

The Role of Multiplex Realtime PCR in Detecting Acute Diarrhoea Pathogens

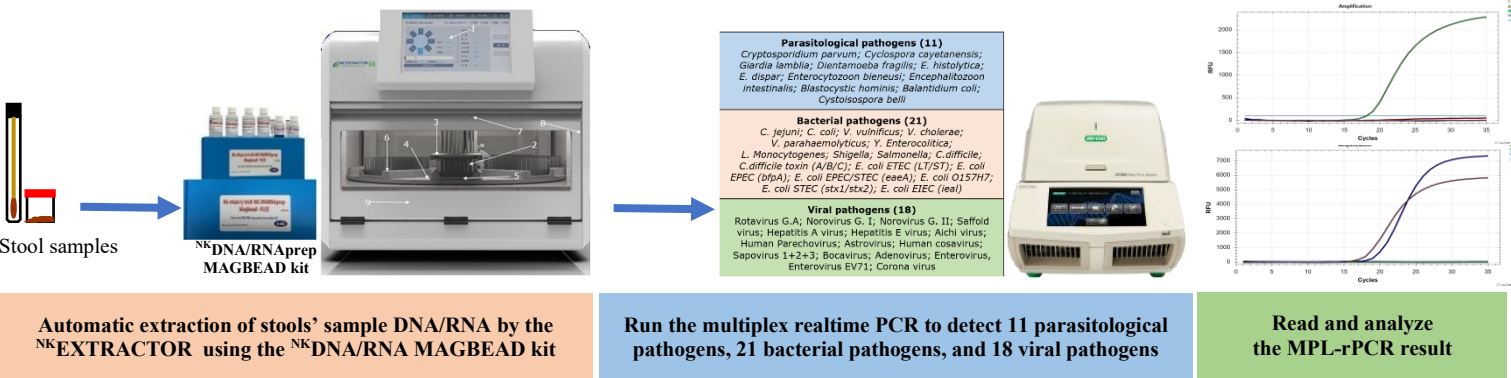
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BACKGROUND

Gastrointestinal infections are primary causes of acute diarrhea. Detecting and identifying the specific causative agent is often challenging due to the limitations of classical microbiological methods and the vast diversity of pathogens, each requiring distinct conditions and diagnostic methods that many laboratories cannot accommodate

AIMS OF THE STUDY

This study aims to apply Multiplex Real-time PCR developed by *Vietnam Research and Development Institute of Clinical Microbiology* to simultaneously detect 18 viruses, 21 bacteria, and 11 parasites, therefore provides clinicians with rapid and accurate diagnostic support for timely treatment



Picture 1: The multiplex real-time PCR method to detect the micro-organisms causing acute diarrhea from stool samples. This method include 3 steps: (1) Automatic extraction of the stools' sample DNA/RNA by NKEXTRACTOR using the NKDNA/RNA MAGBEAD; (2) Run the multiplex realtime PCR to detect 11 parasitological pathogens, 21 bacterial pathogens, and 18 viral pathogens; (3) Read and analyze the MPL-rPCR results

RESULTS

A total 1,992 samples were analyzed, with the majority of cases belonging to the 0–4 age group (66.7%). The overall positivity rate reached 63.5% (1,265/1,992 cases). Notably, single-pathogen infections were identified in 48.4% of cases, while multi-pathogen co-infections were: 15.1%. Among the 1,265 positive cases, viruses were the most frequent contributors (795 cases), followed by bacteria (685) and parasites (95). *Rotavirus* Group A (31.4%), *Adenovirus* (16.0%), and *Norovirus* GII (14.6%) were the most prevalent pathogens among the viral group. In the bacterial group, Diarrheagenic *E. coli* (DEC) dominated at 20.9%, followed by *Clostridium difficile* (10.9%) and *Salmonella* (10.5%). In parasitological group, *Blastocystis* was the leading parasite agent (44.2%).

