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Original Article

Rapid detection of gastrointestinal pathogens using a multiplex polymerase chain reaction gastrointestinal panel and its role in antimicrobial stewardship

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Mixed detections

Abstract *Objectives:* The FilmArray gastrointestinal panel (FAGIP) is widely used to detect infectious diarrhoea due to its outstanding sensitivity compared to conventional methods, but there is geographic variation, such as in the distribution of pathogens, among populations.
Methods: This was a retrospective study that analysed patients with acute diarrhoea who underwent FAGIP tests from all age groups during 2022. We compared positive rates of FAGIP between paediatric (n = 245) and adult patients (n = 242) of different origins. The targeted therapy rate and antimicrobial agent use rate were also analysed.
Results: Among the 487 stool samples evaluated, the overall, community-origin (CO), and nosocomial (NC) positivity rates of paediatric patients were significantly higher than those of adults (73.9 % vs. 43.0 %, $p = 0.000$; 76.2 % vs. 51.7 %, $p = 0.000$; 50.0 % vs. 19.7 %, $p = 0.000$). *Salmonella* was the most frequently detected pathogen (35.9 %) in children, while the predominant pathogen in adult patients was toxin A/B-genic *Clostridioides difficile* (13.2 %). There was a significantly lower antimicrobial agent use rate after FAGIP results were

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available (79.1 % vs. 64.5 %, $p = 0.000$) and a higher rate of targeted therapy towards *C. difficile* infection in adults than in children (84.4 % vs. 69.0 %, $p = 0.011$).
Conclusion: Paediatric diarrhoea patients showed higher positivity rates than adult patients. Application of FAGIP for acute diarrhoea might lower unnecessary antimicrobial use.

Introduction

With the increased use of multiplex polymerase chain reaction (PCR) for stool examination, patients suffering from acute infectious diarrhoea can receive more accurate treatment promptly. This new technology has not only shortened the examination time compared to conventional methods but also enhanced our stewardship of antimicrobial agents.[1] The FilmArray Gastrointestinal panel (FAGIP), for example, is a multiplex PCR commercial kit used for syndromic testing for infectious diarrhoea with thorough quality control under internal validation and correction.[1,2] Despite its false positivity (2.8 %) or cross-reaction rates (0.42 %),[3] FAGIP is still valuable for detecting causative pathogens and informing appropriate treatment.

According to the literature, adult and paediatric diarrhoea patients have different distributions of pathogens, with enteropathogenic *Escherichia coli* (EPEC) and *Clostridioides difficile* being most frequently isolated in the former, while EPEC and enteroaggregative *E. coli* (EAEC) are common in the latter.[4] Geographic variation has been observed in the diarrhoeal pathogens from Africa, Asia, and South-Central America.[5] It is important to establish local epidemiological data among all age groups as a reference for the empirical treatment of acute infectious diarrhoea.

A previous study on the detection of common diarrhoea-causing pathogens in northern Taiwan by multiplex PCR demonstrated that the multiplex PCR method was more sensitive than conventional methods, and it detected co-infection with more than one pathogen.[6] The study was beneficial for paediatric cohorts because conventional methods could have yielded undetectable results.[6] However, the study did not show the distribution of pathogens in different age groups. Consequently, research with a comprehensive description of pathogen distributions according to age groups is urgently needed.

Given the incomplete data and inconsistent results among countries from previous studies, we aimed to analyse the distribution of detected pathogens from paediatric and adult patients with a clinical diagnosis of acute infectious diarrhoea using FAGIP and compare positive rates with those of conventional methods. Variations among age groups, ward types, and patient origins were also assessed to establish local epidemiological patterns of acute infectious diarrhoea in Taiwan. The clinical impact concerning antibiotic stewardship was studied by comparing the targeted therapy rate between adult and paediatric patients. The antimicrobial use rate and difference in types of antimicrobials used before and after availability of the FAGIP results were also analysed.

Materials and methods

Participants

From 1 January to 31 December 2022, medical charts from the emergency department, outpatients, and inpatients were searched by the laboratory information system of the China Medical University Hospital (CMUH, Taichung, Taiwan) via the FAGIP order. Patients with acute diarrhoea who underwent FAGIP tests were included, and a detailed history and examination results were collected retrospectively. Informed consent was waived due to the negligible risk to patients. All collected data were anonymized. The institutional review board (IRB) approved the collection of data from each patient. This study was approved by the IRB of the CMUH (IRB number: CMUH112-REC1-045).

Definition, diagnosis, inclusion, and exclusion criteria

Diarrhoea was defined as an unformed or liquid appearance of stool that was increased in frequency to over 200 g per day, and acute duration was defined as less than two weeks, according to Harrison's Principles of Internal Medicine, 21st edition.[7] Patients identified as having acute diarrhoea caused by noninfectious conditions such as medication or food, without classical clinical presentation of diarrhoea caused by infection, or insufficient description of clinical presentations in the chart, were excluded from the analysis. Patients under eighteen years old were categorized into the paediatric group, and those aged eighteen years or above were categorized into the adult group. Mixed infection referred to more than one pathogen detected from a stool sample. Nosocomial (NC) diarrhoea was defined as diarrhoeal events that occurred at least three days after admission; community-origin (CO) diarrhoea episodes were defined as diarrhoea episodes that developed in nonhospitalized patients or within 3 days of hospitalization, as recommended.[8] Targeted therapy for *Campylobacter* and *C. difficile* infection (CDI)-related diarrhoea was defined as the application of regimens suggested by guidelines after the FAGIP result was available. For example, azithromycin, a carbapenem, a fluoroquinolone, or doxycycline was used to target to treat infections caused by *Campylobacter* species; oral vancomycin, fidaxomicin, or metronidazole was used to treat CDI.[9–11] If *Salmonella* species were identified, antibiotic treatment was initiated if patients fulfilled the documented risk factors, such as age over 12 months or less than

50 years, immunocompromised status, haemodialysis, or fever with diarrhoea over 9 times per day that required hospitalization; otherwise, hydration with supportive care was performed without antibiotic use.[7,10] Two well-trained clinicians specializing in adult and paediatric infectious diseases independently reviewed all the collected medical charts. Standard checklists following the definition described above were used for participant inclusion or exclusion.

