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Short Communication

Diagnosis of Acute Gastrointestinal Infections in Hospitalized Patients: A Molecular-Based Screening Approach

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ABSTRACT

Molecular multiplex methods can improve the diagnosis of gastrointestinal infections, by detecting in a short period of time and simultaneously several bacterial, viral and parasitic pathogens. We report our laboratory molecular-based screening approach for acute gastrointestinal infections diagnosis in hospitalized patients.

Keywords: Molecular microbiology; Gastrointestinal infections; Multiplex molecular diagnosis

INTRODUCTION

Diarrheal disease remains a significant health concern also in the developed countries, because of the frequent need to hospitalize the critical patients, young children and the elderly [1-2]. Current guidelines for the laboratory diagnosis of acute gastrointestinal infections are not well defined [3].

Conventional culture-based methods for bacterial enteric pathogens underestimate the presence of gastrointestinal pathogens [4-7] with a high proportion of fecal samples (>90%) diagnosed as negative [8]. The poor etiological diagnosis of acute gastrointestinal infections is likely due not only to the low sensitivity of the standard coproculture, generally directed to microbiological detection of only 3 species (*Salmonella spp*, *Campylobacter spp* and *Shigella spp*), but also to the use of unsuitable fecal samples [8]. In fact, patients with acute gastrointestinal infections typically produce frequent diarrheal stools that are watery, soft, or unformed, while patients who produce formed stool are not likely to be infected. The rapid and accurate detection of the pathogens that cause acute gastrointestinal infections is crucial for appropriate therapy, and mandatory for the infection control and to prevent the disease's spread [1,9]. Recently, the development and implementation of multiplexed molecular panels allowed clinical laboratories to more rapidly and sensitively diagnose gastrointestinal infections [9].

We report our recent laboratory experience in the management of stool samples from hospitalized patients with acute infectious gastroenteritis suspect, after the introduction of a multiplexed molecular assay.

Between April 2017 and October 2018, our laboratory received a total of 501 standard coproculture requests (e.g. for *Salmonella spp*, *Shigella spp*, *Campylobacter spp*) from hospitalized patients with suspicion of acute gastrointestinal infections. The median age of the patients was 56 years, and 46.6% were male.

Stool samples were provided in 2 tubes: A) a sterile tube with fresh stool, for appropriateness sample evaluation based on Bristol stool chart; B) FecalSwab™ (Copan Italia SpA, Brescia, Italy) tube, suitable for both molecular and cultural tests. Only diarrheal samples were evaluated and we performed first the Allplex™ GI Assay (Seegene, Seoul, Korea) instead of the standard coproculture ordered (Figure 1). Allplex™ GI Assay is a multiplex PCR real-time system, made up of 4 different panels that allow the detection, in as little as 6 h, of up to 25 targets, including: 13 bacteria (*Shigella spp.*/Enteroinvasive *Escherichia coli* (EIEC), *Campylobacter spp.*, *Yersinia enterocolitica*, *Vibrio spp.*, *Clostridium difficile* toxin B, *Aeromonas spp.*, *Salmonella spp.*, stx1/stx2 (Shiga toxin genes), eaeA for enteropathogenic *Escherichia coli* (EPEC), lt/st for enterotoxigenic *E. coli* (ETEC), E. coli O157, aggR for enteroaggregative *E. coli* (EAEC) and hypervirulent *Clostridium difficile*; 6 viruses: Norovirus GI, Norovirus GII, Rotavirus A, Adenovirus, Astrovirus, Sapovirus; 6 parasites: *Giardia lamblia* (GL), *Entamoeba histolytica* (EH), *Cryptosporidium spp.* (CR), *Blastocystis hominis* (BH), *Dientamoeba fragilis* (DF), *Cyclospora cayetanensis* (CC).

