Real-time PCR for the diagnosis of *E. coli* meningitis in infants: A diagnostic accuracy study

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BACKGROUND

Escherichia coli is the most common Gram-negative organism associated with meningitis in infants[1], with an annual incidence rate of around 0.14/1,000 live births in Ireland since 2014 (95% CI, 0.03 to 0.23/1,000)[2]. Prompt diagnosis and treatment are essential to achieving good outcomes in affected infants. Cerebrospinal fluid (CSF) culture of *E. coli* is considered the gold standard for the diagnosis of *E. coli* meningitis (ECM), however this can be frequency negative due to prior antimicrobial exposure or the difficulty in obtaining adequate volumes of CSF for culture and other routine analyses. Other parameters frequently considered useful adjuncts for ECM diagnosis are CSF white cell count (WCC) and the presence of *E. coli* bacteraemia[1]. Few commercial systems are available to detect *E. coli* in clinical specimens; the BioFire FilmArray Meningitis/Encephalitis Panel only detects K1capsular type strains of *E. coli*[3]. However, other *E. coli* types are also known to cause ECM and with the increasing use of intrapartum antibiotic prophylaxis, it is likely that diverse clones that exhibit decreased susceptibility to many first-line antimicrobials will emerge as more common causes of ECM[4]. Therefore, there is an urgent need for a rapid and sensitive method to detect all *E. coli* strains directly from CSF specimens.

AIM

To describe the performance of an in-house developed real-time PCR assay, for the detection of all strains of *E. coli* in CSF, introduced into the panel of PCR tests offered nationally for invasive bacterial pathogens on 1st January 2014 by IMSRL.

METHODS

Samples: First CSF sample received from infants (aged under 90 days) referred to the IMSRL for *E. coli* PCR over the seven year period, 01/01/2014 to 31/12/2020.

Data: CSF sample culture result, any concomitantly *E. coli* positive sites and CSF WCC, if known. Case data was also reconciled with ECM data entered on CIDR.

Analysis: Performed in Microsoft Excel and Diagnostic accuracy test using MedCalc statistical software (version 19.0; available at www.medcalc.org).

RESULTS

- Primary CSF samples from 876 infants (Fig. 1)
- 62.7% male (n=549), 34% female (298), 3.3% gender unknown (n=29)
- Median age 20 days (range 0-90days; IQR 42 days)
- 44 CSFs tested positive for *E. coli* DNA (5%; Fig. 1 & Table 1)
  - 17 were culture positive (median Ct 28.41; Fig.2)
  - 27 were culture negative (median Ct = 37.17; Fig.2)
    - Concomitant *E. coli* bacteraemia in 56% of PCR positive/culture negative infants (Table 2)
  - Pleocytosis present in 88% of PCR positive infants
- 830 CSF samples tested negative for *E. coli* DNA (Fig. 1 & Table 1)
  - 1 was culture positive (PCR test negative - suboptimal volume (<200µl) of CSF received, collected 2 days after date of diagnosis‡)
  - 829 were culture negative
    - Concomitant *E. coli* bacteraemia in 14.5% (Table 2)
  - Pleocytosis present in 38% of PCR negative infants
- Compared with culture the PCR test exhibited 97% accuracy, 94% sensitivity & 97% specificity (Table 2)

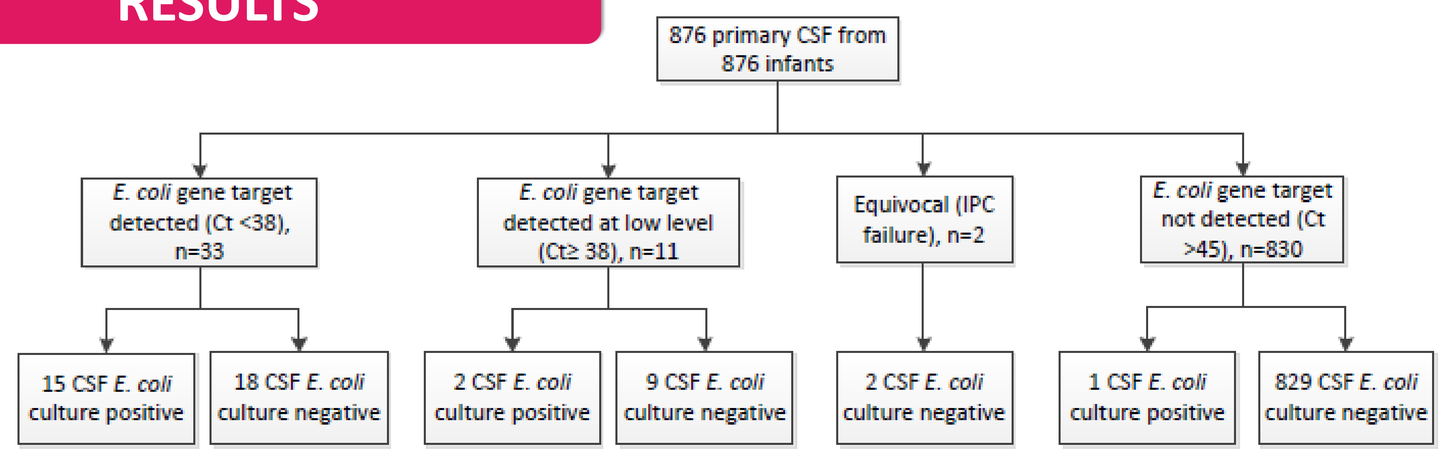

Fig. 1. Flow-chart of CSF samples received and tested with the in-house developed *E. coli*-specific real-time PCR assay.