Data extraction and processing

Demographic data, including age, sex, specimen source, and ward type, were extracted. The whole-year positivity rates for paediatric and adult patients were also calculated. Detected pathogens were recorded to determine their distribution and mono- or mixed-infection rate calculations. The positivity rate between FAGIP and conventional methods and the antimicrobial prescription adjustment of clinicians were analysed for clinical impact and antibiotic stewardship. The reasons for discontinuing antibiotic use after the availability of FAGIP results were also documented during chart review.

Sample collection, transportation, and FAGIP testing procedure

FAGIP has been available at CMUH since August 2021. All FAGIP procedures were performed in the central lab of CMUH, which was accredited by The College of American Pathologists (CAP). The commercial Biofire FAGIP kit (BioFire Diagnostics, LLC., Salt Lake City, USA) applied in this study detects 22 pathogens, including 13 bacteria, 4 parasites, and 5 viruses. All procedures were performed according to the manufacturer's instructions.

To prepare for FAGIP testing, at least 1 g of stool sample from patients diagnosed with acute infectious diarrhoea was collected in FecalSwab™ Cary Blair transport medium (COPAN Diagnostics Inc., Murrieta, USA) and sent to the central laboratory of CMUH within 2 h. Two hundred microlitres of the specimen was extracted from the medium, added to the buffer solution, and fully mixed with hydration fluid from the injection vial kit. After submitting the specimen-buffer solution and hydration fluid to the FAGIP platform, nucleic acid extraction, reverse transcription, amplification, and analysis were conducted automatically. The results were available approximately 1 h after every run. A valid result would be "Detected", "Not detected" or "Not applicable", as confirmed by the software when the specimen run was complete and passed the internal control. A "Not applicable" result reflected the detection of EPEC with Shiga-like toxin-producing *E. coli* (STEC) or *E. coli* O157 with undetected STEC.[3]

Conventional methods for pathogen detection

If ordered by clinicians, conventional methods used to determine infectious aetiology were also extracted from medical records for the patients examined using FAGIP. Stool samples were collected in a Buffer Glycerol Saline Stool Cup (CREATIVE LIFE SCIENCE CO., LTD., New Taipei City, Taiwan) and were used to inoculate BBL™ Trypticase™ Soy Agar with 5 % Sheep Blood/Levine Eosin Methylene Blue Agar (Nippon Becton Dickinson Company, Ltd., Tokyo, Japan) and Xylose lysine deoxycholate (XLD)/Hektoen enteric (HE) Agar Plate (BioStar™, New Taipei City, Taiwan) for *Shigella* and *Salmonella* culture. For *Vibrio* culture, the BBL™ CultureSwab™ Plus Collection & Transport System for Aerobes & Anaerobes (BD, Franklin Lakes, New Jersey, USA) was used for stool collection, and thiosulfate-citrate-bile salts-sucrose (TCBS) AGAR PLATE (BioStar™, New Taipei City, Taiwan) and BBL™ Trypticase™ Soy Agar with 5 % sheep blood/leafine EMB agar (Nippon Becton Dickinson Company, Ltd., Tokyo, Japan) were applied for inoculation. The plates were cultured at 35 °C in an incubator with 5 % CO_2 for 18—24 h. Similarly, the BBL™ CultureSwab™ Plus Collection & Transport System for Aerobes & Anaerobes (BD, Franklin Lakes, New Jersey, USA) was used for stool collection and transport and was used to inoculate Campylobacter Isolated Agar (Campy Preston Blood-Free Medium; CREATIVE LIFE SCIENCE CO., LTD., New Taipei City, Taiwan). The plates were cultured at 42 °C in an incubator for 72 h for *Campylobacter* culture. The bacterial colonies from culture plates were assessed using the Bruker matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) biotyper Compass (Bruker Daltonics, Billerica, Massachusetts, USA) platform for identification.

For the diagnosis of CDI-related diarrhoea, Xpert® *C. difficile*/Epi (Cepheid®, Sunnyvale, California, USA) was used for testing. Each stool sample was collected into a sterile cup, sent to the central lab, and submitted within 24 h for automatic analysis following the manufacturer's instructions.[12]

Statistical analysis

Continuous variables are presented as the median and interquartile range (IQR), and categorical variables are shown as n (%). The Mann—Whitney *U* test was performed to compare medians between the paediatric and adult groups. The chi-square test was performed to analyse independent dichotomous variables. The McNemar test was used for paired nominal data analysis. A *P* value less than 0.05 was defined as statistically significant. SPSS Statistics, version 22 software (IBM® SPSS® Statistics, Illinois, Chicago, US) and Microsoft 365 Excel (Microsoft Corporation, Redmond, Washington DC, USA) were applied for statistical analysis.

Results

Clinical characteristics

There were 581 stool samples tested using FAGIP at CMUH. After excluding cases that did not fulfil the definition of acute diarrhoea and cases of medication-related diarrhoea (Table 1), 487 stool samples collected from 468 patients were divided into paediatric (n = 245) and adult groups (n = 242) (Fig. 1). Each stool sample sent for examination reflected an independent episode of acute diarrhoea. The median age of the paediatric group was 2.0 years, ranging from 1.0 to 4.0 (IQR), and was 56.0 years for the adult group, ranging from 34.0 to 70.0 (IQR). Inpatients accounted for the

Table 1 Analysis of excluded cohorts.

Characteristic	No. (% of patients)		
	Overall (n = 94)	Paediatrics (n = 27)	Adults (n = 67)
Positive rate (%)	19 (20.2)	10 (37.0)	9 (13.4)
Clostridioides difficile toxin A/B	9 (9.6)	4 (14.8)	5 (7.5)
Salmonella	3 (3.2)	2 (7.4)	1 (1.5)
EPEC	7 (7.4)	5 (18.5)	2 (3.0)
EAEC	1 (1.1)	0	1 (1.5)
Entamoeba histolytica	1 (1.1)	0	1 (1.5)
Norovirus GI/GII	1 (1.1)	1 (3.7)	0
Sapovirus	1 (1.1)	1 (3.7)	0

EPEC: Enteropathogenic *Escherichi coli*; EAEC: Enteroaggregative *E. coli*.

