged as the most prevalent protozoan, followed by *Entamoeba* spp. (4.52%), *Cryptosporidium* spp. (4.17%), and *Cyclospora cayetanensis* (3.18%). Parasitic infections showed seasonal trend, with higher rates during humid rainy seasons compared to dry winter months. Genotyping of *G. lamblia* isolates revealed two distinct assemblages: *Giardia enterica* (assemblage B) and *Giardia duodenalis* (assemblage A). Assemblage B was twice as prevalent as assemblage A. Within assemblage A, subtype AII was more common than AI. A potential correlation between assemblage A and symptomatic giardiasis was observed. The community-based survey of asymptomatic individuals revealed a higher prevalence of *G. lamblia* (10.3%) compared to symptomatic cases. Asymptomatic carriage was notably prevalent in the 2-20 years age group. Assemblage B remained the dominant genotype in asymptomatic cases, consistent with findings from symptomatic co-infections. The developed multiplex PCR-RFLP method demonstrated high diagnostic sensitivity (94.2%), specificity (100%), and accuracy (97.1%) for giardiasis diagnosis. This approach enabled simultaneous identification and differentiation of assemblages A and B, as well as subtyping of AI, AII, BIII, and BIV. Genetic analysis of virulence genes and MTZ metabolic pathway genes revealed higher allelic diversity and non-synonymous single nucleotide variations (SNVs) in assemblage B compared to assemblage A. This genetic diversity may contribute to the adaptability and MTZ inactivation capability of assemblage B parasites. This study provides comprehensive insights into the epidemiology and genetic characteristics of enteric protozoan parasites, particularly *G. lamblia*, in eastern India. The high prevalence of *G. lamblia* in both symptomatic and asymptomatic populations underscore its public health importance. The dominance of assemblage B and its extensive genetic variability suggest potential implications for parasite adaptability and drug resistance. The observed genetic diversity in virulence and MTZ metabolism genes highlights the need for continued surveillance of drug resistance and the development of alternative treatment strategies.

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