A diagnostic algorithm to optimize detection of *Shigella* spp. and enteroinvasive *E. coli*

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**INTRODUCTION**

*Shigella* spp. and enteroinvasive *Escherichia coli* (EIEC) are causative agents of bacillary dysentery. A unique property shared by *Shigella* spp. and EIEC is a 220-kb plasmid (pINV) which contains virulence factors involved in invasion of intestinal epithelial cells. Diagnosis of infection caused by *Shigella* spp. or EIEC is hampered due to low sensitivity of culture and lack of unique geno-/phenotypic properties, rendering discrimination of these pathogens a challenge. This study describes a diagnostic algorithm using both molecular and conventional methods to optimize detection of *Shigella* spp. and EIEC.

**MATERIALS AND METHODS**

- A total of 4426 stool samples from patients with presumed infectious gastroenteritis were prospectively screened using real-time multiplex PCR (qPCR) for enteropathogens, including *Shigella*/EIEC (*ipaH* gene). Subsequently, *ipaH* qPCR positive stools were cultured on selective media, directly and after enrichment in GN broth (Figure 1).Suspicious colonies were identified using biochemical methods and serotyping (*S. sonnei* Phase I/Phase II, *S. flexneri*, *S. boydii*, *S. dysenteriae* EIEC polyvalent 7 - O28ac, O112ac, O124, O136, O144, and EIEC polyvalent 8 - O29, O143, O152, O164).
- Additional qPCRs targeting the O-serotype specific *wzx* gene for *Shigella sonnei*, *Shigella flexneri* type 1-6 and *Shigella dysenteriae* type 1 were performed on all *ipaH* PCR positive stools, incubated GN broths and MC (MacConkey) plate streaks.
- Furthermore, conventionally identified isolates of *Shigella*/EIEC were confirmed with these qPCRs.

**RESULTS**

- In total, 41/4426 (0.9%) stool samples were *ipaH* positive. The algorithm was applied to 38 samples (35 patients).
- Of the 38 *ipaH* positive samples 31 (82%) were confirmed with O-serotype specific PCR (Table 1). Culture confirmed the diagnosis in 4 additional samples. The 3 remaining samples had relatively high *ipaH* Cᵢ values (all Cᵢ > 30).
- A total of 18/38 (47%) *ipaH* positive samples were confirmed by guided culture. In 28/38 (74%) cultures *ipaH* PCR positive plate streaks were found.
- *S. sonnei*, *S. flexneri*, and EIEC were detected in 13 (34%), 17 (45%), and 3 (8%) samples, respectively. Two samples were mixed infections (5%). *Plesiomonas shigelloides* was detected in one sample and one sample remained inconclusive (Table 1).
- Cᵢ values of samples in which culture remained negative were significantly higher (p < 0.001) compared to culture positive samples (Figure 2). This difference remained significant (p < 0.001) when regarding *ipaH* PCR positive plate streaks as culture positive samples.
- PCR after enrichment in GN broth did not lower *ipaH* PCR Cᵢ values compared to PCR on direct sample (p = 0.59).
- A total of 25/34 (74%) patients had travelled to a high-risk region for developing infectious diarrhea.

**CONCLUSIONS**

- The diagnostic algorithm enables fast and specific diagnosis of *Shigella* spp. and EIEC infections.
- The majority of *ipaH* positive specimens contained either *S. sonnei* or *S. flexneri*, whereas detection of EIEC appeared low.
- The GN enrichment step did not improve culture yield.