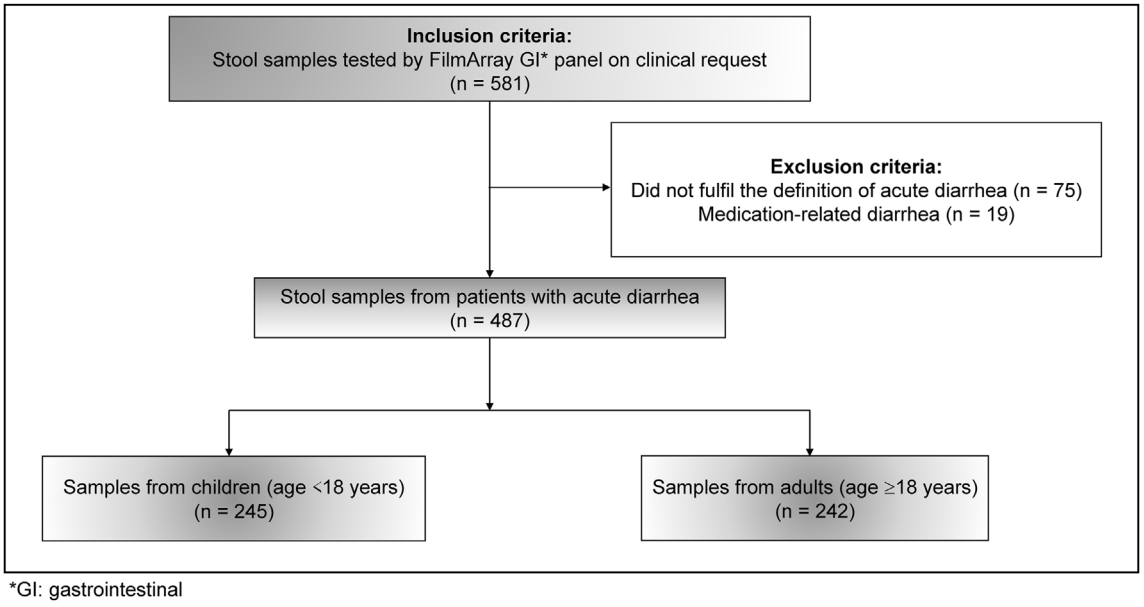

Figure 1. Algorithm of inclusion and exclusion criteria for clinical samples and grouping.

majority (83.3 % and 89.3 % in the paediatric and adult groups, respectively) of samples in both groups. The proportion of intensive care unit (ICU) adult patients was significantly higher than that of paediatric patients (17.1 % vs. 7.4 %, $p = 0.001$). In contrast, samples were collected in the emergency department (ED) from children more often than from adult patients (9.4 % vs. 1.7 %, $p = 0.000$). The overall, CO, and NC diarrhoea positivity rates were 1.7, 1.5, and 2.5 times higher, respectively, in the paediatric patients than in the adult patients (73.9 % vs. 43.0 %, $p = 0.000$; 76.2 % vs. 51.7 %, $p = 0.000$; 50.0 % vs. 19.7 %, $p = 0.000$). Similarly, the overall mixed detection rate was 3.3 times higher in paediatric patients than in adult patients (26.1 % vs. 7.9 %, $p = 0.000$) (Table 2).

Distribution of detected pathogens among different age groups

Table 3 summarizes the distribution and differences in pathogens among the paediatric and adult patients. *Salmonella* was the most frequently detected pathogen (35.9 %) in the paediatric group, followed by EPEC (18.8 %), *C. difficile* (toxin A/B, 15.9 %), and *Campylobacter* (12.2 %). In contrast, the predominant detection in the adult group was toxin A/B (13.2 %), followed by *Campylobacter* (10.7 %) and EPEC (8.7 %). There were significant differences between the paediatric and adult patients in the detection of *Salmonella* (35.9 % vs. 7.0 %, $p = 0.000$), EPEC (18.8 % vs. 8.7 %, $p = 0.001$), and Astrovirus (1.6 % vs. 0 %, $p = 0.046$).

The monthly distribution of pathogens detected in adult and paediatric patients is shown in Fig. 2A and B. In adults, *Campylobacter* (8.3 %—33.3 %, from the lowest to highest), toxin A/B (13.6 %—66.7 %), and *Salmonella* (6.7 %—33.3 %) showed no seasonal change and were detected throughout the year. On the other hand, EPEC was primarily isolated from June to November (16.7 %—41.7 %), and norovirus GI/GII often presented from January to July (9.1 %—33.3 %). In paediatric patients, *Salmonella* predominated the whole year (17.9 %—72.7 %) and was frequently isolated from July to November. There was no seasonal change in the identification of *Campylobacter* species (5.9 %—25.0 %), *C.*

Table 2 Demographic data for overall, paediatric, and adult groups.

Characteristic	No. (% of patients)			*p* value
	Overall (n = 487)	Paediatric (n = 245)	Adult (n = 242)	
Age (years), median [IQR]	17.0 [2.0–56.0]	2.0 [1.0–4.0]	56.0 [34.0–70.0]	NA
Male (%)	257 (52.8)	129 (52.7)	128 (52.9)	0.958
Source of sample (%)				
Outpatients	40 (8.2)	18 (7.3)	22 (9.1)	0.483
Emergency department	27 (5.5)	23 (9.4)	4 (1.7)	0.000
Inpatients	420 (86.2)	204 (83.3)	216 (89.3)	0.055
General ward	368 (87.6)	189 (92.6)	179 (82.9)	0.415
ICU	52 (12.4)	15 (7.4)	37 (17.1)	0.001
Positivity rate (%)				
Overall	285 (58.5)	181 (73.9)	104 (43.0)	0.000
Community origin	261 (65.4)	170 (76.2)	91 (51.7)	0.000
Nosocomial	24 (27.3)	11 (50.0)	13 (19.7)	0.000
Mixed detection (%)				
Overall	83 (17.0)	64 (26.1)	19 (7.9)	0.000
Two pathogens	65 (13.3)	52 (21.2)	13 (5.4)	0.000
Three pathogens	14 (2.9)	10 (4.1)	4 (1.7)	0.109
Four pathogens	2 (0.4)	0	2 (0.8)	0.154
Five pathogens	1 (0.2)	1 (0.4)	0	0.320
Six pathogens	0	0	0	NA
Seven pathogens	1 (0.2)	1 (0.4)	0	0.320

