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Role of multiplex PCR in the management of acute infectious diarrhea in a hospital setting – a single center retrospective study

Abstract

Background and Aim

Isolation measures in patients with acute diarrhea produce enormous costs of materials and personnel. Aim of this retrospective study was to evalute the percentage of unnecessary isolations and the impact of multiplex PCR stool testing on isolation days before and after introduction into clinical practice.

Methods: Between July 2020 and July 2022 in total 2373 stool specimens of patients with acute diarrhea and a course no longer than 14 days were analyzed using BioFire® FilmArray® Gastrointestinal (GI) Panel. Number of isolation beds per day were compared before and after the introduction of Multiplex PCR testing.

Results: In 65% of all specimen examined no pathogen was detected. Single-room isolation was a necessary in only 22% of all cases. Isolation days on the gastroenterological ward decreased from 635 in 2019 to 384 in 2020, a reduction of 39,5%.

Conclusion: In acute infectious diarrhea Multiplex PCR is a usefull tool to rapidly identify the causative agent and exclude the necessity of isolation in the majority of cases. In spite of all medical and economical advantages Multiplex PCR testing has not been established widely so far.

Keywords: Gastrointestinal, acute diarrhea, ELISA, Clostridioides strains

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Introduction

Acute infectious diarrhea is one of the most frequent diseases worldwide caused by a broad spectrum of viral and bacterial pathogens as well as parasites.[1] In most cases the course is self-limiting and the patients are treated in an outpatient setting. According to the Federal Office of Statistics 254,000 patients in Germany had to be treated in hospital because of a complicated course in 2017.[2] If a patient is admitted to hospital because of acute infectious diarrhea isolation of the patient will be necessary until the causative pathogen is identified. These isolation measures produce enormous costs of materials and personnel every year since not every hospital is equipped with a specialized isolation ward. The identification of pathogens from stool specimen applying conventional methods can take up to several days until isolation can be broken. In 2020 we introduced BioFire® FilmArray® Gastrointestinal (GI) Panel, a multiplex PCR testing tool for 22 pathogens, in the diagnostic algorithm of acute infectious diarrhea. The FilmArray GI Panel test consists of automated nucleic acid extraction, reverse transcription, amplification, and analysis, with results available in 1h per run per specimen.[3]

The aim of this retrospective observational study was primarily to determine the percentage of unnecessary isolations in patients admitted to hospital with acute diarrhea and a possible decline of isolation days after introduction of multiplex PCR testing.

Methods

Patients admitted to our emergency department were suspicious of acute infectious diarrhea when they presented with more than three loose or watery stools per day or more frequent passage than normal for the individuals lasting less than 14 days.

Between July 2020 and July 2022 in total 2373 stool samples of patients with acute diarrhea admitted to our hospital were analyzed using BioFire® FilmArray® Gastrointestinal (GI) Panel, biomerieux, France. The FilmArray reagent pouch stores all the necessary reagents for sample preparation, reverse transcription-PCR, PCR, and detection in a freeze-dried format. The sample is collected in Cary-Blair transport media. Prior to a run, the user injects hydration solution and filtered sample combined with sample buffer mix into the pouch. The FilmArray extracts and purifies all nucleic acids from the sample and performs a nested multiplex PCR.

During the first-stage PCR, the FilmArray performs a single, large volume, massively multiplexed reaction. Last, individual singleplex second-stage PCR reactions detect the products from the first stage PCR. Using endpoint melting curve data, the FilmArray software automatically generates a result for each target in a single report.[3]

If Clostridioides were tested positive in BioFire® FilmArray® Gastrointestinal (GI) Panel an ELISA assay was conducted to proof the presence of the toxin itself.

