Community-acquired Bacteremia in Human Immunodeficiency Virus-infected Children in Harare, Zimbabwe

NATHOO, KUSUM J. MBCHB, MRCP; CHIGONDE, SAMUEL MLT; NHEMBE, MARGARET SRN; ALI, MOHAMED H. MBCHB; MASON, PETER R. PHD, MRCPATH

From the Departments of Paediatrics and Child Health (KJN, MN), Medical Microbiology (SC, PRM) and Medical Laboratory Technology (PRM), University of Zimbabwe Medical School, Harare Central Hospital (MHA), and the Biomedical Research and Training Institute, Harare, Zimbabwe (PRM).

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Address for reprints: Professor P. R. Mason, Biomedical Research and Training Institute, P.O. Box CY1753, Causeway, Harare, Zimbabwe. Fax 263-4-307898; E-mail zapp@healthnet.zw.
Abstract

Background: HIV infection is common in mothers and their children in Zimbabwe, and HIV-infected children are particularly susceptible to bacterial infections. There is little information on the etiology and outcome of HIV-related bacteremia in African children.

Methods: Blood cultures from 309 hospitalized children in Zimbabwe, of whom 168 were diagnosed as having HIV, were examined for pathogens. The association among significant bacteremia, HIV infection and mortality was assessed in these children.

Results: The most common isolates were coagulase-negative staphylococci (31 children, 25 clinically significant), Staphylococcus aureus (22 children) and Streptococcus pneumoniae (20 children). Nontyphoidal Salmonella (10 children), Escherichia coli (4 children) and Klebsiella sp. (4 children) were the most frequent Gram-negative bacteria. Two children had Rhodococcus equi pneumonia. HIV-infected children showed increased risk of bacteremia (odds ratio (OR) = 2.68), especially if younger than 18 months of age (OR = 2.94), and high risk of enterobacteremia (OR = 15.76). There was no significant association of bacteremia with nutritional status. Mortality was 17% overall but was higher in HIV-infected children up to 6 months of age (OR = 2.81) and in bacteremic children of any age (OR = 2.03).

Conclusions: Prompt recognition of pathogens and early administration of appropriate antimicrobials is important in reducing the morbidity and mortality associated with bacteremia in HIV-infected children in Africa.
INTRODUCTION

Abnormal antibody responses, polyclonal hypergam-maglobulinemia, defects in leukocyte function and defects in cytokine secretion occur in HIV-infected children.1-3 Such children are at increased risk of recurrent and life-threatening bacterial infections4, 5 and early detection of bacteremia by blood culture in these children is important. Recent epidemiologic investigations have given conflicting findings on the age-related risk of HIV-associated bacteremia. In developed countries the risk of community-acquired bacterial invasive disease in HIV-infected children has been shown to rise rapidly in children older than 12 months of age4, 6 whereas there is no difference in risk in younger children. This contrasts with the situation in Africa, where bacteremia is frequently found in HIV-infected children younger than 12 months.7 A number of retrospective studies have identified Streptococcus pneumoniae, Salmonella enteritidis and other enterobacteria as significant causes of morbidity in children infected with HIV,5-7 although many other organisms may be isolated from blood.4, 8

In Zimbabwe more than 30% of pregnant women have antibodies to HIV 9 and HIV infection is thought to be responsible for the increase in perinatal and pediatric mortality that has been experienced since the 1980s.10, 11 Acute respiratory tract infections are among the most common conditions in hospitalized children, and a high proportion of these have bacteremia, with almost equal numbers of Gram-positive and Gram-negative organisms being isolated.12 Malnutrition and clinical HIV infection are significant risk factors for mortality in children with acute respiratory illness 12 and multiply resistant Gram-negative organisms, particularly Klebsiella sp., are the most frequent isolate from postmortem pediatric blood cultures from children and neonates.13, 14 It is against this background that we conducted a prospective, 18-month study in pediatric wards at Harare Hospital, with the objectives of collecting data on the etiology and antimicrobial susceptibility of bacteremias in children from a community where HIV infection is common, and on the impact of concurrent HIV infection on the mortality associated with these bacteremias.