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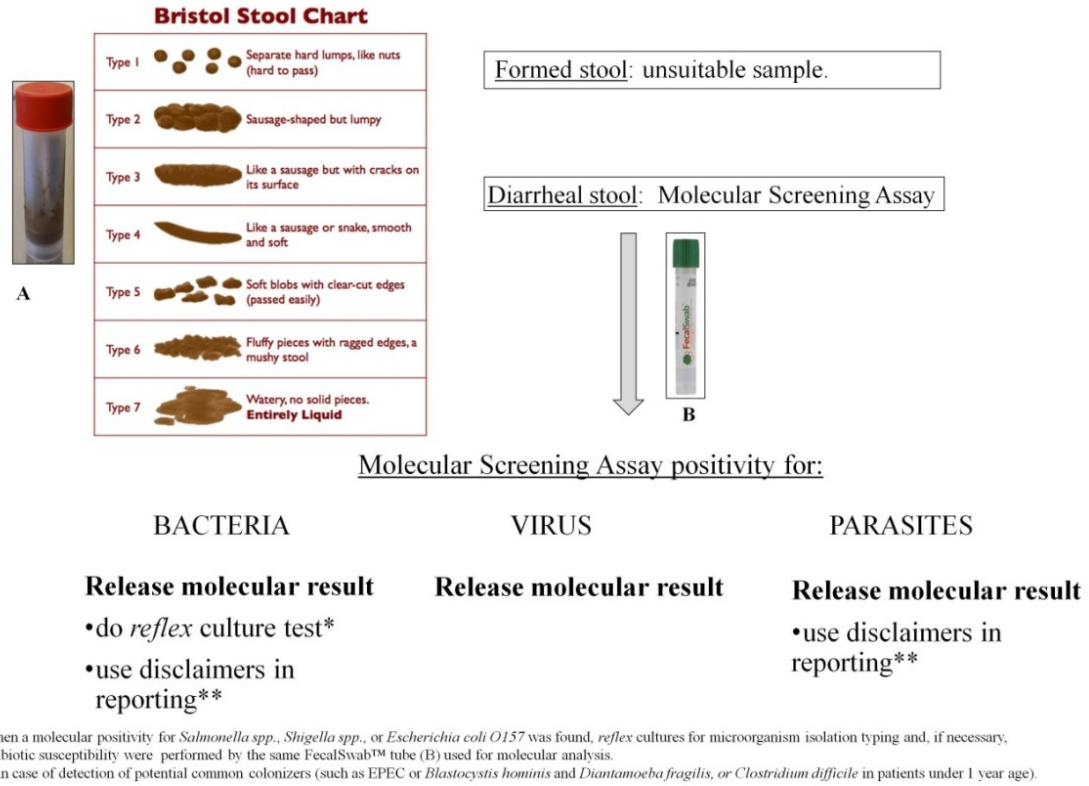

Figure 1: Hospitalized community-acquired infectious diarrhea: Molecular based screening approach and interpretative algorithm proposal.

In case of any molecular positivity, we followed the algorithm shown in Figure 1. In particular, when molecular positivity for a cultivable germ was detected, a reflex culture test for the microorganism isolation, typing and antibiotic susceptibility test was performed directly by the same FecalSwab™ tube used for molecular analysis; when molecular positivity for viruses or for parasites was detected, we released the result without further investigations.

Table 1: Single or multiple detections by Allplex GI assay in diarrheal stools from 484 hospitalized patients with acute gastroenteritis: Number and types of co-infection (n 40).

Parameters	**No of Patients**	**n targets**
Single target	144	144
Two target	35	70
Three target	3	9
Four target	2	8
Total	184	234
Co-detection		**n**
Adenovirus/NorovirusGI		1
Blastocystis hominis/NorovirusGI		1
Blastocystis hominis/NorovirusGII		1
Blastocystis hominis/Rotovirus		2

