Evaluating the impact of the BioFire FilmArray in childhood meningitis: an observational cohort study

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Abstract

Background

Multiplex PCR assays have the potential to reduce antibiotic use and shorten length of inpatient stay in children with suspected Central Nervous System (CNS) infection by obtaining an early microbiological diagnosis. The clinical impact of the implementation of the BioFire FilmArray Meningitis/ Encephalitis (ME) Panel on the management of childhood meningitis was evaluated at the John Radcliffe hospital in Oxford and Children's Health Ireland at Temple Street in Dublin.

Methods

Children who had lumbar punctures performed as part of a septic screen were identified retrospectively through clinical discharge coding and microbiology databases from April 2017 to December 2018. Anonymised clinical and laboratory data were collected. Comparison of antibiotic use, length of stay and outcome at discharge was made with a historical cohort in Oxford (2012-2016), presenting prior to implementation of the FilmArray.

Results

The study included 460 children who had a lumbar puncture as part of an evaluation for suspected CNS infection. Twelve bacterial *cases were* identified on the FilmArray that were not detected by conventional bacterial culture. Bacterial culture identified one additional case of bacterial meningitis, caused by *Escherichia coli*, that had not been identified on the FilmArray.

Duration of antibiotics was shorter in children when FilmArray was used than before its' implementation; enterovirus meningitis (median 4 days vs 5 days), human parechovirus meningitis (median 4 days vs 4.5 days) and culture/FilmArray negative CSF (median 4 days vs 6 days).

Conclusions

The use of a FilmArray can identify additional bacterial cases of meningitis in children that had been negative by traditional culture methods. Children with viral meningitis and culture negative meningitis received shorter courses of antibiotics and had shorter hospital stays when FilmArray was used. Large studies to evaluate the clinical impact and cost effectiveness of incorporating the FilmArray into routine testing are warranted.

Introduction

The introduction of bacterial polysaccharide-protein conjugate vaccines has led to a significant reduction in the incidence of childhood bacterial meningitis (1,2). Over the last two decades, there has been a rapid increase in the diagnosis of viral meningitis in infants and young children (3,4). This apparent increase appears to be driven by the replacement of traditional culture methods with highly sensitive molecular diagnostic tools such as polymerase chain reaction (PCR) assays (5).

The use of multiplex PCR assays, which rapidly identify viral causes of meningitis, could lead to reduction in healthcare costs (e.g. shortened hospital length of stay), improvement in antimicrobial stewardship (e.g. early discontinuation of antibiotics) and reduction in pain (e.g. minimising investigations such as repeat blood cultures/lumbar punctures). However, data from a U.K surveillance study shows that the uptake of PCR assays to detect viral pathogens in the evaluation of meningo-encephalitis is highly variable across National Health Service (NHS) laboratories (6). The indication to test for viruses in CSF varies is dependent on the age of the patient, evidence of pleocytosis, specific clinician request or seasonality.

The BioFire FilmArray Meningitis/Encephalitis PCR panel (ME PCR Panel, FilmArray, BioFire Diagnostics, Salt Lake City, UT) can detect 14 common bacteria, viruses and yeast from CSF, requires 0.2mls of CSF and delivers a result within an hour. However, uncertainty exists on how best to

integrate the test into the routine diagnostic algorithm, financial cost, concern regarding false positive bacterial results (mainly with *Streptococcus pneumoniae* and group B Streptococcus) and false negative viral results (mainly with HSV) in children (7).

We evaluated the impact of the BioFire FilmArray on the clinical management of children with suspected meningo-encephalitis in two large tertiary paediatric hospitals in England and Ireland.

Methods

Study population

The study was conducted at the John Radcliffe Hospital, Oxford and Children's Health Ireland at Temple Street in Dublin. Any child less 16 years old who had a lumbar puncture obtained as part of a septic screen between April 2017 and December 2018 was eligible. Cases were retrospectively identified through local microbiology databases and hospital discharge coding data.

A study proforma was devised to collect anonymised demographic, clinical, laboratory and management data from hospital records. The study was classified as service evaluation and therefore did not require parental consent. CSF samples at both hospitals were tested on site using the BioFire FilmArray ME Panel. All microbiological samples were tested according to local standard operating procedures.