MATERIALS AND METHODST\n
Patients. The children included in the study were admitted to the pediatric wards at Harare Hospital between June, 1993, and December, 1994. Harare Hospital is a referral center for municipal clinics and health centers in the surrounding provinces, and children are referred by these local clinics/health centers or are brought in by their relatives. Children younger than 8 years of age with an axillary temperature of 38°C or greater or with clinical evidence of pneumonia, meningitis or acute nonfocal infection were eligible for enrollment. Because of financial and manpower constraints, only those children admitted between 8 a.m. and 2 p.m. on Mondays to Wednesdays during the study period were enrolled. Because none of the children in this study had been referred from another hospital and blood cultures were collected within 12 h of admission, all bacteremias were defined as community-acquired.4

Clinical data. The following demographic and clinical data were recorded: age; sex; weight and temperature on admission; total white blood cell count; clinical indications of HIV infection; clinical diagnosis and outcome (death or discharge from hospital). Verbal consent was obtained
from the mother or caretaker for the collection of blood specimens. Precounseling for HIV serology was given at this time, and post-test counseling was given when results were available. There were no refusals of consent.

Specimens. Blood cultures were taken as soon as possible after admission, before administration of antibiotics or catheterization. Health care staff responsible for collecting blood samples were cautioned specifically about the need for care in preparation of the skin before collection to avoid contamination. The skin was cleansed with povidone-iodine and up to 5 ml of blood were collected by a sterile technique. Between 1 and 3 ml of blood were inoculated into 20 ml of brain-heart infusion broth containing sodium polyanethol sulfonate (Paediatric Septi-Check®, Roche Diagnostics), following precautions recommended by the manufacturers. The remaining blood was inoculated into a plain tube for collection of serum, and both specimens were sent to the laboratory on the same day for processing.

Laboratory. BCB-Slides® (Roche Diagnostics), comprising a combination of MacConkey, malt and chocolate agars, were attached to the blood culture bottle, following the protocol supplied by the manufacturer. According to the information supplied by Roche Diagnostics, this combination of broth and agars is suitable for the isolation of enterobacteria, fungi and a wide variety of Gram-positive and Gram-negative bacteria, including fastidious organisms such as Haemophilus influenzae. The bottle assembly was incubated at 37°C for up to 7 days, with daily flooding of the media slides. When there was evidence of growth, with turbidity of the broth and colonies on the media, the organisms were subcultured and identified with standard microbiologic assays. Antimicrobial susceptibilities were determined with discs impregnated with antibiotics commonly available in Zimbabwe.

HIV infection. The clotted blood specimen was centrifuged, and the serum was used to detect antibodies to HIV with a synthetic HIV-1 and HIV-2 peptide enzyme-linked immunosorbent assay (ICL Dipstick®, Thailand). Antibody-positive sera were retested to detect the presence of HIV p24 antigen (Abbott, Diagnostics Division). The definition of HIV infection that we used was the presence of HIV antibody and either age >18 months or the presence of p24 antigen or any two of the following clinical features: generalized lymphadenopathy; oropharyngeal candidiasis; hepatosplenomegaly; pneumonia; and failure to thrive.15

Nutritional status. Nutritional status was assessed by weight for age, using NCHS standards, into normal (80% or more of expected weight for age), moderate malnutrition (60 to 79% expected weight for age) or severe malnutrition (<60% expected weight for age).

Statistical methods. Clinical and laboratory data were stored according to an Epi-Info® package. Odds ratios (OR) with 95% confidence intervals (CI), and probabilities by chi square or Fisher's exact test as appropriate were calculated from these data.

