Short Communication

A retrospective study of factors which determine a negative blood culture in Cambodian children diagnosed with enteric fever

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Background: Blood cultures are used to confirm a diagnosis of enteric fever but reported sensitivities can be as low as 40%.
Aims: To determine the factors associated with a negative blood culture in Cambodian children with suspected enteric fever.
Methods: In a retrospective study of hospitalised Cambodian children given a discharge diagnosis of enteric fever, the following factors associated with a negative blood culture were analysed: age, blood culture volume, prior antibiotic therapy, duration of illness and disease severity.
Results: In 227 hospitalised Cambodian children with a discharge diagnosis of enteric fever, it was confirmed in 70% by a positive blood culture. There was no association between a negative blood culture and younger age, lower blood volumes for culture, prior antibiotic therapy, a late presentation or milder disease.
Conclusions: Although blood culture sensitivity was higher than expected, alternative simple, rapid and sensitive tests are needed for diagnosing enteric fever.

Keywords: Enteric fever, Typhoid, Cambodia, Children, Blood culture, Salmonella, Paediatric, Angkor Hospital for Children

Introduction

Enteric fever (EF) is caused by *Salmonella enterica* serovar Typhi (*S.* Typhi) and serovar Paratyphi A.[1] In 2000 there were an estimated 27 million illnesses and 217,000 deaths from EF worldwide.[2] The typical clinical syndrome in children and young adults includes fever, malaise, headache, myalgia, anorexia, vomiting, cough, constipation or diarrhoea. Children under 5 years of age may present with a non-specific febrile illness. Complications include gastro-intestinal bleeding, intestinal perforation, pneumonia, cholecystitis, encephalopathy, chronic carriage and relapse.[1] In hospitalised patients in low- and middle-income countries, fatality rates of 2% (0–15%) have been reported.[3] EF is treatable with antibiotics but multidrug resistance (MDR, resistant to ampicillin, trimethoprim-sulphamethoxazole and chloramphenicol) and intermediate susceptibility to ciprofloxacin is common in many areas.[1] Recent studies in Cambodia have shown that *S.* Typhi are commonly MDR with intermediate susceptibility to ciprofloxacin.[4,5]

Enteric fever may be confused with other common causes of febrile illness in endemic areas (including malaria, dengue, chikungunia, leptospirosis and rickettsial infections), and confirming the diagnosis is difficult. The principle method available is blood culture with a reported sensitivity of 40–80%.[6,7] Although bone-marrow culture is more sensitive, it is rarely used.[6] A number of rapid diagnostic tests that detect antibodies against Salmonella antigens in the blood are available with sensitivities reported to vary between 43% and 100% and specificities of 58–100%, e.g. Typhidot-M®, IDL Tubex®-TF and the LifeAssay

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Test-It™ lateral flow test.[7] EF is a common diagnosis among children attending Angkor Hospital for Children, a charitably funded paediatric hospital in Siem Reap, Cambodia.[5,8] It was observed that despite the availability of blood cultures many children diagnosed with EF had a negative result. This study sought to explore why blood culture was negative in children with EF. It was hypothesised that a negative blood culture could be associated with a smaller volume of blood sent for culture, presentation after the second week of illness, milder disease or prior partial treatment with antibiotics in the community.[7]

Methods

This was a retrospective study of children admitted to Angkor Hospital for Children (AHC) in Siem Reap town and its Satellite Clinic at Sotr Nikom District Hospital, Siem Reap province between 1 January 2010 and 31 July 2012 inclusive. Children admitted to AHC with a history of fever routinely have a blood culture taken on admission. The blood culture bottles were weighed before and after inoculation of blood to determine the volume of blood added. Isolated micro-organisms were identified by standard microbiological methods.[8] Regular internal quality control of all media has been in place since 2007.

