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Infectious gastroenteritis: comparison of conventional and molecular methods for detection of pathogens

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Introduction

Infectious gastroenteritis is a disease with an important impact worldwide. Millions of patients are affected every year, with rare cases developing more serious illness. Specific therapy is only possible if the pathogen is known. Conventional diagnostic consists in several methods specific for singular pathogens. Two newly developed multiplex PCR assays allow the simultaneous detection of a broad range of pathogens. The accuracy and usefulness of these assays in a diagnostic laboratory was evaluated by comparison with conventional methods.

Methods

126 routine stool specimens from patients with gastroenteritis were examined with two commercially available mulitplex-PCR assays, and with conventional methods as requested. Conventional detection consisted in culture on selective media for bacteria and in specific EIA for Adenovirus, Rotavirus, C. difficile Toxin, E. coli VTEC/EHEC, Giardia and Entamoeba histolytica. For Noroviruses, real time-PCR on LightCycler was used. Cryptosporidium spp. is found by microscopic examination.
The Seeplex Diarrhea ACE (Seegene, Seoul, Korea) assay includes three parallel amplification reactions by dual priming oligonucleotides and detection by auto-capillary electrophoresis. For a more consistent result as compared to conventional methods, only signals >30 were considered. The xTAG® GPP (Luminex, Toronto, Canada) assay is based on a single one-step (RT)-PCR followed by target specific primer extension, hybridisation to specific xTAG beads and laser detection (Luminex technology).

Results

The pathogens found in 126 routine stool specimens are shown in figure 1. This reflects the pathogens normally found to cause infectious gastroenteritis in Switzerland. For 8 specimens, only one of three methods was positive (pathogens found: 3 Y. enterocolitica, 2 Noroviruses, 1 Campylobacter spp., 1 Shigella spp., 1 C. difficile Toxin). Pathogen detection with two of three methods was possible in 6 cases, and all three methods gave an equally positive result in 30 stool samples. In 12 stool samples, 2 different pathogens could be detected simultaneously.

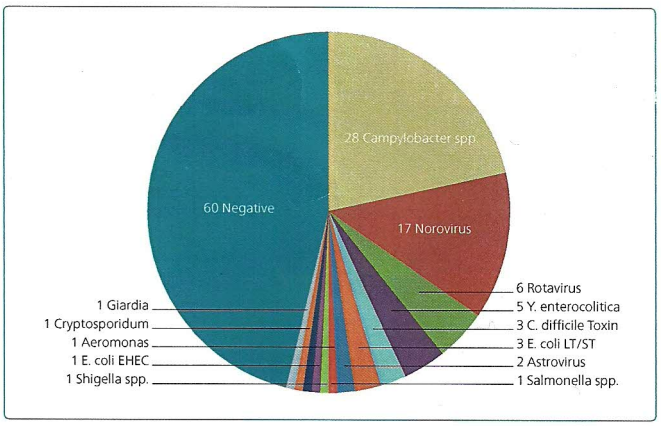

Figure 1: Pathogens detected in 126 routine stool samples by any of the mulitplex PCR assays or conventional methods

	xTAG® GPP (Luminex)	Diarrhea ACE (Seegene)	Conventional
Adenovirus	0	0 (+1w)	0 / 3
Astrovirus	ND	2	ND
Norovirus	15	17 (+3w)	4 / 22
Rotavirus	6	6 (+4w)	0 / 7
Aeromonas spp.	ND	1 (+3w)	2 / 19
Campylobacter spp.	26	27 (+1w)	26 / 122
Clostridium perfringens	ND	8 *	ND
Salmonella spp.	1	1	1 / 123
Shigella spp.	0	1 (+2w)	0 / 122
Vibrio spp.	0	0	0 / 17
Yersinia enterocolitica	2	2 (+4w)	5 / 19
Clostridium difficile Toxin A/B	3	1	2 / 21
Enterotoxigenic E. coli (ETEC) LT/ST	3	ND	0 / 0
Shiga-like Toxin producing E. coli (VTEC/EHEC) stx1/stx2	1	1 (+2w)	0 / 21
E. coli O157	0	0	0 / 0
Cryptosporidium spp.	1	ND	0 / 0
Entamoeba histolytica	0	ND	0 / 12
Giardia lamblia	1	ND	0 / 7

Table 1: Results obtained with the three methods.
ND: not detectable; (+xw): weak signals with Diarrhea ACE;
**: 5/8 in combination with another pathogen, probably colonization.*

Some of the pathogens were missed by conventional methods, since only the basic stool examination (culture for bacteria) was requested. Campylobacter spp. was not detected with each multiplex assay in 1-2 cases. VTEC was only found by PCR, culture was repeatedly negative. Noroviruses resulted in high positive signals, corresponding to the high viral load in acute disease.

The two multiplex assays gave identical results in 115 of 126 samples, 51 detections and 64 negative results. The ACE assay gave weak positive results in 20 samples, 9 of them in combination with another pathogen. Since only Y. enterocolitica could be found by another method, the chosen cut-off allowed an interpretation more consistent with the symptoms. The detection signals achieved by xTAG® GPP were at least 10x the noise signal, therefore we suggest to use this as cut-off value.

Conclusions

- Multiplex PCR allows a rapid diagnosis of a wide range of infectious gastroenteritis causing pathogens within one assay.
- The definition of a higher cut-off than proposed by the manufacturer allowed a better correlation compared to conventional methods.
- For less frequent pathogens like protozoa further evaluations are needed.
- Country-specific spectra of pathogens should be furthermore considered for the choice of an assay.

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