Clinical data collected from the John Radcliffe Hospital, Oxford were compared to cases from the same centre that were enrolled in the UK Childhood Meningitis and Encephalitis (ChiMES) cohort study from 2012 – 2016 (8). The ChiMES study enrolled children younger than 16 years old who had a lumbar puncture performed for suspected meningo-encephalitis. Participants were enrolled into the ChiMES study after informed written consent was obtained. Data obtained from the ChiMES study provides a historical cohort to allow comparison of clinical management of children with suspected meningo-encephalitis in an era before the introduction of the BioFire FilmArray(8).

FilmArray ME testing in clinical practice

At the John Radcliffe Hospital, Oxford, the BioFire FilmArray is used routinely for samples from all children who have had a lumbar puncture as part of a septic screen irrespective of age of child or CSF white cell count and performed at all times of the day. However, at Children's Health Ireland at Temple Street, the BioFire FilmArray is only used if there is evidence of CSF pleocytosis, if the bacteria is isolated from another site (typically blood or urine), in instances where the child is at increased risk for invasive infection or consultant microbiologist request. It is not

routinely used for investigation of suspected ventriculo-peritoneal shunt infections. CSF samples can be processed overnight if deemed necessary and all positive viral PCR results are sent to the National Virus Reference Laboratory in Ireland for confirmation. At Oxford, no confirmatory viral PCR testing is performed unlike in Dublin where this is performed routinely.

Discordant results

Results were considered discordant if the BioFire FilmArray and gold standard test results (i.e. bacterial culture, single target or alternative multiplex PCR if available) were different. At this point, adjudication using medical notes and laboratory records in order to confirm if this was a genuine discrepancy was conducted by S.K and S.P. Re-testing samples using the BioFire FilmArray was not possible due to the lack of volume available and because samples are often discarded before this could be considered.

Data analysis

The distribution of demographic, clinical presentations and clinical test results between hospitals were presented by median and IQR for continuous variables or frequency and percentage for categorical variables.

The demographic, clinical, laboratory and management data are summarised for both the BioFire FilmArray and ChiMES cohorts using median and IQR for continuous variables or frequency and percentage for categorical variables. The endpoints to assess the impact of BioFire FilmArray introduction are antibiotic duration, length of hospital stay and outcome at discharge (completely recovered or not). Since the antibiotic duration and length of hospital stay had highly skewed disruptions, we dichotomised both variables using a cut-off of 7 days (>7 days, ≤7 days). To adjust for potential confounding effects, logistic regression models were used to test whether the introduction of the BioFire FilmArray was associated with change in antibiotic duration, length of hospital stay and outcome at discharge. The potential confounding factors adjusted in the model included age, place of admission (ward, high dependency unit, paediatric intensive care unit, neonatal intensive care unit and children's decision unit), peripheral white cell count, neutrophil count, c- reactive protein (CRP), cerebrospinal fluid (CSF) protein, CSF glucose, CSF white cell count and CSF red cell count.

We observed a high proportion of patients with missing values for antibiotic duration so we further imputed missing data by multiple imputation (MI) as this is generally preferred instead of dropping data with missing values in order to avoid potential bias (9). The assumption for the MI is that the probability of missing on a variable does not depend on the value of that variable but it may depend on other observed variables (10). Before conducting the MI, we compared the patients with and without missing data on antibiotic duration. The imputation model included all the potential confounding factors in the regression analysis, length of hospital stay and outcome at discharge. Five independent data sets were imputed and were included in the analysis together with the original database which had missing values were removed. The variation in the

imputed values between the MI data sets reflects uncertainty about the missing values. All analyses were conducted using R version 3.6.1, and "mice" package (version 3.6.0) was used to conduct the missing data imputation (11,12).

Results

Overall, 303 children in Oxford (from January 2018 to December 2018) and 69 children in Dublin (from April 2017 to December 2018) who had CSF tested using a FilmArray were included in the study (Table 1). Both centres used different algorithms to integrate the FilmArray. The baseline demographic and clinical characteristics of cases enrolled in each centre are therefore different (appendix Table 1 and appendix Table 2). In summary, children in the Dublin cohort were older (median age 17 months vs 1 month) and had more severe disease (seizures in 29.0% vs 13.8%, haemodynamic shock in 27.5% vs 6.3% and abnormal Computed Tomography head scans in 8.7% vs 2.3%) than in Oxford.

