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BRIEF REPORT

Point-of-care multiplex-PCR enables germ identification in haemolytic uremic syndrome 94 h earlier than stool culture

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Abstract

Haemolytic uremic syndrome often affects children causing a relevant morbidity and mortality. We compared the time to diagnosis by multiplex-PCR and stool culture in 15 children from two centres. Multiplex-PCR accelerated the time to diagnosis by 94 (95% confidence interval, 80–119; $P = 0.0007$) hours. Multiplex-PCR offers a time advantage of stool culture that may aid in earlier identification of outbreak clusters.

Keywords Biofire · Gastrointestinal panel · Shiga-like toxin · Children

Introduction

Much has been written since the initial assessment of clinical human faecal samples for shiga toxin–producing *Escherichia coli* using the multiplex-PCR for gastrointestinal pathogens [1]. These scientific contributions did however focus on infectious diarrhoea per se [2], but not haemolytic uremic syndrome. In addition, the performance of the multiplex-PCR has only been compared to conventional stool analyses for infectious diarrhoea [3]. We thus evaluated the BioFire gastrointestinal panel multiplex-PCR for its performance in the detection of shiga toxin–producing *E. coli* with special focus on time to diagnosis.

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Methods

We retrospectively analysed the clinical records of all patients below the age of 18 years diagnosed with typical haemolytic uremic syndrome that were admitted between 1st October 2017 and 31st March 2018 to two academic medical centres in two different continents. Using predefined abstraction charts, we collected patient data on age, length of hospital stay, admission to and length of intensive care stay, duration of renal replacement therapy if necessary, required time from obtaining stool samples to diagnosis via multiplex-PCR and stool culture as well as mortality. Stool culture was conducted following clinical routines and the BioFire gastrointestinal panel multiplex-PCR has been used following the manufacturer's instructions.

Statistical analysis has been conducted using R (version 3.6.3) [4]. Data were analysed for normality by the Shapiro-Wilk test and the median times to diagnosis were compared via Mood's test for differences in medians from the RVAideMemorie package (version 0.9–75). Agreement between the multiplex-PCR and stool culture was assessed using Cohen's kappa using R's irr-package (version 1.2.0). We bootstrapped bias–corrected, accelerated confidence intervals for differences between medians with the simpleboot-package (version 1.1–7). Differences between medians could only be

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calculated by excluding the case without stool culture as missing data could not be handled by the bootstrap function. Data are presented as median. Statistical significance was assumed with $P < 0.05$.

Results

We included 15 patients with a median age of 26 months (range 9–73) that had a median hospital stay of 17 days (range 2–96). Twelve of these patients (80%) were admitted to the paediatric intensive care unit with a median length of stay of 9 days (range 2–34). All of these patients required renal replacement therapy for a median of 8 days (range 2–12). Two of them were discharged directly from the paediatric intensive care unit and one patient died from a multi-organ dysfunction syndrome.

Pathogen identification was possible in all cases by multiplex-PCR, whereas stool culture identified a causative germ in 13 of 15 patients. Only one stool culture was negative; another patient did not have a stool culture. The multiplex-PCR identified an *E. coli* O157 in 11 cases and shiga toxin–producing *E. coli* in the remaining four cases. In contrast, the stool culture identified *E. coli* O157 in eight cases, *E. coli* O145 in two, shiga toxin-2–producing *E. coli* in another two, and shiga toxin–producing *E. coli* non O157 in the remaining case. This yields a moderate agreement of $\kappa = 0.481$ ($z = 3.02$, $P = 0.00256$).

Multiplex-PCR identified the causative organism after a median duration of 1.47 h (IQR, 1.32–3.82) since admission, whereas stool culture needed a median duration of 96 h (IQR, 72–114). This leads a time advantage of multiplex-PCR of 94 h (95% confidence interval 80–119; $\chi^2 = 11.571$; df = 1; $P = 0.0007$) compared to stool culture.

Discussion

Multiplex-PCR for gastrointestinal pathogens has been described in its use for the detection of causative germs in haemolytic uremic syndrome with its first introduction [1], but its use in children has been focused on infectious diarrhoea [2, 3]. Multiplex-PCR for gastrointestinal pathogens has been described to lower health care costs in children, reduce unnecessary radiologic and interventional procedures, and reduce prescription of antimicrobial chemotherapy [5]. A result that could also be corroborated in adults [6].

The importance of successful pathogen identification is crucial even in the absence of haemolytic uremic syndrome: Children with acute bloody diarrhoea have an increased risk of being infected with shiga toxin–producing *E. coli* and more than 50% of stool cultures in these patients do not detect the causative germ [7]. In line with previous results on infectious diarrhoea [8], multiplex-PCR increased the cumulative pathogen identification in our study compared to stool culture alone. This is crucial for epidemiologic questions as more than a half of cases may have an unknown ethology based on conventional stool testing [9]. Moreover, systematic parallel testing of stool samples with multiplex-PCR resulted in the identification of the causative germ 94 h earlier than conventional stool testing. This time advantage could have relevant public health implications by earlier detection of an outbreak that may be traced back to a certain place—a playground in an outbreak in Germany 2011 [10]—and thus allow its elimination by taking appropriate measures to stop it.

Authors' contributions All authors contributed to the study conception and design, data collection and analysis as well as writing, and approved the submission.

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Data availability Raw data are available from the corresponding author.

Compliance with ethical standards

Conflict of interest Luis Hernán Llano López and Pablo Melonari were invited speakers on webinars and meetings organised by BioFire Diagnostics LLC and Biomérieux Argentina. None of the authors received fees or any other recompensations for their research.

Ethical approval Retrospective analyses are exempt from ethical approval by law per article 36 paragraph 2 Landeskrankenhausgesetz Rheinland-Pfalz.

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