DISCUSSIONS AND CONCLUSION

Detecting the causative microorganisms of diarrhea is a challenge in clinical microbiology laboratories because many pathogens are beyond the laboratory's capabilities. Therefore, the application of MPL-rPCR for multi-pathogen detection is a necessary solution that needs to be implemented. This study showed a high detection rate of pathogens, including viral pathogens such as rotavirus and norovirus. In addition, bacterial and parasitic pathogens that would be undetectable with conventional microbiology methods, such as DEC and Campylobacter, were also detected with the developed MPL-rPCR.

KEY WORDS

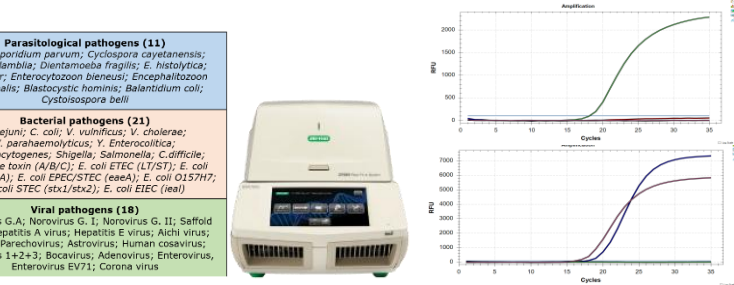
Acute Diarrhea Pathogens; MPL real-time PCR; Diarrheagenic viral pathogens; Diarrheagenic bacterial pathogens; Diarrheagenic parasitological pathogens

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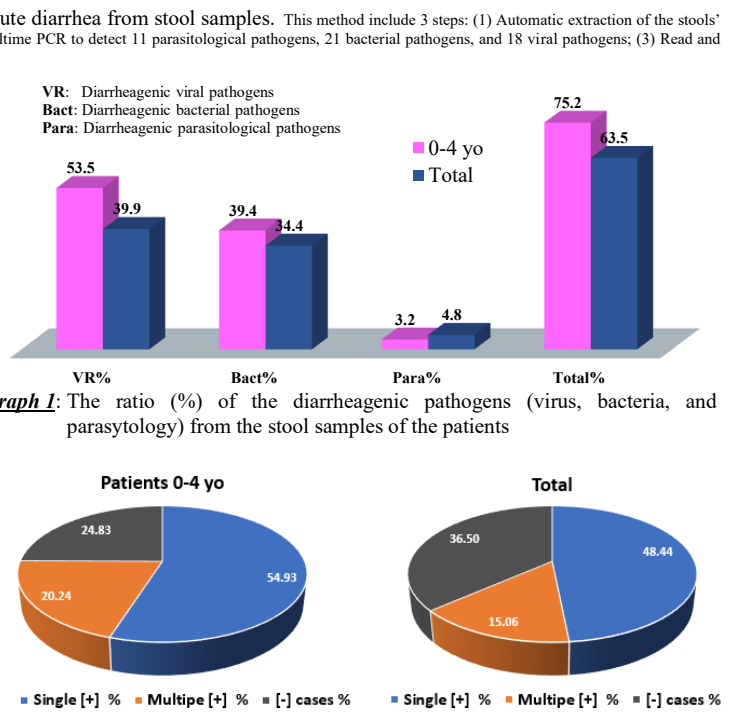
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MATERIALS AND METHODS