Discordant results between Biofire FilmArray and CSF culture

In total, there were 12 bacterial (5 *Neisseria meningitidis,* 3 *Streptococcus pneumoniae, 2 Escherichia coli,* 2 group B streptococcus) and 1 fungal (*C. gattii) case* identified on the FilmArray that were not detected on bacterial culture (appendix Table 3). The lumbar puncture was performed

after antibiotics in all 12 bacterial cases. The bacterial cases were all deemed true positive as they were also identified on PCR testing at the national reference laboratory in England and Ireland respectively. However, the *C. gatti* was a false positive as the clinical syndrome was inconsistent with the result and testing at the reference centre using a specific PCR assay was negative. One additional case of bacterial meningitis was identified by bacterial culture in CSF, caused by *Escherichia coli*, that had not been identified on the FilmArray. In this case, the FilmArray result was deemed to be a false negative as the infant had *E.coli* with pyuria that was cultured from the urine, presenting features consistent with CNS infection and renal imaging showed an abnormal urinary tract. Two children from the overall cohort of 460 cases enrolled had HSV identified on FilmArray. One of these cases had positive confirmatory PCR testing. No additional cases were identified on standard PCR testing that were not identified on FilmArray.

Clinical impact of the introduction of the BioFire FilmArray

The impact of the introduction of the BioFire FilmArray was made by comparing duration of antibiotic use, length of stay and clinical outcome at discharge with cases enrolled in the ChiMES study at Oxford during 2012 -16 (i.e. pre implementation of the BioFire FilmArray).

The ChiMES study recruited 276 children in Oxford between 2013 and 2016. Table 1 summarises the demographics, clinical and laboratory data of the two cohorts. The median length of hospital stay was 4 days (range: 0-226) and 4 days (range: 1-124 days) for the BioFire and ChiMES

cohorts respectively in children with no pathogen isolated. However, children with enterovirus (EV) meningitis (median 2 days vs 3 days) and human parechovirus (HPeV) meningitis (median 3 days vs 3.5 days) had shorter lengths of stay in the BioFire FilmArray compared with the ChiMES cohort.

The median duration of antibiotics was 4 days (range: 0-28) in the BioFire and 6 days (0-118) in the ChiMES cohort in children with no pathogen isolated (Table 2). Children with EV meningitis (median 4 days vs 5 days) and HPeV meningitis (median 4 days vs 4.5 days) received fewer days of antibiotics in the BioFire FilmArray compared to the ChiMES cohort.

As the proportion of children with missing data on antibiotic use was around 50%, we further compared children with and without missing data in the BioFire FilmArray cohort (appendix table 4) and imputed the missing data by MI for regression analysis. Appendix table 4 shows the pooled results by multivariable logistic regression after MI to study the impact of BioFire FilmArray introduction on antibiotics use and hospital stay and presents the logistic regression results for the BioFire and ChiMES cohorts combined. The estimate of OR for hospital stay >7 days increased to 1.4 (95%CI: 0.9-2.3) but the result remains statistically non-significant (p=0.16). We observed a similar result for antibiotics use before and after MI.

Discussion

This study shows that the introduction of BioFire FilmArray reduces antimicrobial use and shortens length of stay in children with suspected meningitis. This impact appears most marked in children with viral meningitis. The BioFire FilmArray identified 12 additional cases of bacterial meningitis that had not been detected on traditional bacterial culture. However, one case of *E.coli* meningitis was detected on CSF culture and not identified using the BioFire FilmArray.

The FilmArray was introduced into the U.K in 2017. A systematic review found only 5 paediatrics studies (4 conducted in the USA and 1 in Australia) which included 624 children that evaluated the diagnostic accuracy of the BioFire FilmArray (7). The largest multicentre trial of the FilmArray, which included 1560 CSF samples in adults and identified 12 out of 16 *S. pneumoniae* results that were false positive. However, after reviewing those discordant results with repeat testing and clinical data, a further 5 out of 12 cases were deemed to be true positive (13). A study in children, identified one out of 4 positive *S. pneumoniae* cases using the FilmArray was a likely contaminant due to mishandling (14). Bacterial culture is the gold standard for the diagnosis of bacterial meningitis but has a low yield after antimicrobial exposure. A study of 62 CSF samples in young infants found that the FilmArray identified a further 7 cases of bacterial meningitis than culture when CSF was taken after antibiotics were administered (15). In our study, we showed no evidence of discordance with bacterial cases identified with the FilmArray. We found one case of *E.Coli* identified on bacterial culture in CSF but not FilmArray. This could be explained by the fact that FilmArray only detects *E.coli* K1

serotype. The K1 serotype accounts for approximately 80% of all isolates in neonatal *E.coli* meningitis (16). Clinicians must therefore have awareness that not all E. coli serotypes are detected by the this Multiplex PCR.

