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Controlled Evaluation of Bactec Peds Plus/F and Bactec Lytic/10 Anaerobic/F Media for Isolation of *Salmonella enterica* Serovars Typhi and Paratyphi A from Blood[▽]

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We compared anaerobic lytic (AL) and pediatric aerobic resin-containing (Peds Plus/F) blood culture media for the isolation of *Salmonella enterica* serotype Typhi or Paratyphi A from children. The yields from AL and Peds Plus/F media were the same with equal volumes of blood, but recovery was faster from AL medium than Peds Plus/F medium (10.7 and 16.4 h, respectively) ($P < 0.001$).

Salmonella enterica serotype Typhi causes an estimated 16 million illnesses per year with 600,000 deaths globally (9). *S. enterica* serotypes Typhi and Paratyphi A are the most-common causes of community-acquired bacteremia in South Asia; children are affected disproportionately, with high mortality under age 5 years (1, 7, 11). Enteric (typhoid) fever cannot be clinically distinguished from other causes of acute febrile illness in areas of endemicity (9). Accurate microbiological diagnosis currently depends on culture of blood, since culture of bone marrow is rarely done now and most children are treated as outpatients. The Bactec Peds Plus/F (PP; BD Diagnostics-Diagnostic Systems, Sparks, MD) bottle, an aerobic blood culture medium with resins, is a commercial pediatric bottle commonly used worldwide. The standard recommendation for culturing blood in a single PP bottle is based on its demonstrated superior recovery of pathogens such as *Streptococcus pneumoniae* and the infrequency of anaerobic pathogens in children in North America (14).

We hypothesized that the optimal pediatric bottle for North America might not be best for South Asia and that an anaerobic lytic bottle might theoretically be better for the recovery of *S. enterica* serotypes Typhi and Paratyphi A in areas of the world where *Salmonella* organisms are the most-common pathogens recovered from blood. Although *Salmonella* bacteria are facultative anaerobes, isolates requiring strict anaerobic conditions for growth have been reported (6). Furthermore, the historic superiority of culture of bone marrow (sensitivity, 80% to 95%) over culture of blood (sensitivity, 60% to 80%) has been attributed to recovery of intracellular bacilli from mononuclear phagocytes (5, 9, 12). Recent studies from Vietnam suggest that ~66% of bacteria in blood are also harbored within phagocytes, and thus, lysis of circulating macrophages in blood might improve the yield of blood cultures (9). Because inadequate blood sampling is frequent in pediatrics and could confound the comparison of media, we undertook a volume-controlled evaluation of PP versus Bactec lytic/10 anaerobic/F (AL) (BD Diagnostics-Diagnostic Systems) media to assess the yield and time to detection of *Salmonella* from the blood of children. For our study site, we chose Karachi, Pakistan, since *S. enterica* serotypes Typhi and Paratyphi A are the most-common causes of pediatric bacteremia in Pakistan (2) and the presence of a state-of-the-art clinical microbiology laboratory at Aga Khan University Hospital enabled the comparison of these media. The study was approved by ethical review boards at both the Children's Hospital Boston and Aga Khan University.

(This work was presented in part as abstract 48 at the 55th Annual Meeting of the American Society for Tropical Medicine and Hygiene, Atlanta, GA, 13 November 2006 [10a].)

Children from ≥1 to 15 years of age with suspected enteric fever (history of ≥3 days of undifferentiated fever, no hospitalization in the previous 3 days, and a documented temperature of ≥38°C) presenting to the Aga Khan University Hospital, a primary care clinic in a low-income community, or to Karachi's large public sector referral children's hospital (National Institute for Child Health) were eligible if assessed by usual providers to require a blood culture and if consent could be obtained during daytime hours by the study doctor. Age-appropriate volumes of blood (3 ml for 1- to 3-year-olds and 4.5 ml for >3- to 15-year-olds) were obtained by a single aseptic venipuncture. Equal volumes of blood were inoculated into each of paired, preweighed PP and AL bottles. When bone marrow cultures were indicated clinically, aspirated bone marrow was divided between a second set of PP and AL bottles.