RESULTS

During the study period blood cultures from 332 children were received in our laboratory, and there were sufficient clinical and serologic data from 309 of these for inclusion in the analysis. The Microbiology Laboratory at Harare Hospital normally receives about 1400 blood cultures
from pediatric wards per year, and the sample represents 15% of the expected pediatric blood cultures during the study period. Pneumonia was by far the most frequent clinical diagnosis (253 children), complicated by diarrhea or dysentery in 26 children, septicemia in 23 children and meningitis in 11 children. In children without clinical pneumonia there were 14 children diagnosed with septicemia, 7 with meningitis and 10 with diarrhea/dysentery. Other clinical diagnoses included measles and malaria. Fiftytwo children (17%) died while hospitalized.

The median age of children included in the study was 5 months (range <1 to 96 months) and 72% of blood cultures (222 of 309) were from children younger than 12 months of age. Positive blood cultures were obtained from 50 of 171 (29%) children up to 6 months, 19 of 51 (37%) children 7 to 12 months, 9 of 33 (27%) children 13 to 18 months and 17 of 54 (32%) children more than 18 months of age. There was no statistical difference in the prevalence of bacteremia in boys (43 of 125, 34%) and girls (52 of 184, 28%).

More than 70% (218 of 309) of the children had antibodies to HIV, and 168 of 309 (54%) met our criteria of HIV infection, with 43 HIV-seropositive children 18 months of age or older, 64 seropositive children younger than 18 months having p24 antigenemia and 61 seropositive children having clinical evidence of HIV infection in the absence of p24 antigenemia. Bacteremia was recorded in 67 of 168 (40%) children with HIV infection, compared with 28 of 141(20%) children without HIV infection (OR 2.68, 95% CI 1.55 to 4.64, P < 0.001). This difference was most striking in children up to 18 months of age, with 51 of 125 (41%) such HIV-infected children having bacteremia, compared with 22 of 116 (19%) similarly aged children without evidence of HIV infection (OR 2.94, 95% CI 1.58 to 5.52, P < 0.001). In children older than 18 months of age, there was no significant difference in the prevalence of bacteremia in those who were infected with HIV (16 of 43, 37%) or were not infected with HIV (6 of 25, 24%).

Nutritional status was determined for 284 children; 140 (50%) had weight for age within normal limits, 103 (36%) were moderately malnourished and 41 (14%) were severely malnourished. In children infected with HIV bactermias were detected in 23 of 66 (35%) children who were not malnourished compared with 29 of 68 (43%) children who were severely malnourished. For the same nutritional status groups, the frequencies of bacteremia in children without HIV were 13 of 74 (19%), 7 of 35 (20%) and 4 of 18 (22%), respectively.

The organisms causing bacteremia are shown in Table 1. Eighteen cultures were regarded as probable contamination with skin flora on the basis of the organism isolated, the time taken for a positive culture (3 days of incubation or more) and absence of either pyrexia (defined as temperature >38°C) or leukocytosis (defined as leukocyte count >15 × 10⁹/liter). In HIV-infected and in noninfected children, staphylococci were the most frequent isolates, with almost equal numbers of coagulase-positive and coagulasenegative organisms. The time taken to detect coagulase-negative staphylococci (CONS) varied, but 22 of 28 (79%) were detected within 2 days, and 21 (96%) of these children had a pyrexia and leukocytosis, or other indication that this was a true infection. Of the 6 CONS isolated only after 3 days of incubation or more, one was from a child with marked pyrexia and leukocytosis and thus was included as significant, but the rest were regarded as contaminants.
The blood culture from one child yielded two organisms, *Strep, pneumoniae* and *Actinomyces israelii*, but all other infections were with single organisms. Blood cultures from two children, each with a clinical diagnosis of pneumonia and evidence of HIV infection, grew *Rhodococcus equi*, and both of these children died. Among the Gram-negative bacteria, *Salm. enteritidis* serotypes and *Escherichia coli* were the most frequent isolates. Enterobacteria were isolated more frequently from HIV-infected (17 of 168, 10%) than from noninfected (1 of 141, 0.7%) children (OR 15.76, 95% CI 2.39 to 663.30, P < 0.001). A single isolate of *Haemophilus influenzae* type b was obtained, and two other blood cultures grew *Haemophilus parainfluenzae*. The antimicrobial resistance patterns of frequent isolates are shown in Table 2.