HSV encephalitis causes 70% case fatality in untreated patients and up to 30% fatality with a high risk of permanent neurological sequalae even in treated patients (17). There have been multiple small studies which have reported false negative HSV results on the FilmArray (18–20). In our study, we found no evidence that the BioFire FilmArray missed any cases of HSV meningo-encephalitis. However we only had 2 cases of HSV encephalitis in our cohort. In total, we identified 15 cases in which HHV-6 was identified on the BioFire FilmArray. In each of these cases, HHV-6 was unlikely to have caused a CNS infection, as clinical features were inconsistent with meningo-encephalitis. In addition, all the samples were heavily blood stained which likely reflects viraemia rather than CSF infection. In an analysis of 15 patients with a positive HHV-6 CSF PCR assay (also using the FilmArray), Green et al. report that only in 1 case of HHV-6 was thought to be likely significant, in a 17 year old HSCT patient with altered mental consciousness and seizures (21). The routine use of a FilmArray will likely identify viral infections in CSF, such as HHV-6, when there is presence of large numbers of red cells in CSF and in the context of viraemia, chromosomally integrated HHV-6 and often not clinically relevant. In these instances, careful discussion between the laboratory and clinical team is warranted to understand the significance of a positive FilmArray result, whether clinical features are consistent with viral meningo-encephalitis and any host susceptibility factors that make genuine infection more likely.

To our knowledge, this is the largest clinical case series of childhood meningitis evaluating the implementation of the BioFire FilmArray ME panel. Tansarli and colleagues, in a systematic review of the diagnostic accuracy of the BioFire FilmArray, showed that the highest proportion of false positive results were due to *S. pneumoniae* and followed by GBS (7). These findings were noted in the earliest studies to use the BioFire FilmArray in clinical practice. Our data did not reveal any false positive bacterial results. This could be due to careful validation of the assay at Oxford and Dublin before introducing into clinical practice, which included adherence of strict precautions to minimise contamination. Data from our study are consistent with smaller paediatric studies which show low false positivity rates with EV or HPeV (14,22,23).

We were able to compare the added value of using the BioFire FilmArray with a historical cohort of children recruited to the ChiMES study in Oxford. This provided a unique insight by showing that using the BioFire FilmArray had reduced antibiotic duration and length of hospital stay in children with viral meningitis. We have shown that using the BioFire FilmArray reduced median duration of antibiotics by 2 days in children with culture negative suspected meningitis.

This study has limitations. The FilmArray has only been introduced in the U.K in a few centres from 2017 and so we were only able to collect data from two tertiary paediatric centres which had minimal previous experience of using the assay. This study therefore has a small number of cases of bacterial meningitis and only two cases of HSV encephalitis making it implausible to derive any robust sensitivity or specificity analyses. The retrospective nature and relatively small number of cases made it not possible to do a cost analysis i.e. through saved bed days and reduced

antibiotic usage. The ChiMES study provided a historical cohort of children in Oxford with suspected/proven meningitis who were enrolled into a research study. This historical cohort is therefore subject to selection bias as parents were required to give written consent to be enrolled into the study. There are therefore inherent differences to the BioFire FilmArray and ChiMES cohort which are outlined in table 3. Finally, there was no residual sample left to repeat CSF testing in one patient with discordant results (E. coli) and adjudication was done by two co-authors who retrospectively reviewed laboratory data and clinical notes.

Often, infants with EV or HPeV meningitis do not have CSF pleocytosis (6,24). A diagnostic algorithm that relies on using the FilmArray only in cases of CSF pleocytosis will therefore miss the high proportion of cases of viral meningitis in this age group. Data from this study, support considering the implementation of the FilmArray in all CSF samples irrespective of the presence of pleocytosis, in infants under 90 days where the burden of enteroviral and parechovirus meningitis is highest. However, it could be argued that identifying a viral agent in those cases may not impact on clinical care, as most of these patients typically recover rapidly with good outcome. CSF pleocytosis is a prominent feature of bacterial meningitis. It would therefore be expected that the commonest causes of childhood bacterial meningitis would be identified on the FilmArray, even following antibiotic administration (25,26).