Paired bottles were transported within 3 h of collection to the Aga Khan Hospital's clinical microbiology laboratory, where they were weighed to the nearest 0.01 g before being loaded into a Bactec 9240 series automated blood culture instrument. Contaminants were assessed according to published criteria (13). Paired volumes were considered to be equal if within 50% of each other for volumes of <5 ml and within 20% of each other for volumes of ≥5 ml (10) The gold standard for diagnosis of enteric fever was isolation of *S. enterica* serotype

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TABLE 1. Comparative yields of *Salmonella* from PP and AL blood culture bottles

Salmonella enterica serovar	No. of isolates detected by:			*P* value[a]
	Both bottles	PP bottles only	AL bottles only	
Typhi	30	7	2	NS
Paratyphi A	6	0	1	NS
Both	36	7	3	NS

[a] NS, not significant ($P > 0.05$).

Typhi or Paratyphi A from either bottle or both, since *Salmonella* bacteria are always pathogenic when recovered from blood. *Salmonella* isolates were identified biochemically and were typed with specific antisera (2). Statistical analyses were performed with STATA 9 statistical software; McNemar's modified chi-square test was used to assess yield and the Wilcoxon matched-pairs signed-rank test for time to detection.

Of 817 paired blood cultures submitted from 817 patients over 12 months, 46 (5.6%) grew pathogens (39 *S. enterica* serotype Typhi and 7 *S. enterica* serotype Paratyphi A) and 36 (4.4%) grew contaminants (including coagulase-negative staphylococci and gram-positive rods). No isolates of *Streptococcus pneumoniae* or *Haemophilus influenzae* were found. The two media were comparable for the recovery of *S. enterica* serotypes Typhi and Paratyphi A (Table 1). The median volume of blood cultured in the PP and AL bottles was 1.86 ml (intraquartile range, 1.53 to 2.33) and 1.96 ml (intraquartile range, 1.67 to 2.45), respectively. All isolates were recovered from <5 ml of blood (maximum of 4.90 ml in the PP and 4.12 ml in the AL), so Table 1 represents comparable volumes within 50%. If only sets that are within 20% are considered to be equal volumes, 17 isolates grew in both bottles and 2 each in PP or AL only. When recovered from both bottles, the median times to detection of *S. enterica* serotype Typhi and *S. enterica* serotype Paratyphi A were 16.4 h (interquartile range, 11.8 to 22 h) for PP and 10.7 h (interquartile range, 8.8 to 16 h) for AL, respectively ($P < 0.001$). Of the 10 patients who had both bone marrow and blood cultures, 2 had *S. enterica* serotype Typhi isolated only from bone marrow and not from blood in either PP or AL bottles; the other 8 were negative. Multidrug (ampicillin, chloramphenicol, and co-trimoxazole) resistance was found in 15 of 39 (38.5%) isolates of *Salmonella enterica* serotype Typhi and none of the 7 isolates of *S. enterica* serotype Paratyphi A. All isolates were susceptible to ceftriaxone and ciprofloxacin by current CLSI interpretive criteria (3), although resistance to nalidixic acid was found in 18 (46.2%) *S. enterica* serotype Typhi isolates and 2 (28.6%) *S. enterica* serotype Paratyphi A isolates.

In conclusion, we found that *S. enterica* serotypes Typhi and Paratyphi A were the most frequent isolates from blood in Pakistani children with suspected enteric fever, which suggests that our clinical criteria for enrollment were specific for enteric fever in this population. We found that the AL bottle was not superior to PP for the recovery of *S. enterica* serotypes Typhi and Paratyphi A, although it achieved results faster. Because we enriched our sample with those suspected clinically to have enteric fever and enrolled at two hospitals during 12 months, our sample size for *S. enterica* serotype Typhi was larger than is usually reported for any one organism in a study designed to compare two different media (15). One must choose a medium formulation based on the total spectrum of pediatric bloodstream pathogens in a population. Since the anaerobic lytic medium and other lytic media that contain saponin have been shown to have reduced recovery of *S. pneumoniae* (4), and pneumococci do cause pediatric bacteremia in Pakistan (8), we conclude that PP would be a better overall choice for the evaluation of pediatric bacteremia in Pakistan, as it is in North America.

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