ORIGINAL ARTICLE INFECTIOUS DISEASES

Combined stool-based multiplex PCR and microscopy for enhanced pathogen detection in patients with persistent diarrhoea and asymptomatic controls from Côte d'Ivoire

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Abstract

Infectious diarrhoea ranks among the leading causes of morbidity worldwide. Although most acute diarrhoeal episodes are self-limiting, the diagnosis and treatment of persistent diarrhoea (≥2 weeks) are cumbersome and require laboratory identification of the causative pathogen. Stool-based PCR assays have greatly improved the previously disappointing pathogen detection rates in high-income countries, but there is a paucity of quality data from tropical settings. We performed a case–control study to elucidate the spectrum of intestinal pathogens in patients with persistent diarrhoea and asymptomatic controls in southern Côte d'Ivoire. Stool samples from 68 patients and 68 controls were obtained and subjected to molecular multiplex testing with the Luminex® Gastrointestinal Pathogen Panel (GPP), microscopy and rapid antigen detection tests for the diagnosis of diarrhoeagenic pathogens. Overall, 20 different bacteria, parasites and viruses were detected by the suite of diagnostic methods employed. At least one pathogen was observed in 84% of the participants, and co-infections were observed in >50% of the participants. Enterotoxigenic *Escherichia coli* (32%), *Giardia intestinalis* (29%) and *Shigella* species (20%) were the predominant pathogens, and *Strongyloides stercoralis* (10%) was the most prevalent helminth. Pathogen frequencies and numbers of co-infections were similar in patients and controls. Although the Luminex® GPP detects a broad range of pathogens, microscopy for helminths and intestinal protozoa remains necessary to cover the full aetiological spectrum in tropical settings. We conclude that highly sensitive multiplex PCR assays constitute a useful screening tool, but that positive results might need to be confirmed by independent methods to discriminate active infection from asymptomatic faecal shedding of nucleic acids.

Keywords: Bacteria, Côte d'Ivoire, diagnosis, helminths, intestinal protozoa, microscopy, multiplex PCR, persistent diarrhoea, stool, viruses
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Introduction

Infectious diarrhoeal diseases constitute the fourth most common contributor to the global burden of disease, accounting for a burden of 89.5 million disability-adjusted life-years [1]. Low-income and middle-income countries are

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particularly affected, and infectious diarrhoea ranks among the three leading causes of mortality in children under the age of 5 years [2,3]. The aetiology of diarrhoea, abdominal pain and related digestive disorders may include >40 infectious pathogens, which can be grouped into bacteria, parasites (helminths and intestinal protozoa), and viruses [4]. Although many diarrhoeal episodes are self-limiting and of only light or moderate severity, a considerable proportion of patients may develop either severe acute disease or long-lasting symptoms such as persistent diarrhoea (duration: ≥2 weeks) [5]. Differentiation between several aetiological agents based on clinical presentation alone is difficult and error-prone. Hence, laboratory examinations are important to identify the causative agents of infectious diarrhoeal diseases. However, no currently available laboratory technique can diagnose the many potentially implicated pathogens with high accuracy. It follows that detection rates of intestinal pathogens are disappointing if conventional diagnostic methods are employed and only a narrow spectrum of pathogens is targeted. Prior research has shown that the causative infectious agent may remain undetected in up to 80% of stool samples obtained from patients with diarrhoea [6].

The development of molecular diagnostic methods, such as PCR assays, has considerably increased the diagnostic sensitivity for the detection of many intestinal pathogens [7]. Recently, several multiplex PCR panels that may detect multiple diarrhoeagenic pathogens concurrently in a single stool sample have become available, and they improve the diagnostic yield in symptomatic patients with digestive disorders. The Luminex® xTAG Gastrointestinal Pathogen Panel (GPP; Luminex® Corporation, Austin, TX, USA) is a technique that uses fluorescent beads to allow for the concurrent detection of 15 pathogens: nine bacteria, three viruses, and three intestinal protozoa [8]. The Luminex® GPP is licensed for use in the USA, Canada, and Europe, where it is being increasingly employed in hospitals [9,10] and travel clinics for the diagnosis of acute diarrhoea [11,12]. So far, however, experience with a multiplex Luminex® platform for the diagnosis of intestinal pathogens in the tropics is scant. Additionally, it remains to be elucidated how this multiplex assay performs if applied to stool specimens from asymptomatic individuals, especially in settings of high endemicity, where multiple pathogens are the norm rather than the exception. It is conceivable that a combination of different diagnostic tests may lead to more accurate results [13]. Most commercially available multiplex PCR assays do not cover an extensive panel of parasitic pathogens, despite the fact that these are considered to be important causative agents of diarrhoeal diseases in the tropics [4]. Thus, a combination of diagnostic methods has been proposed for the comprehensive assessment of diarrhoea and related digestive disorders in such settings.

Here, we report findings from a study that employed a suite of diagnostic methods, including the Luminex® GPP, parasitological microscopic examinations, and rapid antigen detection tests, for a detailed diagnostic work-up of patients presenting with persistent diarrhoea. The study was carried out in the southern part of Côte d'Ivoire towards the end of 2012, and also included asymptomatic controls.

Materials and methods

Ethics statement

Individuals aged ≥12 months with residency in Dabou, southern Côte d'Ivoire who had provided written informed consent (parents/guardians signed for individuals aged <18 years) were eligible to participate. Study approval was given by the institutional research commissions of the Swiss Tropical and Public Health Institute (Basel, Switzerland), the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (Abidjan, Côte d'Ivoire), and the Directorate of the Hôpital Méthodiste in Dabou. The study is registered in Current Controlled Trials (identifier: ISRCTN86951400). Within 2 days of enrolment, study participants were informed about their individual diagnostic test results based on microscopic analysis of stool samples and rapid diagnostic tests (RDTs). In the case of a parasitic infection, free treatment was offered, i.e. albendazole (400 mg) against soil-transmitted helminths, ivermectin (200 μg/kg) against *Strongyloides stercoralis*, praziquantel (40 mg/kg) against *Schistosoma mansoni*, and metronidazole against symptomatic *Giardia intestinalis* (400 mg three times daily for 5–7 days). Because the PCR tests were conducted several weeks after enrolment of study subjects in a reference laboratory abroad, these results did not guide the clinical management of infected individuals.

Study location, design, and population

Our exploratory study was part of a multi-country assessment to identify suitable settings for a subsequent prospective, non-experimental case–control study to be implemented in four countries. The purpose of the current investigation was to deepen our understanding of the aetiology and clinical presentations of persistent digestive disorders in the prospective study site in Côte d'Ivoire. The research is coordinated by the European research network with the acronym NIDIAG, which aims to develop simple and cost-effective diagnostic–treatment algorithms for three clinical syndromes (i.e. persistent digestive disorders [4], persistent fever [14] and neurological disorders [15]) in tropical settings.

The current study was carried out over a 2-week period in October 2012 in Dabou, a small town in south Côte d'Ivoire,

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