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Contribution of the FilmArray® Gastrointestinal Panel in the laboratory diagnosis of gastroenteritis in a cohort of children: a two-year prospective study

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ABSTRACT

This study represents a 2-year picture of the epidemiology of enteric pathogens in children suffering from gastroenteritis using the FilmArray® Gastrointestinal Panel (FA-GP), a multiplex molecular assay that allows to simultaneously detect a large panel of pathogens independently of the etiological suspicion and to evaluate its potential contribution to the diagnosis compared to the conventional methods.

A total of 1716 stool samples, collected from children with clinical suspicion of bacterial and/or viral gastroenteritis attending the University Hospital of Parma, was submitted to the FA-GP and, when an adequate aliquot was available, to electron microscopy (n = 1163) for virus detection and to an enterovirus-targeting real-time PCR (n = 1703). Specimens with positive results for *Salmonella*, *Yersinia enterocolitica*, *Vibrio*, diarrheagenic *Escherichia coli/Shigella*, *Campylobacter*, *Plesiomonas shigelloides* and/or parasites by the FA-GP were also submitted to conventional diagnostic methods.

The FA-GP gave positive results in 958 (55.8%) cases, 64.8% from inpatients: 647 (67.5%) contained a single agent and 311 (32.5%) multiple agents, for a total of 1374 pathogens. Enteropathogenic *E. coli*, rotavirus, norovirus, toxigenic *Clostridioides difficile*, and sapovirus were the most commonly detected pathogens. A total of 812 additional agents (344 of which as single pathogen) was detected by the FA-GP and not included in the clinical suspicion. The overall recovery rate of the conventional methods from stools that resulted positive by the FA-GP was 38.6% for bacteria, 50% and 84.2% for *Giardia intestinalis* and *Cryptosporidium*, respectively, and ranged from 3.7% to 64.6% for viruses, if excluding all electron microscopy-negative astroviruses. Enterovirus, an agent not targeted by the FA-GP, was revealed in 9.6% (164/1703) of the examined samples, and in 52 cases it was the only agent detected.

The results of this study allowed to extend the range of detectable pathogens independently of the clinical suspicion, to detect co-infections in almost one third of children positive for at least one agent and to show that conventional methods would have missed more than half of the enteric agents detected by the FA-GP.

1. Introduction

Pathogen-induced acute gastroenteritis (AGE) is one of the leading cause of childhood morbidity and mortality worldwide (Fischer Walker et al., 2013; Liu et al., 2012). A broad range of pathogens has been recognized as the etiology of clinically indistinguishable AGE (Hennessy et al., 2004; Humphries and Linscott, 2015; Sidoti et al., 2015).

Conventional diagnostic methods for routine detection of enteric pathogens are time consuming, labor-intensive, often lack sensitivity and specificity, and leave undiagnosed cases (Simpson et al., 2003; Zhang et al., 2015). In the past few years, PCR based detection has greatly reduced the diagnostic gap (Amar et al., 2007; Simpson et al., 2003). Through the use of multiplex PCR assays, which enable parallel testing for multiple pathogens reducing the workload and the time to result, the true burden of gastroenteritis and the role of mixed infections can be better evaluated. However, epidemiological studies using multiplex molecular assays targeting enteric pathogens have been reported for bacterial or viral etiologies separately (Gómez-Duarte et al., 2009; Khamrin et al., 2011; Nazeer et al., 2013). More recently with the

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introduction of the syndromic multiplex PCR systems, the use of the culture-independent FilmArray® Gastrointestinal Panel (FA-GP, BioFire Diagnostics, USA; bioMérieux, France) has allowed to simultaneously detect a wider range of enteric pathogens (Buss et al., 2015; Murphy et al., 2017; Spina et al., 2015). However only a limited research considering the broad representation of the enteric pathogen community in children was reported (Stockmann et al., 2016). In this report we describe the results of a two-year hospital-based surveillance activity (January 2016-January 2018) for a large spectrum of diarrheagenic pathogens detected by the FA-GP in children with the clinical suspicion of bacterial and/or viral gastroenteric infection. Another important aim of the present study was to assess whether the prospective use of the FA-GP, independently of the clinical suspicion, could be useful to identify unsuspected infections as well as unsuspected co-infections in order to fill the diagnostic gap, and to improve clinical care and public health surveillance.

2. Materials & methods

2.1. Samples and patients

From 14th January 2016 to 14th January 2018, all stool samples (1716) sent to our laboratory for bacterial and/or viral diagnostic purposes from as many children (age range: 1 day - 13 years and 8 months, mean age: 3 years, 6 months and 25 days) suffering from gastroenteritis (onset of symptoms was not available) and attending the University Hospital of Parma, Northern Italy (1147 inpatients and 569 outpatients), were prospectively submitted to the FA-GP, independently of the etiological suspicion, within two days from specimen collection (stored at 4 °C). When an adequate aliquot was available, the samples were submitted to electron microscopy (EM, 1163 cases) and to an enterovirus-targeting real-time PCR (1703 cases).

In particular, 470 samples (27.4%) arrived with a clinical suspicion of bacterial infection, 358 (20.9%) with a clinical suspicion of viral enteric infection and 888 (51.7%) for both bacterial and viral suspicion.

The samples analysed in this study were sent to the University Hospital of Parma for routine diagnostic purposes, and the laboratory diagnosis results were reported in the medical records of the patients as answer to a clinical suspicion; ethical approval at the University Hospital of Parma is required only in cases in which the clinical samples are to be used for applications other than diagnosis.