The FilmArray delivers a binary result and provides no quantitative data. In our opinion, it is therefore crucial that molecular diagnostic testing such as the FilmArray are used in settings where there are strong collaborative links between clinicians and laboratory teams to interpret results

carefully. This may include use of a pathogen specific real-time PCR test, or a repeat CSF sample especially if symptoms started less than 72 hours preceding the LP if there is a high index of suspicion of HSV. Laboratories should follow strict SOP's to minimise the risk of contamination with respiratory pathogens such as *Streptococcus pneumoniae*. Finally, data from this study are a reminder for clinicians to undertake LP's to rule out meningitis at the earliest opportunity, if possible before antibiotic administration, to achieve early discontinuation of antibiotics and discharge.

The FilmArray has the potential to significantly improve the clinical management of children with suspected and proven meningitis. Bacterial meningitis and HSV meningo-encephalitis are rare but can both cause significant morbidity and mortality. Future studies evaluating the diagnostic accuracy of the FilmArray therefore need to include significant numbers of cases given the paucity of both these syndromes in single centres. There are emerging paediatric data, from retrospective single centre studies, that the FilmArray can contribute to hospital cost savings through shorter intensive care admissions and less antibiotic usage (27,28). However, future prospective multicentre trials, should consider the cost implications of using the FilmArray, in different diagnostic algorithms across healthcare systems. Larger multicentre studies should prospectively evaluate the feasibility of integrating the FilmArray into routine clinical care given the potential clinical benefits to children and clinicians.

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	Oxford (N=303)	Dublin (N=69)
Age		
n (%).	303 (100.0%)	69 (100.0%)
Median in year (Range)	0.1 (0.0-15.9)	1.5 (0.0-15.0)
0-3m	207 (68.3%)	27 (39.1%)
3-12m	34 (11.2%)	7 (10.1%)
1-5y	35 (11.6%)	11 (15.9%)
5-11y	18 (5.9%)	15 (21.7%)
11-16y	9 (3.0%)	9 (13.0%)
Sex		
Female, n (%)	132 (43.6%)	27 (39.1%)
Duration of admission		
n (%).	297 (98.0%)	69 (100.0%)
Median(Range)	4.0 (0.0-226.0)	6.5 (0.3-273.0)
Temperature		
n (%)	167 (55.1%)	68 (98.6%)
Median(Range)	38.0 (34.4-40.8)	38.0 (35.0-40.5)
Seizure		
Yes, n (%)	41 (13.5%)	20 (29.0%)
Missing, n (%)	9 (3.0%)	1 (1.4%)
Shock		
Yes, n (%)	19 (6.3%)	19 (27.5%)
Missing, n (%)	8 (2.6%)	1 (1.4%)
Respiratory Distress		
Yes	59 (19.5%)	10 (14.5%)
Missing	8 (2.6%)	1 (1.4%)
Abdominal distension		
Yes, n (%)	3 (1.0%)	4 (5.8%)
Missing, n (%)	8 (2.6%)	3 (4.3%)
Bulging		
Yes, n (%)	8 (2.6%)	4 (5.8%)
Missing, n (%)	9 (3.0%)	1 (1.4%)
Irritability		

Yes, n (%)	64 (21.1%)	53 (76.8%)
Missing, n (%)	9 (3.0%)	1 (1.4%)
Lethargy		
Yes, n (%)	77 (25.4%)	60 (87.0%)
Missing, n (%)	9 (3.0%)	1 (1.4%)
Reduced feeding		
Yes, n (%)	102 (33.7%)	46 (66.7%)
Missing, n (%)	9 (3.0%)	1 (1.4%)

Appendix Table 1: Demographic and presenting features of children who had an LP at the John Radcliffe Hospital, Oxford and Temple Street

