Evaluation of real-time PCR based assay for bacterial gastroenteritis pathogens

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INTRODUCTION AND PURPOSE

Annually 80 million tourists traveling to tropical and subtropical areas contract traveler's diarrhea (TD). Approx. 40 to 80% of cases are caused by bacteria. Standard clinical diagnostic tests can identify only a few of the bacteria causing TD. We developed a quantitative polymerase chain reaction (qPCR) assay to identify all major bacterial pathogens. The aim was to achieve low-cost, high-throughput, multiplex qPCR assay for simultaneous detection of eight bacterial pathogens in stool samples: *Salmonella*, *Yersinia*, *Campylobacter, Shigella* or enteroinvasive *Escherichia coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and enteropathogenic *E. coli* (EPEC).

This study investigated performance of new multiplex qPCR test compared with culture and reference molecular methods. The aim was to assess technical feasibility to clinical screening purposes.

MATERIALS AND METHODS

A set of 1168 stool samples from patients with suspected bacterial diarrhea were evaluated by the Amplidiag™ Bacterial GE assay (Mobidiag Ltd.). Two extraction instruments were used for sample preparation step: A) 400 µl of patient samples in eSwab (Copan, Italy) tube was extracted with Bullet Stool Kit using Bullet (DiaSorin, Ireland) instrument according to manufacturer's instruction. B) A 150-200mg, pea-size, amount of stool sample was suspended to 600 µl of 1x PBS, heated at 100 °C for 10 min and centrifuge 15 sec at 500 x g. 300 µl of supernatant with 250 µl of BLB (Roche) buffer were vortexed and 500 µl added to the extraction using MagNA Pure 96 DNA and Viral NA Large Volume Kit and MagNA Pure 96 Instrument (Roche) instrument. 5 µl of DNA extracts were transferred to the Amplidiag™ Bacterial GE assay analysis according to manufacturer's instruction. Target identification and control analysis were analyzed and reported automatically by the Amplidiag™ Analyzer software. See workflow demonstration in Figure 1.

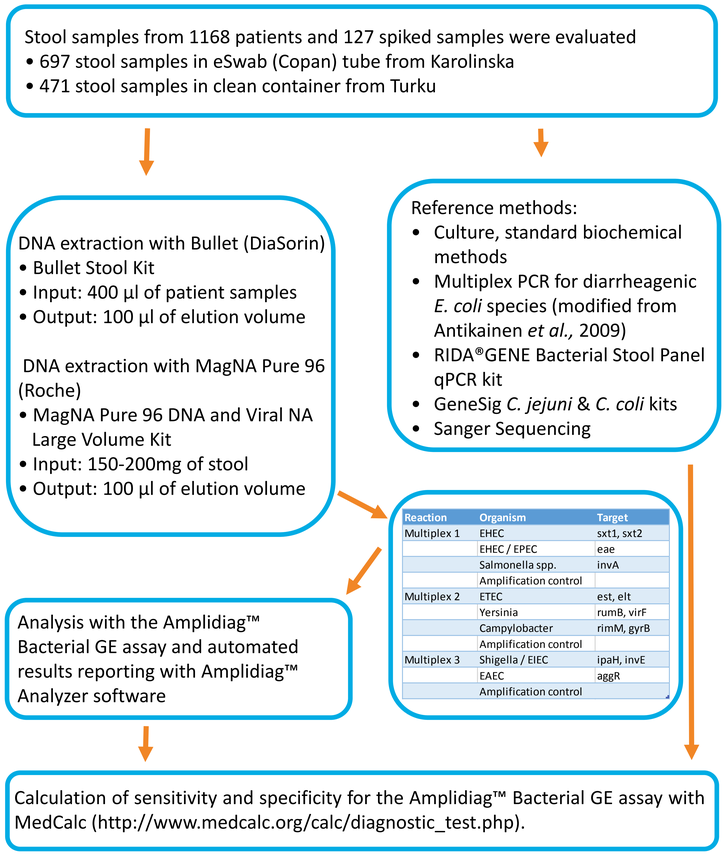

Figure 1. Workflow of sample analysis.

RESULT

The results demonstrated good performance of the Amplidiag™ Bacterial GE real-time PCR assay in comparison to golden standard. No false negative results, compared with culture alone, were detected. This was a key finding to justify screening by real-time qPCR method. As expected, small number of false positive or negative findings were detected utilizing reference molecular methods. All results which could not be repeated and confirmed by reference methods were determined as false negative or positive. False positive results were probably mainly caused by inability to repeat and verify low abundant findings by reference methods, except one case with unambiguous result between *C. coli* and *C. upsaliensis*.

Table 1. Sensitivity and specificity of the Amplidiag™ Bacterial GE assay

Description	Salmonella	Yersinia	Campylobacter	Shigella/EIEC	EHEC	EHEC/EPEC	ETEC	EAEC
Sensitivity (95% CI)	**100 (89.3-100)**	**100 (85.6-100)**	**98.9 (94.2-99.8)**	**98.0 (89.3-99.7)**	**100 (81.3-100)**	**99.1 (95.1-99.9)**	**93.1 (77.2-99.0)**	**100 (95.0-100)**
Specificity (95% CI)	**99.7 (99.2-99.9)**	**99.9 (99.5-100)**	**99.3 (98.7-99.7)**	**99,3 (99.6-100)**	**99.9 (99.6-100)**	**98.8 (98.1-99.3)**	**99,7 (99.3-100)**	**99.8 (99.4-100)**
Positives	33	24	94	50	18	113	29	72
Negatives	1326	1335	1254	1336	1347	1262	1357	1314
True positives	33	24	93	49	18	112	27	72
False positives	4	2	9	1	1	17	4	3
False negatives	0	0	1	1	0	1	2	0
Total samples	1359	1359	1348	1386	1365	1375	1386	1386
PPV (95% CI)	89.2 (74.6-96.9)	92.3 (74.8-98.8)	91.2 (83.9-95.9)	98.0 (89.3-99.7)	94.7 (73.9-99.1)	86.8 (79.7-92.1)	87.1 (70.1-96-3)	96.0 (88.7-99.1)
NPV (95% CI)	100 (99.7-100)	100 (99.7-100)	99.9 (99.6-100)	99.9 (99.6-100)	100 (99.7-100)	99.9 (99.6-100)	99.9 (99.5-100)	100 (99.7-100)

CONCLUSIONS

This study suggests that screening of most common, or important, pathogens of bacterial diarrhoea is technically possible without compromising sensitivity for important pathogens, at least in low endemic settings. A confirmatory identification, and antimicrobial susceptibility, for positive results by standard biochemical methods is still relevant for some of the pathogens. In future, economical studies related to overall impact on the clinical process should be carried out to position this approach among other diagnostic workflows.

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