IQR: interquartile range; NA: not applicable; ICU: intensive care unit.

difficile toxin A/B (4.5 %–23.5 %), or EPEC (5.9 %–31.3 %). Norovirus GI/GII was primarily detected from November to May (7.1 %–44.4 %).

Differences between CO and NC diarrhoea groups

When dividing the pathogens into CO and NC groups, *Salmonella* (38.6 %), EPEC (20.2 %), and toxin A/B (14.8 %) were most frequently detected in the paediatric CO group; *Campylobacter* (14.2 %), toxin A/B (12.5 %), and EPEC (11.4 %) were predominant in the adult CO group. Among NC infections, toxin A/B was the most frequently detected in both the paediatric and adult groups (27.3 % vs. 15.1 %, respectively) (Table 4).

Analysis of antibiotic stewardship

Fig. 3A demonstrates the antimicrobial agent use rate before and after the FAGIP result was available in the subgroup that fulfilled the criteria of negative FAGIP and no further indications for antibiotic use. Antimicrobials were given as empirical therapy for 79.1 % of cases before obtaining the FAGIP report, but this decreased to 64.5 % after the results were available, a significant decline ($p = 0.000$). For those with empirical antibiotic therapy that was discontinued after FAGIP results were available, no side effects, treatment failure, or other culprits of diarrhoea could be observed. The distribution of different antimicrobials used before and after FAGIP results available is laid out in Table 5. Overall, there was significantly decreased use of cephalosporins (52.7 %–35.5 %, $p = 0.000$) and carbapenems (5.5 %–4.5 %, $p = 0.031$) and increased prescription of oral fluoroquinolones (15.5 %–19.1 %, $p = 0.031$) and others (3.6 %–6.4 %, $p = 0.008$). Ceftriaxone (28.2 %–6.4 %, $p = 0.000$) and flomoxef (9.1 %–0.9 %, $p = 0.012$) were the main antibiotics that were substantially reduced, but an increased percentage of oral cefixime use was observed (0–19.1 %, $p = 0.000$). When divided into paediatric and adult groups, the results showed similar patterns as in the overall group, with reduced ceftriaxone (41.0 %–5.1 %, $p = 0.000$ for paediatrics; 21.1 %–7.0 %, $p = 0.013$ for adults) but increased cefixime (0–33.3 %, $p = 0.000$ for paediatrics; and 0–11.3 %, $p = 0.008$ for adults) use. Comparisons between adult and paediatric patients for the targeted therapy rate for *Campylobacter* and toxin A/B showed a significantly higher proportion in the adult patients than in the paediatric patients in the targeting of toxin A/B (84.4 % vs. 69.0 %, $p = 0.011$) (Fig. 3B).

Comparison between FAGIP and conventional methods

To compare the positivity rates between FAGIP and conventional methods, *Campylobacter*, toxin A/B, and *Salmonella* were selected for analysis. Positivity rates were significantly higher by FAGIP than by conventional methods for the detection of *Campylobacter* and *Salmonella* (11.5 % vs. 1.5 %, $p = 0.000$, and 21.6 % vs. 12.6 %, $p = 0.000$, respectively) (Fig. 4A). Positivity based on conventional methods, including for *Salmonella*, *Shigella*, and *Campylobacter* cultures and the toxin A/B nucleic acid amplification test (NAAT) in 2021, showed a low average positivity rate (4.7 %, 0.0 %, and 0.3 %, for *Salmonella*, *Shigella*, and *Campylobacter* species, respectively), except for NAAT (12.8 %) (Fig. 4B).

Table 3 Distribution of detected pathogens among overall, paediatric, and adult groups.

Target	No. (% of patients)			*p* value
	Overall (n = 487)	Paediatric (n = 245)	Adult (n = 242)	
Bacteria (%)				
Campylobacter	56 (11.5)	30 (12.2)	26 (10.7)	0.604
Clostridioides difficile (toxin A/B)	71 (14.6)	39 (15.9)	32 (13.2)	0.399
Plesiomonas shigelloides	5 (1.0)	4 (1.6)	1 (0.4)	0.182
Salmonella	105 (21.6)	88 (35.9)	17 (7.0)	0.000
Vibrio (*parahaemolyticus, vulnificus* & *cholerae*)	4 (0.8)	1 (0.4)	3 (1.2)	0.309
Vibrio cholerae	1 (0.2)	1 (0.4)	0	0.320
Yersinia enterocolitica	2 (0.4)	2 (0.8)	0	0.159
Enteroaggregative *E. coli* (EAEC)	20 (4.1)	12 (4.9)	8 (3.3)	0.376
Enteropathogenic *E. coli* (EPEC)	67 (13.8)	46 (18.8)	21 (8.7)	0.001
Enterotoxigenic *E. coli* (ETEC) *lt/st*	11 (2.3)	7 (2.9)	4 (1.7)	0.371
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	3 (0.6)	1 (0.4)	2 (0.8)	0.555
E. coli O157	2 (0.4)	1 (0.4)	1 (0.4)	0.993
Shigella/Enteroinvasive *E. coli* (EIEC)	1 (0.2)	0	1 (0.4)	0.314
Parasites (%)				
Cryptosporidium	5 (1.0)	4 (1.6)	1 (0.4)	0.182
Cyclospora cayetanensis	0	0	0	NA
Entamoeba histolytica	0	0	0	NA
Giardia lamblia	0	0	0	NA
Virus (%)				
Adenovirus F40/41	1 (0.2)	1 (0.4)	0	0.320
Astrovirus	4 (0.8)	4 (1.6)	0	0.046
Norovirus GI/GII	32 (6.6)	19 (7.8)	13 (5.4)	0.289
Rotavirus A	1 (0.2)	1 (0.4)	0	0.320
Sapovirus	3 (0.6)	2 (0.8)	1 (0.4)	0.570

NA: not applicable.