Children with a discharge diagnosis of typhoid or paratyphoid fever (ICD-10 code A01.0 or A01.1) were identified from the hospital electronic database. This diagnosis was recorded by the clinician responsible for the care of the child during that admission. Medical records were retrieved for each child and information collected regarding age, gender, prior antibiotic use, duration of symptoms before admission, severity of disease and complications. The hospital electronic database and laboratory records were used for admission blood tests, blood-culture results and volume of blood cultured. When a child's medical records were unobtainable, it was still possible to retrieve information from the hospital's electronic database and laboratory records.

Each child's medical record was reviewed by VK (a senior paediatrician with more than 7 years' experience of paediatric practice in Cambodia) and allocated into three diagnostic categories according to how typical of EF the clinical and laboratory features were. The categories were: blood culture-confirmed EF; probable EF (compatible clinical picture, white cell count normal or low, mild abnormality of alanine transaminase, and appropriate clinical response to treatment) or possible EF (compatible clinical picture and appropriate clinical response to treatment); and less typical EF (clinical picture and/or response to treatment not typical of classic EF). The severity of disease in patients with EF (classified as mild, moderate or severe) was assessed using a morbidity score.[9]

Statistical analysis

Data were analysed using STATA (Statacorp, Texas) version 12.0. Categories were compared with the Kruskall–Wallis test or ANOVA. The study aimed to analyse more than 200 children with a discharge diagnosis of EF.

Ethics approval

Ethics approval was obtained from the AHC Institutional Review Board.

Results

A total of 227 children attending AHC during the study period were given a discharge diagnosis of EF. There were 160 (70%) with a positive blood culture (all *S.* Typhi). Among the 67 blood culture-negative children, 29 (13%) were considered to be probable EF (17, 8%) or possible EF (12, 5%) and in 38 (17%) the clinical picture was less typical of EF. The demographic, clinical and laboratory data are summarised in Tables 1A and B according to each category. The age and gender distribution, routine blood tests and observations on admission were generally similar between each group. Vomiting was more common in the culture-positive group and abdominal pain less common in the group with features less typical of EF. There were no significant differences between the three groups in the median duration of symptoms before presentation at hospital, a history of prior antibiotic therapy, the volume of blood inoculated for culture, a moderate or severe disease score and the occurrence of complications. Relapses occurred only in the children who were blood culture-confirmed. No death caused by EF occurred during the study.

Discussion

This study highlights the difficulties of confirming the diagnosis of EF in an endemic area even when blood culture facilities are available. In this paediatric hospital, almost one-third of children with a discharge diagnosis of EF had a negative blood culture. Careful review by an experienced paediatrician of the case sheet of each patient suggested that about one-half had features typical of EF. It is well recognised that EF may not always present with the text-book syndrome and this is particularly so in very young children.[10] It is difficult to ascertain if blood cultures are less likely to be positive in young children with EF owing to the lack of a gold standard test to confirm the diagnosis. A recent study at this centre found that febrile children with a negative blood culture but positive blood PCR for *S.* Typhi were frequently very young with a short illness history.[11] Conversely, over-diagnosis of EF has been a problem highlighted

Table 1A Comparison of the demographic and clinical features of 227 hospitalised children with a discharge diagnosis of enteric fever according to the category determined by a senior paediatrician [numbers (%) or median (25th and 75th centiles)]

Variable	Blood culture positive EF (n=160)	Probable or possible EF (n=29)	Less typical EF (n=38)	P-value
Age, yrs	8.0 (5.5–11.6)	8.9 (7.0–11.1)	6.5 (4.6–10.9)	0.24
Male	75 (46.9)	19 (65.5)	19 (50.0)	0.18
Duration of illness, days	6.0 (5–10)	6.5 (3–8)	5.0 (3–7)	0.07
Taken medicine this week	113 (70.6)	24 (82.8)	29 (76.3)	0.31
Taken antibiotics this week	38 (23.8)	6 (20.7)	5 (13.2)	0.16
Diarrhoea	48 (30.0)	5 (17.2)	12 (31.6)	0.84
Vomiting	54 (63.5)	10 (11.8)	21 (24.7)	0.02
Abdominal pain	117 (73.1)	23 (79.3)	19 (50.0)	0.02
Headache	27 (16.9)	7 (24.1)	11 (28.9)	0.08
Cough	45 (28.1)	12 (41.4)	14 (36.8)	0.18
Temperature, °C	38.5 (37.7–39.4)	38.2 (37.0–39.0)	38.4 (37.5–39.0)	0.08
Pulse, beats/min	112 (100–126)	114 (100–128)	115 (101–127)	0.71