	BioFire FilmArray (N=303)	**ChiMES (N=276)**
Gender		
Male	171 (56.4%)	172 (62.3%)
Female	132 (43.6%)	104 (37.7%)
Age, median (range)	0.1 (0.0-15.9) [n=303]	0.2 (0.0-15.6) [n=276]
Age group		
0-3m	207 (68.3%)	168 (60.9%)
3-12m	34 (11.2%)	53 (19.2%)
1-5y	35 (11.6%)	33 (12.0%)
5-11y	18 (5.9%)	13 (4.7%)
11-16y	9 (3.0%)	9 (3.3%)
Hb (g/d), median (range)	121.0 (3.0-241.0) [n=294]	11.7 (7.1-118.0) [n=275]
WCC x109/L, median (range)	11.6 (0.3-42.4) [n=294]	10.8 (1.6-40.7) [n=275]
Lymphocytes x109/L, median (range)	3.6 (0.1-23.9) [n=294]	3.5 (0.4-12.3) [n=269]
Neutrophil x109/L, median (range)	5.3 (0.0-35.0) [n=293]	4.5 (0.3-37.4) [n=272]
Platelet x109/L, median (range)	331.5 (5.2-808.0) [n=294]	361.0 (19.0-900.0) [n=274]
CRP (mg/L), median (range)	14.2 (0.1-467.2) [n=284]	9.1 (1.8-422.0) [n=270]
Duration of admission (days)	4.0 (0.0-226.0) [297]	4.0 (1.0-124.0) [n=276]
Place of admission		
Ward	227 (74.9%)	221 (80.4%)
HDU	13 (4.3%)	13 (4.7%)
PICU	35 (11.6%)	19 (6.9%)
NICU	0 (0.0%)	0 (0.0%)
MAU	6 (2.0%)	0 (0.0%)
CDU	22 (7.3%)	22 (8.0%)
Duration of antibiotics, median (range)	4.0 (0.0- 28.0) [n=193]	6.0 (0.0-118.0) [n=274]
Outcome at discharge		
Recovered fully at discharge	245 (86.3%)	170 (61.6%)

Recovered with sequelae at discharge	21 (7.4%)	84 (30.4%)
Transferred at discharge	7 (2.5%)	22 (8.0%)
Still admitted	3 (1.1%)	0 (0.0%)
Died	8 (2.8%)	0 (0.0%)
Transferred with sequelae	0 (0.0%)	0 (0.0%)

Table 1 Demographic, laboratory and clinical management data between BioFire Oxford and ChiMES Oxford cohort.

	Oxford (N=303)	Dublin (N=69)
Hb (g/L)		
n (%)	294 (97.0%)	69 (100.0%)
Median (Range)	12.1 (0.3-24.1)	11.4 (7.5-16.0)
WCC x10^9/L		
n (%)	294 (97.0%)	69 (100.0%)
Median (Range)	11.6 (0.3-42.4)	11.0 (1.3-48.3)
Lymphocytes x10^9/L		
n (%)	294 (97.0%)	69 (100.0%)
Median (Range)	3.6 (0.1-23.9)	2.6 (0.4-27.2)
Neutrophil x10^9/L		
n (%)	293 (96.7%)	69 (100.0%)
Median (Range)	5.3 (0.0-35.0)	6.3 (0.5-40.9)
Platelet x10^9/L		
n (%)	294 (97.0%)	68 (98.6%)
Median (Range)	331.5 (5.2-808.0)	334.5 (100.0-714.0)
CRP (mg/L)		
n (%)	284 (93.7%)	67 (97.1%)
Median (Range)	14.2 (0.1-467.2)	10.0 (1.0-500.0)
CruSS		
Not, n (%)	291 (96.0%)	61 (88.4%)
Normal, n (%)	11 (3.6%)	7 (10.1%)
Abnormal, n (%)	1 (0.3%)	1 (1.4%)
CT head		
Not, n (%)	263 (86.8%)	53 (76.8%)
Normal, n (%)	33 (10.9%)	10 (14.5%)
Abnormal, n (%)	7 (2.3%)	6 (8.7%)
MRI		
Not, n (%)	275 (90.8%)	46 (66.7%)
Normal, n (%)	8 (2.6%)	9 (13.0%)
Abnormal, n (%)	12 (4.0%)	13 (18.8%)
Missing, n (%)	1 (0.3%)	1 (1.4%)