Discussion

This study showed that the positivity rate for NC diarrhoea was lower than that for CO diarrhoea. According to Polage et al., noninfectious diarrhoea accounts for approximately 80.0 % of NC cases. The causes include medications, enteral feeding, and underlying diseases such as inflammatory bowel disease. In addition, bowel ischaemia and hypoalbuminaemia lead to diarrhoea, as observed in hospitalized patients. For NC diarrhoea caused by infectious disease, *C. difficile*-related diarrhoea was the most common aetiology, accounting for 10—20 % of cases,[8] and our findings were similar (15.1 % for adult and 27.3 % for paediatric patients; Table 4). Other pathogens, including pathogenic strains of *Klebsiella oxytoca* and enterotoxin-producing *Clostridium perfringens*, can also be detected after antibiotic exposure.[8] In immunocompromised populations, cytomegalovirus (CMV) needs to be taken into consideration due to its potential to cause severe morbidities.[8,13] Obviously, FAGIP cannot detect any of the above aetiologies other than *C. difficile* and should be reserved for CO diarrhoea. Clinicians must rule out noninfectious causes of diarrhoea, discontinue unnecessary antibiotics, and test for *C. difficile* or CMV based on the patient's immune status to elucidate the cause of diarrhoea.

The pathogens detected between paediatric and adult patients showed different distributions of general condition or specifying CO diarrhoea, with *Salmonella* and EPEC being the most common aetiologies of diarrhoea in the former and toxins A/B and *Campylobacter* predominating in the latter (Tables 3 and 4). Data from the USA revealed that the pathogens most detected by FAGIP were *C. difficile*, EPEC, and EAEC.[1] However, that study did not describe differences between age groups. In nationwide surveillance on community diarrhoeal disease in Taiwan, the overall most prevalent strain of enteropathogen was norovirus GI/GII, which accounted for 33.9 % of positive detections, followed by rotavirus A (17.9 %) and *Campylobacter* (16.7 %). When dividing patients into age groups to illustrate distributions of pathogens, children frequently present *C. difficile* (20.7 %)-related diarrhoea, whereas norovirus (39.2 %) is the main culprit in adults.[14] A study from China suggested that the most common viral pathogen of gastroenteritis in children was norovirus (20.5 %).[15] The results from these studies are quite different from our findings. Geographic variations and changing epidemiology might contribute to

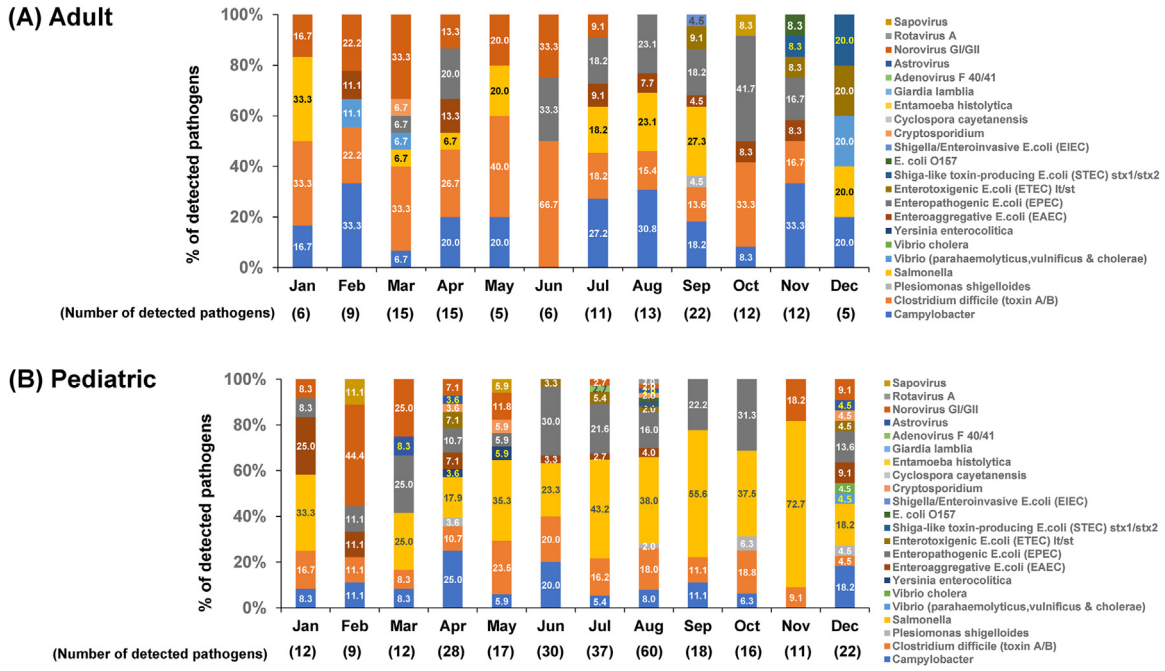

Figure 2. Monthly distribution of pathogens from (A) adult and (B) paediatric patients in 2022.

these differences. Continued surveillance of pathogens causing diarrhoeal diseases is pivotal for guiding empirical treatment, and clinicians need to rely on local epidemiological data to choose appropriate therapies.