Blastocystis hominis/Salmonella spp.	2
Campylobacter spp/Blastocystis hominis	2
Campylobacter spp/Enteropathogenic *Escherichia coli* (EPEC)	2
Campylobacter spp/Enteropathogenic *Escherichia coli* (EPEC)/Giardia lamblia/Enteroaggregative E coli (EAEC)	1
Campylobacter spp/*Diantamoeba fragilis*	1
Campylobacter spp/*Giardia lamblia*	1
Campylobacter spp/Norovirus GII	1
Clostridium difficle toxin B/Enteropathogenic *Escherichia coli* (EPEC)/*Salmonella* spp	1
Clostridium difficle toxin B/Adenovirus	1
Cryptosporidium spp/Saprovirus	1
Dientamoeba fragilis/*Clostridium difficle* toxin B	1
Dientamoeba fragilis/Enteroaggregative *E. coli* (EAEC)	1
Dientamoeba fragilis/Norovirus GI	1
Dientamoeba fragilis/Rotavirus	1
Enteroaggregative *E. coli* (EAEC)/Enteropathogenic *Escherichia coli* (EPEC)/*Salmonella* spp.	1
Enteroaggregative *E. coli* (EAEC)/*Salmonella* spp.	1
Enteropathogenic *E. coli* (EPEC)/Adenovirus	1
Enteropathogenic *Escherichia coli* (EPEC)/*Clostridium difficle* toxin B	2
Enteropathogenic *Escherichia coli* (EPEC)/*Diantamoeba fragilis*	2
Enteropathogenic *Escherichia coli* (EPEC)/Enteroaggregative *E. coli* (EAEC)	2
Enteropathogenic *Escherichia coli* (EPEC)/Rotavirus	2
Enteropathogenic *Escherichia coli* (EPEC)/*Salmonella* spp.	1
Enterotoxigenic *E. coli* (ETEC)/*Clostridium difficle* toxin B	1
Enterotoxigenic *E. coli* (ETEC)/Norovirus GI	1
Norovirus GI/Campylobacter spp./Enteropathogenic Escherichia coli (EPEC)/*Giardia lamblia*	1
Norovirus GI/*Blastocystis hominis*/*Dientamoeba fragilis*	1
Rotavirus/*Cryptosporidium* spp.	1
Rotavirus/Norovirus GII	1
Total of co-detection	40

Seventeen out of 501 samples were rejected as formed stool. The remaining 484 diarrheal samples were analyzed in first instance by Allplex™ GI Assay: 299 samples (62%) were negative and 184 samples (38%) were positive; a total of 231 organisms were detected (Figure 2), with 40/184 sample being positive for multiple targets (Tables 1 and 2). For 55/484 samples (11%), 14

for *Salmonella spp*, 38 for *Campylobacter spp*, 3 for *Shigella*/EIEC, conventional microbiology cultures confirmed molecular results (Figure 2). For 7/484 samples (1%), 1 for *Aeromonas spp*., 3 for *Yersinia enterocolitica*, 3 for *E. coli* O157), conventional microbiology cultures confirmed molecular results (Figure 2), even that the standard coproculture request would have missed these positivity's.

Table 2: Summary of microorganisms detected by Allplex GI assay (n 231) in diarrheal stools from 484 hospitalized patients with acute gastroenteritis, and comparison with reflex culture method for cultivable bacteria. *when a molecular positivity for *Salmonella spp*., *Shigella spp*., or *Escherichia coli* O157 was found, reflex cultures for microorganism isolation typing and, if necessary, antibiotic susceptibility were performed by the same FecalSwab ™ tube (B) used for molecular analysis. ** in case of detection of potential common colonizers (such as EPEC or *Blastocystis hominis* and *Dientamoeba fragilis*, or *Clostridium difficile* in patients under 1 year age).

Targets	Allplex GI (n 231)	Reflex culture method (n59)	Concordance (%)
Adenovirus	3	/	/
Aeromonas spp.	1	1	100
Astrovirus	4	/	/
Blastocystis hominis	26	/	/
Campylobacter spp.	38	38	/
Clostridium difficle toxin B	21	/	/
Cryptosporidium spp.	3	/	/
Dientamoeba fragilis	14	/	/
E. coli O157	3	3	100
Enteroaggregative *E. coli* (EAEC)	11	/	/
Enterohaemohagic *Escherichia coli* (EHEC) stx1/ stx2	4	/	/
Enteropathogenic *Escherichia coli* (EPEC)	29	/	/
Enterotoxigenic *E. coli* (ETEC)	4	/	/
Giardia lamblia	14	/	/
Norovirus GI	4	/	/
Norovirus GII	12	/	/
Rotavirus	18	/	/
Salmonella spps.	14	/	100
Saprovirus	2	/	/
Shigella spp./Enteroinvasive *Escherichia coli* (EIEC)	3	3	100
Yersinia enterocolitica	3	3	100

Culture test for the diagnosis of infections caused by non-O157 strains of E. coli are not available in most of the laboratories. Therefore, 48/484 samples (10%), 29 for EPEC, 4 for ETEC, 4 for EHEC and 11 for EAEC, we have missed by the coproculture alone.