Table 1B Comparison of laboratory features and clinical outcome in 227 hospitalised children with a discharge diagnosis of enteric fever according to the category determined by a senior paediatrician [number (%) or median (25th and 75th centile)]

Variable	Blood culture positive EF (n=160)	Probable or Possible EF (n=29)	Less typical EF (n=38)	P-value
Haemoglobin, g/dl	10.1 (8.9–10.1)	10.6 (9.6–12.0)	10.9 (9.8–11.8)	0.01
White cell count, 10^9/L	7.5 (5.5–9.6)	6.5 (4.8–10.7)	8.1 (4.7–11.2)	0.74
Neutrophil count, 10^9/L	5.2 (3.4–6.7)	4.5 (2.9–7.3)	5.2 (2.5–8.8)	0.78
Platelet count, 10^9/L	260 (173–344)	209 (107–314)	251 (191–330)	0.05
Volume of blood cultured, ml	2.1 (1.8–2.5)	2.1 (1.8–2.5)	2.1 (1.9–2.5)	0.72
Mild disease*	86 (53.8)	20 (69.0)	22 (57.9)	0.27
Complicated disease†	24 (15.0)	5 (17.2)	4 (10.5)	0.58
Relapse	19 (11.9)‡	0 (0)	0 (0)	0.01

* Mild *vs* moderate or severe disease based on typhoid morbidity score (data available for 224 cases); † complicated disease defined as the presence of gastro-intestinal bleeding (the presence of visible blood in the stool); intestinal perforation, encephalopathy (delirium, decreased level of consciousness or coma); haemodynamic shock (systolic blood pressure <90 mmHg and/or diastolic blood pressure <60 mmHg associated with tissue hypoperfusion); hepatitis (as indicated by jaundice with hepatomegaly and/or abnormal levels of ALT (>200 IU/L); a clinical diagnosis of cholecystitis (right upper quadrant pain and tenderness without evidence of hepatitis); pneumonia (respiratory symptoms with abnormal chest radiograph shadowing) or pleural effusion; the need for a blood transfusion (data available for 224 cases); ‡ two of the 19 relapses were blood culture-positive and 17 were diagnosed clinically.

in other areas, often because of over-reliance on a Widal test result.[12]

If the culture-negative patients were true cases of EF, the 70% blood culture sensitivity was higher than is commonly appreciated. The median volume of blood taken was only 2.1 mls, reflecting the young age of many of the children studied. There was no evidence that lower blood volumes were sent for culture in those children with negative blood cultures. Furthermore, recent antibiotic therapy was not more common in the children with a negative culture, although this may not have been accurately reported by the caregivers. There was no evidence of longer duration of symptoms before presentation or significant differences in disease severity. Relapse was seen only in the blood culture-positive children. Whether this was because of more careful follow-up in the culture-positive children could not be determined.

This analysis is limited by being retrospective, by the relatively small number of children with a clinical diagnosis of EF but a negative blood culture, and the lack of an adequate reference standard diagnostic test for EF. There may be additional children with EF and negative blood cultures in which the diagnosis of EF was not considered, leading to incorrect ICD-10 coding and subsequent exclusion from the study. This analysis suggests that blood culture can be more sensitive than is often appreciated but also highlights the need for an additional simple and affordable point-of-care diagnostic test with high sensitivity and specificity, such as those which detect antibodies against specific Salmonella antigens in blood, to help confirm the diagnosis and guide treatment.

Disclaimer Statements

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Conflicts of interest None to declare.

Ethics approval Ethics approval was obtained from the AHC Institutional Review Board.

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