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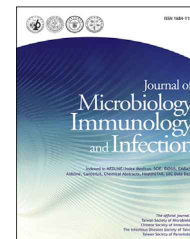

Original Article

Epidemiology, clinical features, and microbiology of patients with diarrhea in community clinics in Taiwan

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Abstract *Objective:* To investigate the clinical features and microbiology of patients with diarrheal diseases in Taiwan.
Methods: From March 2014 to October 2014, patients with diarrheal diseases referred from the community clinics were enrolled into our prospective study. Demographics and clinical features of the participants were acquired. Stool samples were examined by the Luminex Gastrointestinal Pathogen Panel assay. Data were analyzed by SAS version 9.4.
Results: A total of 545 patients were enrolled into this study. Male and adults accounted for 52.3% and 82.6% of patients, respectively. The median age was 36 years. Enteropathogen(s) was identified in 43.3% of patients and 8.5% of them had more than one agent in their stool samples. Viruses, especially norovirus GI/GII, were the predominant agents of gastroenteritis. Moreover, *Campylobacter* species was the most common bacterial agent. Bloody stool was frequently reported in patients with bacterial diarrhea ($P = 0.002$); contrarily, watery stool was significantly associated with viral diarrhea ($P < 0.0001$). Regional variation and seasonality of microbiological distribution were also observed.
Conclusion: In Taiwan, viruses were the predominant pathogens among patients with diarrheal diseases who visited community clinics. The therapeutic strategies for diarrheal patients should be based on the epidemiological and clinical characteristics.
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Introduction

Diarrheal disease remains an important medical conundrum and causes significant morbidity and mortality.[1–4] In the past decades, the distribution of enteropathogens among diarrheal patients has been reported by various studies.[2,5–13] However, most of these studies have focused on a selected population of patients who had presented to general practitioners or visited/admitted to hospital-based medical care[2,5–9]; only few reports describe general practice (GP)-based investigations of diarrheal diseases among all patients visiting community clinics.[10–13] Similarly, in Taiwan, most if not all studies concerning diarrheal diseases have focused on a specific pathogen, particular patient population, or patients with severe disease,[14–16] and no GP-based study could be found in the literature.

Conventional laboratory diagnosis of infectious diarrhea is currently based on the combined use of different tests, such as culture, microscopy, antigen detection, and real-time PCR assays. In addition to variable sensitivity and specificity, these identification methods are often laborious, time-consuming, and sometimes require skilled technicians.[17,18] In recent years, the Luminex Gastrointestinal Pathogen Panel (xTAG® GPP), one of the multiplex molecular assays, is increasingly used in clinical practice to identify common enteropathogens among patients with diarrheal diseases.[3,4,19] This test has a short turnaround time (4–5 h) and can detect 15 common enteropathogens and toxins causing infectious diarrhea in a single stool sample.[3,17]

In 2013, we conducted a pilot study using xTAG® GPP to investigate enteropathogens among diarrheal patients who visited community clinics at Taichung county. A total of 250 stool samples were collected and enteropathogens were detected in 17 of these samples (10 *Salmonella* species, 5 *Campylobacter* species, and 2 *Cryptosporidium* species). According to these preliminary data, we conducted the current study to investigate the epidemiology, clinical manifestations, and microbiology of gastroenteritis among patients of all ages who visited community clinics in Taiwan.

Materials and methods

Patients, settings, and definitions

From March 2014 through October 2014, we invited community clinics (1 clinic/200,000 population) from 4 different counties in Taiwan, including Hualien (eastern), Tainan (southern), Taichung (western), and New Taipei City (northern), to participate in our prospective study. All patients presented with an episode of diarrhea reported by the responsible clinics were enrolled. A questionnaire was given to the participants to record their basic demographics, co-morbidities, and associated symptoms at the time of stool specimen collection. Diarrhea was defined as a passage of three or more loose or liquid stools per day. The type of diarrhea was categorized as acute if <2 weeks, persistent if 2–4 weeks, and chronic if >4 weeks in duration.[20] Mixed infection was defined if more than one pathogen were identified from a stool sample. Medical history of using antibiotics, steroids, or herbs before the diarrheal episode was included in the questionnaire. A question about diarrheal illness among family members, friends, or co-workers (cluster history) was also inquired. Patients who took long-term stool softener, were treated with chemotherapeutic agents, had a history of pathological changes in their intestines (especially inflammatory bowel diseases), or were recruited into another clinical trial in recent one month were excluded from the current study. According to the testing results of stool specimen, patients were divided into two groups, group 1 (negative test result) and group 2 (positive result). This study was approved by the Institutional Review Board of China Medical University Hospital (CMUH104-REC2-009). Informed written consent was obtained from all participants or their guardians in accordance with the Declaration of Helsinki.

Specimen collection and identification of enteropathogens

The stool sample was initially collected in a sterile container, maintained in Cary–Blair transport medium (Becton Dickinson, Franklin Lakes, NJ, USA), and submitted to the central laboratory (Ruei Fu Shih Medical Lab., Taichung, Taiwan) within one day for enteropathogen analysis. Before nucleic acid extraction, each stool sample was pretreated and processed by the following steps: 100 μl stool sample, 1 ml NucliSENS easyMAG Lysis Buffer (bioMérieux, Lyon, France®) and 10 μl xTAG® MS2 were added into a Bertin SK38 Soil Grinding Lysis Bead Tubes, then vortex 5 min and incubated the tube at room temperature for 10–15 min. After centrifuging 2 min at 14000 rpm, 200 μl supernatant was removed for nucleic acid extraction. The DNA/RNA extraction of each sample was performed by QIAamp MinElute Virus Spin Kit (QIAGEN, Valencia, CA®).

Extracted and purified nucleic acid of each sample was underwent multiplex amplification by xTAG® GPP. Each PCR tube contained 10 μl extracted nucleic acid, 15 μl master mix, 2.5 μl xTAG® RNase-free water, 7.5 μl xTAG® OneStep Buffer, 2.5 μl xTAG® GPP Primer Mix, 0.5 μl xTAG® BSA (10 mg/mL), and 2.0 μl xTAG® OneStep Enzyme Mix. The multiplex amplification was carried out for 20 min followed by 38 cycles of 30 s at 95 °C for denaturation, 30 s at 58 °C for annealing, and 30 s at 72 °C for extension, and 2 min at 72 °C for the final extension.

Thereafter, each PCR product was added with 75 μl of xTAG® 0.22 SAPE which was diluted by xTAG® Reporter Buffer (contains 0.15 M NaCl). Then 5 μl final product was added with 20 μl xTAG® GPP Bead Mix for bead hybridization (3 min at 60 °C and 45 min at 45 °C). The data acquired by the Luminex® 100/200™ System (Austin, TX, USA) were analyzed by the xTAG Data Analysis Software (TDAS).

Statistics

Continuous variables were presented as the mean ± standard deviation or median (interquartile rang, IQR), and categorical variables were reported as a number and percentage. Bivariate statistical methods were used to explore data features. Multiple logistic regression analysis was done to evaluate the odds ratios (ORs) and their

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