Several studies have discussed the clinical application of multiplex PCR, and xTAG® GPP and FAGIP are the two commonly used commercial methods. The reported positivity rate is 43.3 % for the former and 35.3 % for the latter.[1,14] Our study demonstrated a higher overall 58.5 % rate. A possible explanation is the composition of the study populations. The number of paediatric patients vs. adult patients was 245 vs. 242 but 95 vs. 450 in the study conducted by Chi et al.; notably, the positivity rates in the two groups were 73.9 % vs. 43.0 % in our study and 51.6 % vs. 41.6 % in theirs.[14] It is obvious that the detection rate was higher in paediatric patients than in adult patients. In addition, the overall mixed detection rate was 17.0 %, and it was significantly different between paediatric and adult patients in our study. Traditionally, causative microorganisms leading to infection must fulfil Koch's postulates, but nonculturable or less abundant pathogens might be ignored.[16] FAGIP is more sensitive than conventional methods,[1,3,17] which might increase the rates of positivity and mixed detection. Other associated factors are the diversity of the gut microbiota and the interaction between pathogens and the host immune system. Kulka et al. reported that the intestinal microbiota is quite different between healthy children and adults, with diversity being higher in adults than in children.[18] It is well known that the microbiota can regrow and eliminate invading pathogens after infectious events, which is called pathogen clearance, a process that facilitates recovery of the inflamed intestine.[19] Tay et al. showed that the microbiota in the host gut generates colonization resistance by interacting with epithelial cells, which prevents the infecting pathogens from increasing colonization. Invading pathogens can disrupt the diversity of the gut microbiota by mediating the host immune response, which facilitates overgrowth of the pathogens.[20] Moreover, synergy between infecting microbes and resident microbiota, such as *Klebsiella pneumoniae* and *Proteus mirabilis*, by induction of virulence factors stimulates the inflammatory response and leads to coinfections.[20] Taking this evidence together, it is not surprising that paediatric patients are prone to infection by more than one pathogen or to delayed clearance, leading to a higher positive rate than in adult patients, owing to the lower abundance of the gut microbiome. Further studies are needed to gain a more detailed understanding and confirm the mechanisms of coinfection.

The clinical impact of FAGIP was well recognized by Cybulski Jr et al., who described more targeted antibiotic use than conventional culture methods.[1] Our study also focused on the issue of antibiotic stewardship and found that the targeted therapy rate for *Campylobacter* was slightly lower in adult patients than in paediatric patients, though a significantly higher value was observed for targeted therapy against *C. difficile* (Fig. 3B). According to the literature, *Campylobacter* gastrointestinal tract infection is usually self-limited, but fatal conditions sometimes occur, especially in children younger than 5 years old.[21] The median age of the paediatric patients in our study was 2.0 years old, ranging from 1.0 to 4.0, which might explain their higher targeted therapy rate than adult patients had. For CDI, paediatric patients usually present with a shorter clinical course and fewer complications than adult patients. In contrast, adults tended to have more comorbidities attributed to CDI, more severe complications, and more recurrent episodes than children.[22,23] As a result, the more targeted therapies in adults in this study was a reasonable result. In addition, we observed a significant decrease in the

Table 4 Distribution of pathogens from community-origin (CO) and nosocomial (NC) acute diarrhoea patients.

Characteristic	No. (% of patients)			
	Paediatric (n = 245)		Adult (n = 242)	
	CO (223)	NC (22)	CO (176)	NC (66)
Bacteria (%)[a]				
Campylobacter	30 (13.5)	0	25 (14.2)	1 (1.5)
Clostridioides difficile (toxin A/B)	33 (14.8)	6 (27.3)	22 (12.5)	10 (15.1)
Plesiomonas shigelloides	4 (1.8)	0	1 (0.6)	0
Salmonella	86 (38.6)	2 (9.1)	17 (9.7)	0
Vibrio (*parahaemolyticus*, *vulnificus* & *cholerae*)	1 (0.4)	0	3 (1.7)	0
Vibrio cholerae	1 (0.4)	0	0	0
Yersinia enterocolitica	2 (0.9)	0	0	0
Enteroaggregative *E. coli* (EAEC)	12 (5.4)	0	7 (4.0)	1 (1.5)
Enteropathogenic *E. coli* (EPEC)	45 (20.2)	1 (4.5)	20 (11.4)	1 (1.5)
Enterotoxigenic *E. coli* (ETEC) *lt/st*	7 (3.1)	0	4 (2.3)	0
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	1 (0.4)	0	2 (1.1)	0
E. coli O157	1 (0.4)	0	1 (0.6)	0
Shigella/Enteroinvasive *E. coli* (EIEC)	0	0	1 (0.6)	0
Parasites (%)[a]				
Cryptosporidium	2 (0.9)	2 (9.1)	1 (0.6)	0
Cyclospora cayetanensis	0	0	0	0
Entamoeba histolytica	0	0	0	0
Giardia lamblia	0	0	0	0
Virus (%)[a]				
Adenovirus F40/41	1 (0.4)	0	0	0
Astrovirus	2 (0.9)	2 (9.1)	0	0
Norovirus GI/GII	18 (8.1)	1 (4.5)	12 (6.8)	1 (1.5)
Rotavirus A	1 (0.4)	0	0	0
Sapovirus	2 (0.9)	0	1 (0.6)	0

[a] As mixed infection was observed in certain stool samples, the sum of the percentages may be above 100 %.

antimicrobial agent use rate after the availability of FAGIP results. Detailed analysis showed significantly decreased intravenous carbapenem and cephalosporin use but more utilization of oral step-down therapy, such as cefixime or fluoroquinolones. According to the literature, early switching from intravenous antibiotics to oral formulation when clinically stable could be beneficial to patients without apparent negative outcomes.[24] However, the study by Hayotte et al. suggested no reduction in antibiotic use in paediatric ICU patients despite a viral aetiology confirmed by FilmArray® Respiratory Panel.[25] These conflicting results are probably due to the different populations studied, disease severity, and other indications for antibiotic use. In a previous study, the bacterial coinfection rate was 25 %, and all subjects were from the ICU with respiratory failure, neurologic failure, or haemodynamically unstable status.[25] In contrast, our study analysed paediatric and adult patients with negative FAGIP detection and excluded those without other indications for antimicrobial use. It is plausible that FAGIP might enhance clinical practice to prevent overuse of unnecessary antibiotics and reduce broad-spectrum antimicrobials to minimize side effects, costs, and the emergence of drug-resistant pathogens.[26]

The positivity rates by FAGIP for the detection of *Campylobacter* and *Salmonella* were significantly higher than those by conventional methods, but not for the toxin A/B group. Both FAGIP and Xpert® *C. difficile*/Epi are based on PCR, but *Campylobacter* and *Salmonella* were tested by the culture method. Xpert® *C. difficile*/*Epi* is sufficiently sensitive to aid in the diagnosis of CDI-related diarrhoea, whereas FAGIP should be reserved for the survey of CO aetiologies. Clinicians should re-evaluate the reasonability of applying FAGIP only for detection of CDI to prevent waste of laboratory resources.