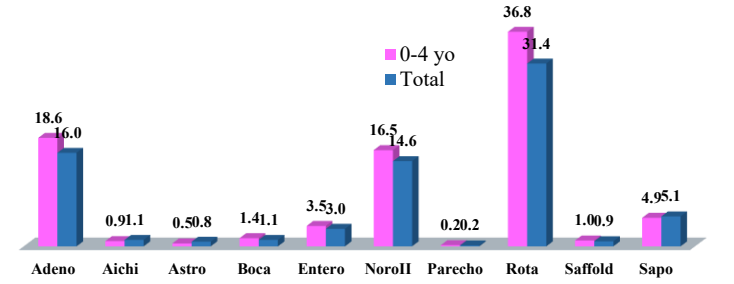
Stool samples from patients clinically diagnosed with acute diarrhea were collected and transported from the hospitals to the laboratory of NK-Biotek company supported by Viet Nam Research and Development Institute of Clinical Microbiology. Right after receiving, the DNA/RNA of the sample was extracted using the NKDNA/RNAprep MAGBEAD kit and the NKDiarrhea Multiplex Real-time PCR kit was subsequently performed to detect the pathogens existed in the patients' stool. This method had applied in some studies in Viet Nam^[1,2,3,4], and in this study we want to analyze the result of this method to detect diarrhea pathogens from a large number of stool samples collected from patients hospitalized by acute diarrhea.



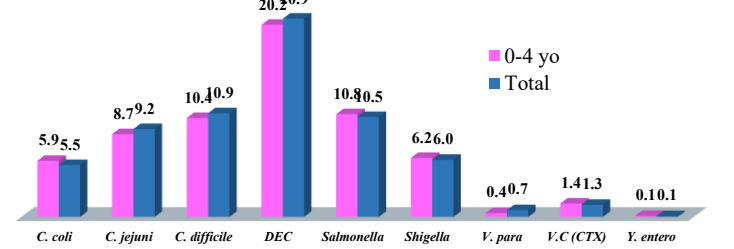
Graph 1: The ratio (%) of the diarrheagenic pathogens (virus, bacteria, and parasitology) from the stool samples of the patients



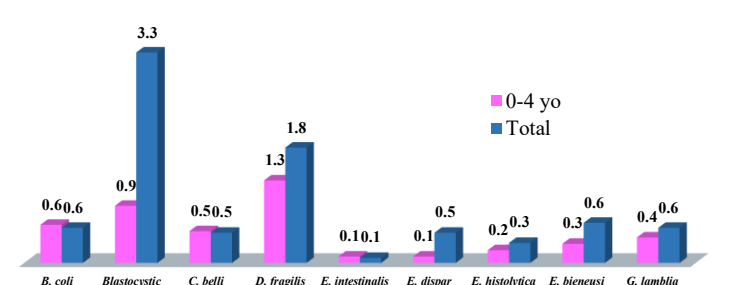
Graph 2: The ratio (%) of the single and multiple diarrheagenic pathogens (virus, bacteria, and parasitology) detected from the stool samples of the patients (sample from 0-4 yo and from all samples) by the MPL-rPCR.



Graph 3: The ratio (%) of the diarrheagenic viral pathogens among the positive cases detected by the MPL-rPCR. Adeno: Adenovirus; Aichi: Aichi virus; Astro: Astrovirus; Boca: Bocavirus; Entero: Enterovirus; NoroII: Norovirus G. II; Parecho: Human Parechovirus; Rota: Rotavirus G.A; Saffold: Saffold virus; Sapo: Sapovirus 1+2+3



Graph 4: The ratio (%) of the diarrheagenic bacterial pathogens among the positive cases detected by the MPL-rPCR. *C. coli*: Campylobacter coli; *C. jejuni*: Campylobacter jejuni; *C. difficile*: Clostridium difficile; *DEC*: Diarrheagenic E. Coli; *V. para*: *Vibrio parahaemolyticus*; *V.C*: *Vibrio cholerae*; *Y. entero*: *Yersinia enterocolitica*



Graph 5: The ratio (%) of the diarrheagenic parasitological pathogens among the positive cases detected by the MPL-rPCR. *B. coli*: Balanitidum coli; *Blastocystic*: *Blastocystis hominis*; *C. belli*: *Cystoisospora belli*; *D. fragilis*: *Dientamoeba fragilis*; *E. intestinalis*: *Encephalitozoon intestinalis*; *E. dispar*: *Entamoeba dispar*; *E. histolytica*: *Entamoeba histolytica*; *E. biennei*: *Enterocytozoon biennei*; *G. lamblia*: *Giardia lamblia*