2.2. FilmArray® Gastrointestinal Panel (FA-GP)

Two hundred microliters of each specimen in Cary Blair transport medium (Faecal swab; Copan, Italy) were submitted to the FA-GP using the FilmArray 2.0 platform, according to manufacturer's instructions. The FA-GP allows the simultaneous detection of adenovirus (AdV) F40/41, astrovirus (AstV), norovirus (NoV) genogroup GI/GII, rotavirus (RV) group A, sapovirus (SaV) genogroups GI, GII, GIV, and GV, *Campylobacter* (*C. jejuni/C. coli/C. upsaliensis*), toxigenic *Clostridium difficile*, recently renamed *Clostridioides difficile* (Lawson et al., 2016), *Plesiomonas shigelloides*, *Salmonella*, *Vibrio* (*V. parahaemolyticus/V. vulnificus/V. cholerae;* with specific detection of *V. cholerae*), *Yersinia enterocolitica*, enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC, with specific detection of *E. coli* O157), *Shigella*/enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), *Cryptosporidium*, *Cyclospora cayetanensis, Entamoeba histolytica*, and *Giardia intestinalis*. The software automatically generates a result for each target in one hour per run (*i.e.* per specimen).

2.3. Conventional methods for bacteria, parasites and viruses

When a positive result for *Salmonella*, *Y. enterocolitica, Vibrio,* diarrheagenic *E. coli/Shigella, Campylobacter*, *P. shigelloides* and/or parasites by the FA-GP was obtained, the specimen was also submitted to conventional diagnostic methods to attempt to isolate and/or identify the pathogen, according to standard procedures (Leber, 2016) as follows: for *Salmonella* and *Shigella* detection the specimen was plated onto Hektoen enteric agar and xylose-lisine-desoxycholate agar directly and after selenite broth enrichment; for *Y. enterocolitica* and *P. shigelloides* onto cefsulodin–irgasan–novobiocin agar; for *Vibrio* onto thiosulfate–-citrate–bile salts agar directly and after alkaline peptone water enrichment; for diarrheagenic *E. coli* (DEC) onto eosin methylene blue agar. Moreover, when STEC was revealed by the FA-GP also a rapid immunoassay for Shiga toxin detection (Immuno*Card* STAT!® EHEC, Meridian Bioscience, USA) was performed. For *Campylobacter* detection, the specimen was submitted to a rapid antigen immunoassay (Immuno*Card* STAT!® CAMPY, Meridian Bioscience) and plated onto *Campylobacter* selective agar. Plates were held for 2 days in the appropriate conditions and suspicious colonies were identified using MALDI-TOF mass spectrometry (Microflex LT, Bruker Daltonics, Germany) (Calderaro et al., 2014a). The *Salmonella* strains identified by MALDI-TOF MS were characterized by serological testing using Statens Serum Institut antisera (Copenhagen, Denmark) while those of *Shigella* and *Y. enterocolitica* by using Denka Seiken (Tokyo, Japan) antisera. For diarrheagenic *E. coli,* Statens Serum Institut and/or Mast Group (Mast Group, Liverpool, United Kingdom) antisera detecting O6, O8, O15, O20, O25, O26, O27, O28ac, O29, O55, O63, O78, O86, O103, O111, O112ac, O114, O115, O119, O121, O124, O125ac, O126, O127, O128ab, O136, O142, O143, O144, O145, O148, O152, O153, O157, O159, O164, O167, O168, and O169 strains were used.

When a parasite was detected by the FA-GP, the same sample and/or additional samples up to three per patient (not further examined by FA-GP, since it is not the gold standard method for the diagnosis of intestinal parasitosis) were submitted to microscopic examination of wet mounts both from fresh and concentrated faeces, according to standard procedures, and to a *G. intestinalis* and *Cryptosporidium* immunochromatographic assay (IC, Immuno*Card* STAT!® *Crypto/Giardia* Rapid Assay, Meridian Bioscience). In case of discordant results between light microscopy and IC, also an immunofluorescence assay (MERIFLUOR® *Cryptosporidium/Giardia*, Meridian Bioscience) was performed according to manufacturer's instructions as previously described (Calderaro et al., 2011, 2010). When diagnostic stages of intestinal parasites were observed, conventional culture for parasites, according to standard procedures, and real-time PCR assays for *E. histolytica/E. dispar* and for *Dientamoeba fragilis* were performed, as previously described (Calderaro et al., 2014a, 2014b).

When a negative result for bacteria and parasites, as well as a toxigenic *C. difficile* positive one by the FA-GP was obtained, further investigation by conventional methods was not performed.

For virus detection, besides EM, performed as previously described using standard techniques (Medici et al., 2005), an enterovirus (EV) real-time RNA amplification (ENTEROVIRUS Q – PCR Alert Kit, ELITechGroup, Turin, Italy), targeting poliovirus 1–3, coxsackievirus A1-A22 and A24, coxsackievirus B1-B6, echovirus 1-9, 11-21, 24-27 and 29-33, and enterovirus 68-71, was used according to the manufacturer's instruction.

2.4. Criteria for the definition of additional agent detected by the FA-GP

An agent detected by the FA-GP was considered as additional if it was: i) a virus detected in a specimen with a request for bacterial investigation; ii) a bacterium detected in a specimen with a request for viral investigation; iii) a parasite; iv) a bacterium detected in a specimen with requests for other bacteria (for EIEC/*Shigella*, undistinguishable by FA-GP, when neither *E. coli* nor *Shigella* were requested); v) an AstV or SaV, since not routinely searched for in the practice before the introduction of FA-GP.