For 121/484 samples (25%), 21 for *Clostridium difficile* toxin B, 18 for Rotavirus, 14 for *Giardia lamblia*, 16 for Norovirus, 4 for Astrovirus, 3 for *Cryptosporidium*, 3 for Adenovirus, 2 for Sapovirus, 26 for *Blastocystis hominis*, 14 for *Diantamoeba fragilis*, we would have missed the positivity diagnoses, because specific diagnostic tests other than standard coproculture have not been ordered. In particular, 7 of the 16 cases of positivity for Norovirus were the expression of a small outbreak occurred in October 2018 in our hospital, promptly reported by the

laboratory (all 16 Norovirus and 21 *Clostridium difficile* toxin B were confirmed by alternative molecular method in use in our laboratory, data not shown). For 17/484 positive samples (3.5%), 14 for *Giardia lamblia*, 3 for *Cryptosporidium* (all confirmed by microscopic examination and/or antigenic test, data not shown), it was soon started the antiparasitic therapy.

Considering that EPEC, *Blastocystis hominis*, *Dientamoeba fragilis*, and *Clostridium difficile* may be common hosts of the gastrointestinal tract, we used specific disclaimers in reporting when these germs were detected (e.g. 4/21 *Clostridium difficile* positives were from patients under 1 year age) [4].

The strength of our work is the proposal of a completely innovative, broad spectrum molecular diagnostic approach, through: first, the evaluation of the appropriateness of the sample, then, the processing only of the faeces not formed with Allplex-GI, and, finally, the execution of the reflex culture test in molecular positive cases, for those microorganisms that need an antibiotic susceptibility test.

As debated in literature [3], the potential limitation of a similar molecular approach could be the risk of an overvaluation of pathogens, especially in asymptomatic patients. Regarding this concern, we strongly reiterate that the molecular approach we suggested should only be applied to the diarrheal faeces of symptomatic and hospitalized patients, thus overcoming the problem of overvaluation in asymptomatic patients.

A cost analysis should take into account some fundamental aspects. Allplex-GI analyzes batches of samples up to 26 per session and gives a result for 25 different targets. The standard coproculture request covers only 3 targets. The comparison that each laboratory, in its own context, should make is among the cost to perform the search for 25 targets simultaneously, taking into account the response times and the highest sensitivity of the molecular method, and cultivation methods, microscopic examination, or molecular monotarget single request, for each of these 25 microorganisms. Noteworthy that, if we had done the standard coproculture alone, we would have missed 176 microbiological diagnoses.