Seasonal changes in gastrointestinal pathogens have been suggested by several investigators, with viral aetiologies predominating in colder months[14,27] and bacterial or parasitic infections more frequently observed in warmer or rainy seasons.[28,29] Our results were slightly different, as norovirus GI/GII predominated in winter but bacterial pathogens such as *Salmonella*, *Campylobacter*, and *C. difficile* toxin A/B showed no seasonal pattern. Geographic variation and population differences might underlie the inconsistent conclusions. Thus, continuous national surveillance is important to update epidemiological data for empirical treatment and infection control policy.

Table 5 Detailed distribution of antimicrobials before and after the FilmArray gastrointestinal panel result was available in the cohort with negative detection without further indications for antibiotics.

Antimicrobials	No. (% of patients)								
	Overall (n = 110)			Paediatric (n = 39)			Adult (n = 71)		
	Before[a]	After[a]	*p* value	Before	After	*p* value	Before	After	*p* value
Cephalosporins	58 (52.7)	39 (35.5)	0.000	22 (56.4)	17 (43.6)	0.063	36 (50.7)	22 (31.0)	0.000
Cefazolin	2 (1.8)	1 (0.9)	1.000	1 (2.6)	1 (2.6)	1.000	1 (1.4)	0	1.000
Cephalexin[b]	0	1 (0.9)	1.000	0	1 (2.6)	1.000	0	0	NA
Cefixime[b]	0	21 (19.1)	0.000	0	13 (33.3)	0.000	0	8 (11.3)	0.008
Flomoxef	10 (9.1)	1 (0.9)	0.012	0	0	NA	10 (14.1)	1 (1.4)	0.012
Cefotaxime	2 (1.8)	0	0.500	2 (5.1)	0	0.500	0	0	NA
Ceftriaxone	31 (28.2)	7 (6.4)	0.000	16 (41.0)	2 (5.1)	0.000	15 (21.1)	5 (7.0)	0.013
Cefoperazone/ sulbactam	9 (8.2)	7 (6.4)	0.625	0	0	NA	9 (12.7)	7 (9.9)	0.625
Cefepime	4 (3.6)	1 (0.9)	0.250	3 (7.7)	0	0.250	1 (1.4)	1 (1.4)	1.000
Penicillins	3 (2.7)	6 (5.5)	0.250	2 (5.1)	2 (5.1)	1.000	1 (1.4)	4 (5.6)	0.250
Amoxicillin[b]	0	1 (0.9)	1.000	0	1 (2.6)	1.000	0	0	NA
Ampicillin	1 (0.9)	0	1.000	1 (2.6)	0	1.000	0	0	NA
Amoxicillin/ clavulanic acid[b]	0	2 (1.8)	0.500	0	0	NA	0	2 (2.8)	0.500
Piperacillin/ tazobactam	2 (1.8)	3 (2.7)	1.000	1 (2.6)	1 (2.6)	1.000	1 (1.4)	2 (2.8)	1.000
Fluoroquinolones	17 (15.5)	21 (19.1)	0.031	1 (2.6)	2 (5.1)	0.250	16 (22.5)	19 (26.8)	0.250
Ciprofloxacin	14 (12.7)	16 (14.5)[b]	0.791	1 (2.6)	2 (5.1)[b]	1.000	13 (18.3)	14 (19.7)[b]	1.000
Levofloxacin	1 (0.9)	4 (3.6)[b]	0.375	0	0	NA	1 (1.4)	4 (5.6)[b]	0.375
Moxifloxacin[b]	2 (1.8)	1 (0.9)	1.000	0	0	NA	2 (2.8)	1 (1.4)	1.000
Carbapenems	6 (5.5)	5 (4.5)	0.031	0	2 (5.1)	0.250	6 (8.5)	3 (4.2)	0.250
Ertapenem	1 (0.9)	0	1.000	0	0	NA	1 (1.4)	0	1.000
Meropenem	5 (4.5)	5 (4.5)	1.000	0	2 (5.1)	0.500	5 (7.0)	3 (4.2)	0.500
Others	4 (3.6)	7 (6.4)	0.008	3 (7.7)	2 (5.1)	0.125	1 (1.4)	5 (7.0)	0.125
Azithromycin[b]	2 (1.8)	0	0.500	2 (5.1)	0	0.500	0	0	NA
Doxycycline[b]	0	1 (0.9)	1.000	0	0	NA	0	1 (1.4)	1.000
Metronidazole[b]	1 (0.9)	6 (5.5)	0.125	0	2 (5.1)	0.500	1 (1.4)	4 (5.6)	0.375
Gentamycin	1 (0.9)	0	1.000	1 (2.6)	0	1.000	0	0	NA

[a] Combination therapy was given in some cases, so the sum of the overall percentages was higher than that in Fig. 3A.
[b] These antibiotics are used as oral formulations.
NA: Not applicable.

FAGIP was used in the ED more often in the paediatric population than in the adult population; in contrast, adult patients in the ICU were more prone to be tested than paediatric patients. In Taiwan, medical services such as the ED are easily available. People may choose any level of health care facility without a referral from a family doctor.[30] In addition, children with diarrhoea sometimes experience febrile presentation, severe illness, or dehydration,[31] which makes parents anxious and prompts them to seek emergency medical help and ask for further examination.[32,33] On the other hand, there were more adults among ICU patients than children at CMUH (average capacity: 200 vs. 90), which might lead to a higher likelihood of FAGIP utilization. Further studies are needed to confirm the correlations between the behaviour of seeking medical advice, disease severity, and FAGIP ordering.