Additionally, the numbers of isolation beds per day on the gastroenterological ward were compared before and after the introduction of Multiplex PCR testing according to the database of the Department of Hygiene and Infection Prevention. A single-room isolation was considered to be necessary if Clostridioides, Norovirus, Adenovirus or Rotavirus were detected according to the Robert Koch Institute (RKI) guidelines.[4]

Results

In 65% of all stool samples no pathogen was detected (Table 1). In 15% of samples Clostridioides were detected harbouring genes to produce toxin A and/or toxin B. In only 45% of these patients a

subsequent ELISA essay was positive for toxin A or B. EPEC was present in 8% of the specimen although the pathogenetic relevance is not clear. Norovirus and Campylobacter were present in 5% of all specimens respectively. All other pathogens were detected in less than 1% the cases. Taken together these results a single-room isolation was necessary in only 22% of all cases. The isolation days on the gastroenterological ward decreased from 635 in 2019 to 384 in 2020, a reduction of 39,5%.

Table 1 Results of Multiplex PCR analysis

Negative	65%	1534
Clostridioides difficile Toxin A/B	15%	365
Enteropathogene E.coli (EPEC)	8%	194
Norovirus GI/GII	5%	128
Campylobacter	5%	108
stx1/2-produz.E.coli (STEC/EHEC)	2%	49
Enteroaggregative E.coli (EAEC)	1%	29
Cryptosporidium	1%	24
Rotavirus A	1%	16
Salmonella	1%	15
Enterotoxische E.coli (ETEC)	1%	14
Giardia lamblia	1%	14
Adenovirus F 40/41	1%	13
Yersinia enterocolitica	0%	10
Sapovirus	0%	10
Astrovirus	0%	4
Shigella/Enteroinvasive E.coli	0%	3

Results of 2373 multiplex PCR analysis from stool specimens of patients with acute diarrhea from 07/2020 – 07/2022, detection of more than one pathogen in a sample possible, red marked: single-room isolation necessary.

Discussion

Laboratory diagnostic of stool samples in acute infectious diarrhea is laborious and time consuming. Cultural methods to detect Yersinia species could take up to 7 days.[5] By applying Multiplex PCR in the diagnostic of stool samples a broad spectrum of pathogens can be examined in a short period of time. Although initial laboratory costs are higher for Multiplex PCR compared to conventional methods 6, we decided to introduce this diagnostic tool in our department in 2020. The spectrum and prevalence of pathogens causing acute infectious diarrhea are different all over the world. The distribution and prevalence of pathogens identified in our department is consistent with other studies from western countries.[3] It is remarkable that only 22% of all cases needed single-room isolation according to RKI guidelines[4] retrospectively. An explanation for the discrepancy between positive proof of toxin producing Clostridioides strains in PCR and negative ELISA as a direct proof of the toxin seems to be the instability of the toxin in stool specimen.[7] In patients with a positive PCR and negative ELISA the clinical course should define adequate therapy and isolation measures.

Multiplex PCR analysis markedly improved clinical sensitivity in patients with acute diarrhea, identified cases with clinical acuity comparable to those identified by culture, and enabled clinicians to make more timely and targeted therapeutic decisions.[8]

In our opinion the overwhelming value of Multiplex PCR in the clinical setting is fast exclosure of isolation necessity. We were able to show a decrease in isolation days after introduction Multiplex PCR in our hospital. The savings caused by a reduction of isolation materials, retention of staff and length of hospital stay outweigh initial higher laboratory costs by far.[6]

To raise the full potential of multiplex PCR based stool analysis it should be conducted as soon as possible, preferably in the emergency room to avoid unnecessary isolation measures on peripheral ward. In spite of all medical and economical advantages Multiplex PCR testing in acute infectious diarrhea has not been widely established in clinical practice so far.

Conclusion

In acute infectious diarrhea Multiplex PCR is a usefull tool to rapidly identify the causative agent and exclude the necessity of isolation in the majority of cases. In spite of all medical and economical advantages Multiplex PCR testing has not been established widely so far.

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Conflicts of interest

We declare there are no conflicts of interest.

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