	Oxford (N=303)	Dublin (N=69)
Echo		
Not, n (%)	301 (99.3%)	68 (98.6%)
Normal, n (%)	0 (0.0%)	1 (1.4%)
Abnormal, n (%)	2 (0.7%)	0 (0.0%)
CSF Neutrophils		
n (%)	195 (64.4%)	0
Median (Range)	0.0 (0.0-3845.6)	-
CSF Lymphocytes		
n (%)	196 (64.7%)	0
Median (Range)	6.0 (0.0-882.7)	-
CSF WCC		
n (%)	291 (96.0%)	69 (100.0%)
Median (Range)	2.0 (0.0-4370.0)	35 (0.0 – 8640.0)
CSF RCC		
n (%)	290 (95.7%)	69 (100.0%)
Median (Range)	39.0 (0.0-104480.0)	8.0 (0.0-1952.0)
CSF Protein		
n (%)	280 (92.4%)	65 (94.2%)
Median (Range)	593.5 (95.0-12849.0)	394.0 (96.0-2465.0)
CSF Glucose		
n (%)	283 (93.4%)	63 (91.3%)
Median (Range)	2.9 (0.1-543.0)	3.3 (0.5-5.7)
CSF Conventional test		
Positive, n (%)	1 (0.3%)	2 (2.9%)
Biofire bacteria		
Positive, n (%)	7 (2.3%)	6 (8.7%)
Biofire virus		
Positive, n (%)	59 (19.5%)	21 (30.4%)
Duration of antibiotics*		
n (%)	193 (63.7%)	65 (94.2%)
Median (Range)	4.0 (0.0-28.0)	7.0 (1.0-27.1)
Duration of antiviral		
n (%)	17 (5.6%)	35 (50.7%)

	Oxford (N=303)	Dublin (N=69)
Median (Range)	3.0 (0.0-142.0)	2.5 (0.3-16.0)
Outcome		
Recovered fully at discharge, n (%)	245 (80.9%)	46 (66.7%)
Recovered with sequelae at discharge, n (%)	21 (6.9%)	10 (14.5%)
Transferred with sequelae, n (%)	0	4 (5.8%)
Transferred at discharge	7 (2.3%)	1 (1.4%)
Still admitted, n (%)	3 (1.0%)	0
Died, n (%)	8 (2.6%)	1 (1.4%)
All/Missing, n (%)	19 (6.3%)	7 (10.1%)

***** Sum of all antibiotic durations

Appendix Table 2. Laboratory data, radiological results and outcomes of meningitis children

Abbreviations: CrUSS (cranial ultrasound), CRP (C reactive protein), CSF (cerebrospinal fluid), CT (computed tomography), Hb (haemoglobin), MRI (magnetic resonance imaging) RCC (red cell count) WCC (white cell count)

	Duration of antibiotics use >7 days		**Duration of hospital stay >7 days**	
	Odds Ratio (95% CI)	P value	Odds Ratio (95% CI)	P value
Study				
BioFire	Ref		Ref	
ChiMES	2.4 (1.4-4.0)	<0.01	1.4 (0.9-2.3)	0.16
Age (per 1 year)	1.1 (1.0-1.1)	0.18	1.2 (1.1-1.3)	<0.01
Gender				
Male	Ref		Ref	
Female	1.0 (0.7-1.6)	0.84	0.9 (0.5-1.4)	0.6
Place of admission				
Ward	Ref		Ref	
HDU	1.2 (0.5-3.4)	0.67	1.3 (0.5-3.7)	0.6
PICU	1.8 (0.6-5.0)	0.32	2.9 (1.4-6.0)	<0.01
MAU	2.4 (0.2-22.8)	0.47	4.0 (0.6-24.9)	0.14
CDU	0.9 (0.4-2.1)	0.83	1.3 (0.5-3.0)	0.6
Blood WCC*	1.3 (0.6-2.6)	0.52	0.8 (0.4-1.6)	0.51
Blood Neutrophils*	0.9 (0.5-1.3)	0.5	0.9 (0.6-1.5)	0.81
CRP*	1.1 (1.0-1.3)	0.06	1.0 (0.8-1.1)	0.67
CSF protein*	1.2 (0.9-1.7)	0.26	1.6 (1.1-2.3)	0.01
CSF glucose*	0.6 (0.3-1.4)	0.25	0.6 (0.3-1.2)	0.17
CSF WCC (>median)	2.9 (1.7-4.9)	<0.01	1.8 (1.0-3.5)	0.07
CSF RCC (>median)	0.6 (0.4-0.9)	0.02	0.7 (0.4-1.2)	0.16
Meningitis				
No	Ref		Ref	
Viral Meningitis	0.5 (0.3-1.0)	0.04	0.7 (0.3-1.4)	0.27
Bacterial Meningitis	2.0 (0.5-8.0)	0.32	1.9 (0.5-6.9)	0.32

* Log transformed values

Appendix Table 4 The Odds ratio for duration of antibiotics use>7days and duration of hospital study>7days in the multivariable adjust logistics regression model using multiple imputation