There are several limitations to our study. First, not all the patients for whom FAGIP was performed were tested using conventional culture or PCR methods simultaneously, which limited the evaluation of false-positive or negative conditions, especially when multiple detections occurred. The reported overall false-negative and -positive rates of FAGIP are 0.9 % and 2.8 %, respectively.[3] FAGIP can only detect pathogens, not susceptibility to antibiotics or resistance genes to predict the failure of therapy. The emergence of cephalosporin-resistant nontyphoid *Salmonella*,[34] for example, should be taken into consideration when third-generation cephalosporins are selected to treat *Salmonella* enteric infection. Second, we did not analyse true infection or asymptomatic carriage when mixed detections revealed toxin A/B with other pathogens, which might overestimate the prevalence of toxin A/B.[9] Further research with a prospective design is needed to verify the false-positive or -negative rate for every pathogen covered by FAGIP to better reflect the true prevalence of mixed detections.

In conclusion, paediatric patients had significantly higher rates of positivity and mixed detection than adult patients, and the distribution of pathogens was different, with *Salmonella* being the most detected pathogen in

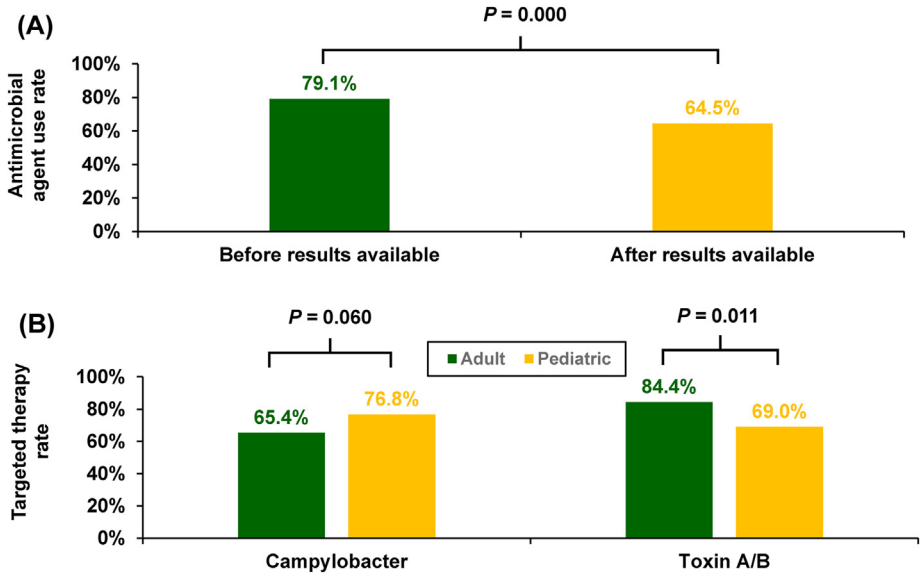

Figure 3. (A) Antimicrobial agent use rate among patients with negative FilmArray gastrointestinal panel (FAGIP) and culture results. (B) Different targeted therapy rates for *Campylobacter* and toxin A/B after FAGIP results were available between adult and paediatric patients.

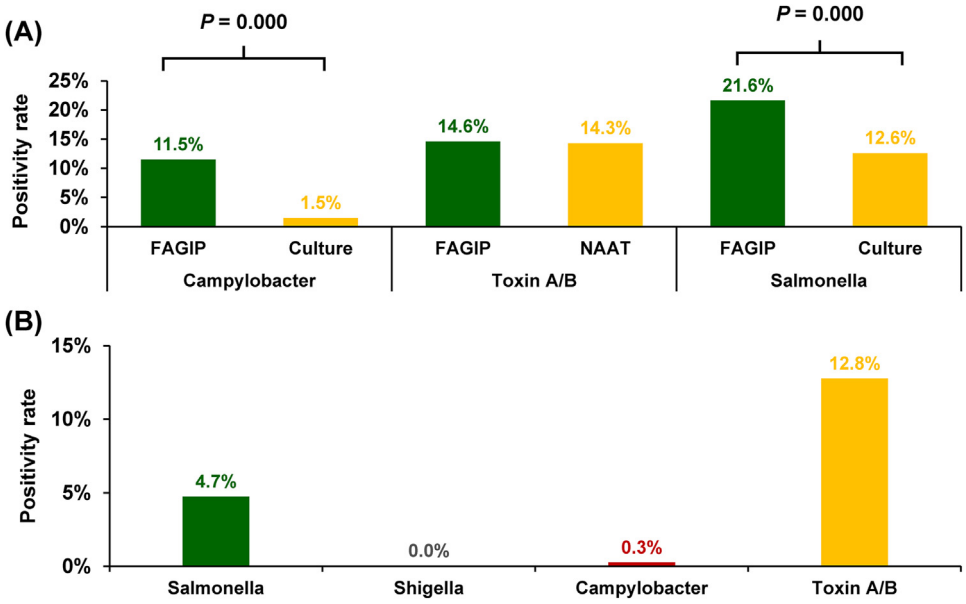

Figure 4. (A) Comparison of positivity rates between the FilmArray gastrointestinal panel (FAGIP) and conventional culture for *Campylobacter* and *Salmonella* and the nucleic acid amplification test (NAAT) for toxin A/B detection. (B) Positivity rates of conventional culture methods for *Salmonella*, *Shigella*, and *Campylobacter* and the nucleic acid amplification test (NAAT) for the detection of toxin A/B used in 2021.

paediatrics and toxin A/B in adults. The rate of CO diarrhoea positivity was higher than that of NC diarrhoea. FAGIP not only presented a better detection rate than certain conventional methods but also lessened unnecessary antimicrobial use. Studies with prospective designs are needed to monitor the dynamic changes in epidemiologic patterns and uncover more clinical impacts of FAGIP in different regions.

Declaration of competing interests

All authors have no conflicts of interest to declare.

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