Table 2. Pre-test probabilities: other relevant microbiological data considered valuable in decision to refer for *E. coli* PCR test

Baseline characteristic	All primary CSF samples	*E. coli* DNA detected (PCR test positive)	*E. coli* DNA not detected (PCR test negative)
CSF samples, each from a separate infant*	876	44	830
CSF White Cell Count (WCC) known	197 (22.5%)	17 (38.6%)	180 (21.7%)
Pleocytosis**	84 (42.6%)	15 (88%)	69 (38.3%)
CSF culture negative infants with concomitant *E. coli* positive other site	n=856	n=27	n=829
Blood culture/PCR result known	651 (76.1%)	25 (92.6%)	626 (75.5%)
Blood culture/PCR positive for *E. coli*	105 (16.1%)	14 (56%)	91 (14.5%)
Blood & NIS culture/PCR result known***	223	26	197
Blood & NIS culture/PCR positive for *E. coli****	192 (86.1%)	15 (57.7%)	177 (89.8%)

*PCR test was inconclusive for 2 CSF samples; **The age-specific cut-off criteria for normal range was taken from the United Kingdom standard for Microbiological Investigation for Cerebrospinal fluids: for neonates aged ≤6 days the normal range for CSF WCC is 0-30 cells x10^6/L & 0-20 cells x10^6/L for children aged 7 days to 4 years. ***NIS - non-invasive site /urine sample culture positive for *E. coli*, assumed indicative of a urinary tract infection (UTI).

Table 1: Diagnostic accuracy table comparing PCR against culture for CSF samples.

Diagnostic Test Evaluation (culture vs. PCR for *E. coli*)		
	Culture positive	**Culture negative**
PCR test positive	17	27
PCR test negative	1‡	829
PCR test equivocal	0	2
Results		
Statistic	**Value**	**95% CI**
Sensitivity (%)	94.44	72.71 – 99.86
Specificity (%)	96.85	95.45 -97.91
Positive Likelihood Ratio	29.98	20.34 – 44.18
Negative Likelihood Ratio	0.06	0.01 – 0.39
Positive predictive value (%)	38.66	29.94 – 48.13
Negative predictive value (%)	99.88	99.2-99.98
Accuracy (%)	96.8	95.41 – 97.86

‡ discordant PCR test negative/culture positive result

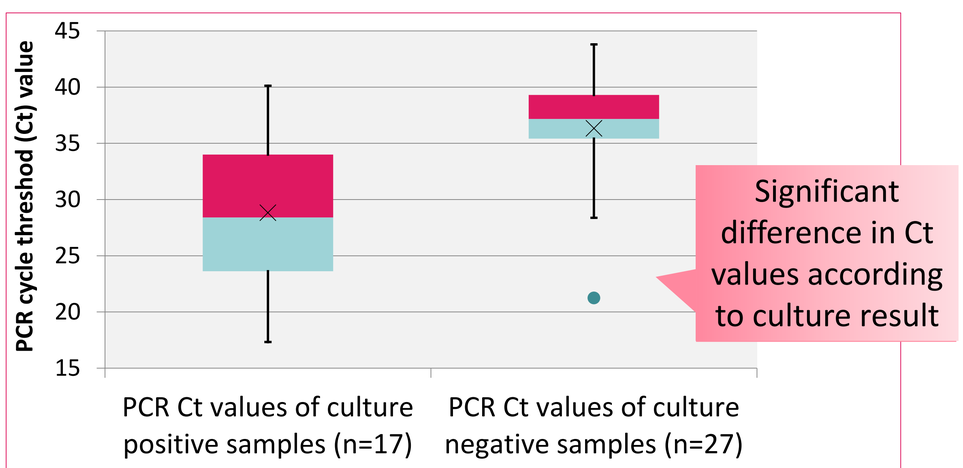

Fig. 2. Box-plot of PCR Ct values according to culture result.
A significant difference between Ct values was observed (p=0.00059)

CONCLUSIONS

- This study demonstrates that the IMSRL *E. coli* PCR assay is sensitive and specific in CSF samples when compared with culture, displaying a 97% accuracy & capable of detecting all (including K1 negative) *E. coli* strains (manuscript in preparation).
- Over the 7 year study period, the incidence of *E. coli* meningitis in this population was low, with a 5% test positivity rate, indicating the requirement for a judicious approach to PCR requesting.
- Co-existing *E. coli* bacteraemia or UTI was not a predictor of meningitis and although pleocytosis was more common in infants with positive *E. coli* PCR results, it is not indicative of *E. coli* meningitis and more likely reflects concomitant UTI.

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