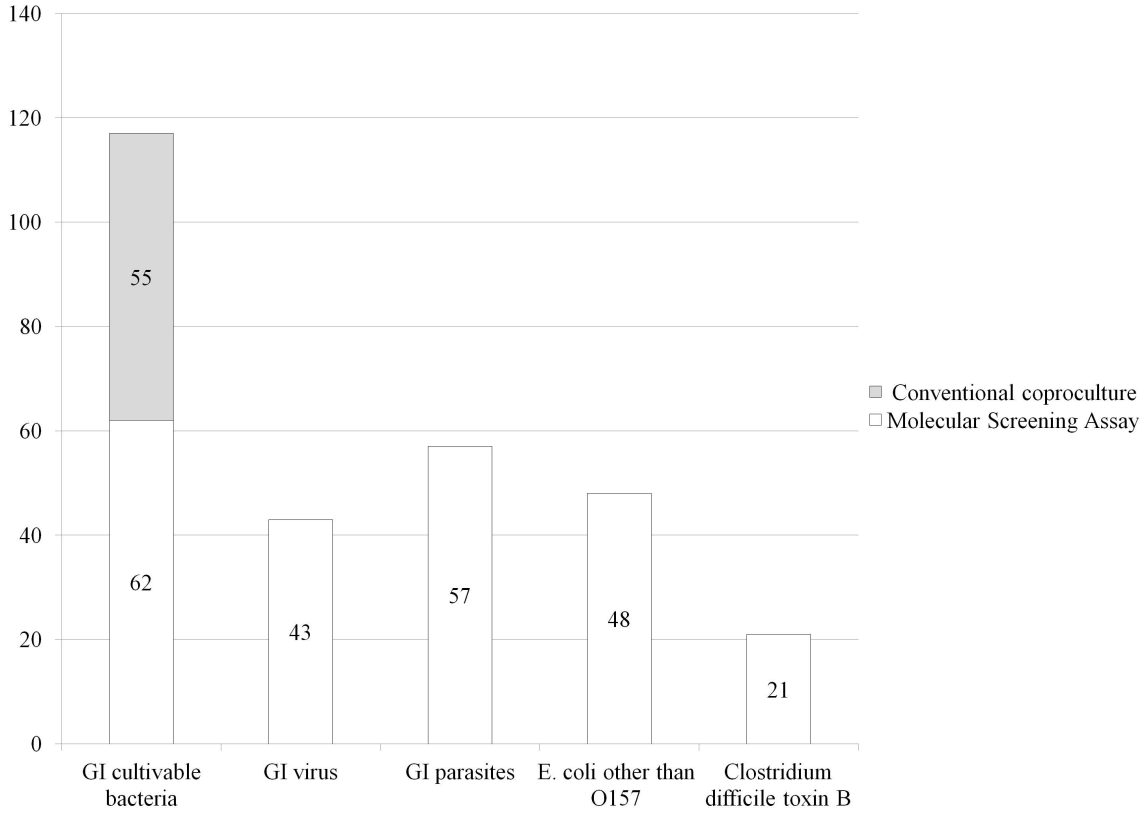

Figure 2: Number of microbiological diagnosis by conventional coproculture alone (n 55) versus molecular screening approach (n 231) in diarrheal stools from 484 hospitalized patients with acute gastroenteritis.

CONCLUSION

In conclusion, our experience support the need to improve the conventional microbiological fecal examination by multiplexed molecular panels to allow clinical laboratories to more rapidly and sensitively diagnose gastrointestinal infections in hospitalized patients.

REFERENCES

1. Martín A, Pérez-Ayala A, Chaves F, Lora D, Orellana M. Evaluation of the multiplex PCR Allplex-GI assay in the detection of bacterial pathogens in diarrheic stool samples. J Microbiol Methods. 2018;144:33-36.
2. Mengelle C, Mansuy JM, Prere MF, Grouteau E, Claudet I. Simultaneous detection of gastrointestinal pathogens with a multiplex Luminex-based molecular assay in stool samples from diarrhoeic patients. Clin Microbiol Infect. 2018;19:E458–465.

3. Macfarlane-Smith LR, Ahmed S, Wilcox MH. Molecular versus culture-based testing for gastrointestinal infection. Curr Opin Gastroenterol. 34:19–24.
4. Hanson KE, Couturier MR. Multiplexed Molecular Diagnostics for Respiratory, Gastrointestinal, and Central Nervous System Infections. CID. 2016;63:1361-1367.
5. Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, et al. Practice guidelines for the management of infectious diarrhea. Clin Infect Dis. 2001;32:331-351.
6. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): A prospective, case-control study. Lancet. 2013;382:209-222.
7. Liu J, Platts-Mills JA, Juma J, Kabir F, Nkeze J, Okoi C et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: A reanalysis of the GEMS case-control study. Lancet. 2016;388:1291–1301.
8. Bullman S, Lucey B, Sleator RD. Molecular diagnostics the changing culture of medical microbiology. Bioengineered Bugs. 2012;3:1-7.
9. Binnicker MJ. Multiplex molecular panels for diagnosis of gastrointestinal infection: performance, result interpretation, and cost-effectiveness. J Clin Microbiol. 2015;53:3723-3728.