As shown in Table 3 the mortality in children with HIV infection was high. In children up to 6 months old the mortality in HIV-infected was 28%, compared with 12% among HIV-noninfected children (OR 2.81, 95% CI 1.17 to 6.85, P < 0.05). Among older children there was also a high mortality in the children with HIV (14 of 82, 17%) compared with children without HIV (4 of 56, 7%), but this difference was not statistically significant. The mortality in bacteremic children was 24% (23 of 95) compared with 14% (29 of 213) in nonbacteremic children (OR 2.03, 95% CI 1.05 to 5.39, P < 0.05).

**DISCUSSION**

Blood cultures from pediatric patients present a number of problems to the clinical microbiologist. The volume of blood that can be withdrawn from children is often small and must be used for a variety of investigations. The amount of blood available for culture is often suboptimal, and the taking of a second sample to confirm the significance of an isolate may not be possible because of the distress this may cause, especially in neonates. Furthermore, the organisms causing bacteremia in children are often different from those in adults, with bacteria of low virulence being of greater importance.16, 17 The latter present even greater difficulty in a community where depressed...
immune function, through HIV infection, may also be prevalent. Blood-borne infections have been associated with serious morbidity and high mortality in HIV-seropositive adults in East and West Africa \(^\text{18, 19}\) but there are relatively few studies on HIV-infected children\(^\text{7, 20}\) despite the fact that HIV infection is the most common cause of morbidity and mortality in children in sub-Saharan Africa.

The HIV seroprevalence in healthy pregnant women in Harare is about \(30\%\) \(^\text{9}\) and while the actual rate of vertical transmission in Zimbabwe is unknown, it is estimated to be just over \(40\%\) in Nairobi. \(^\text{21}\) This suggests that [almost equal to]10 to 12\% of babies born in Harare are infected with HIV. Just over one-half of the children examined in the study were considered to have HIV infection, and this disproportionate representation indicates the high risk for serious disease in HIV-infected children.

As with studies elsewhere we found HIV infection to be a significant risk factor for bacteremia, particularly with enterobacteria. The highest risk was in children up to 18 months of age, a finding that is consistent with a previous report from Africa. \(^\text{7}\) By contrast studies in developed nations have described increased risk of bacteremia only in HIV-infected children more than 12 months old age. \(^\text{4, 6}\) There may be a number of explanations for this, including the use of antiretroviral agents in children in developed countries. The high cost of such agents precludes their use in developing countries, and young children in Africa may therefore develop the immunologic abnormalities associated with HIV infection at an earlier age. Additionally children in developing countries are likely to experience earlier and more frequent exposure to pathogens in their environment.

We did not find an association of bacteremia with clinical evidence of malnutrition, as has frequently been reported elsewhere, \(^\text{12, 22}\) either in children with HIV infection or those without. However, few children in this study were severely malnourished.

Prompt initiation of empiric therapy, before an etiologic diagnosis has been determined, may be crucial for early management of bacteremia in HIV-positive children. This requires data on the “most likely organism, most likely antimicrobial susceptibility.” Studies on HIV-infected adults and children in Africa have emphasized infection with nontyphoidal Salmonella and other enterobacteria. \(^\text{7, 18, 19}\) Although HIV-infected children in our study had a much higher risk of infection with Salm. enteritidis or other enterobacteria than did non-HIV-infected children, Gram-negative bacteria as a whole made up only about one-fourth of our total isolates. As has been described in studies of malnourished children in Africa and Jamaica \(^\text{12, 16, 22}\) isolates of S. aureus, CONS and Strep. pneumoniae were much more frequent. Thus the recommendations for empiric treatment require consideration that >75\% of bacteremias may be with Gram-positive bacteria, even in a community where HIV infection is prevalent. S. aureus is a common cause of bacteremia in adults and children in Zimbabwe, \(^\text{12, 23}\) and isolates are frequently multiply resistant to antibiotics. \(^\text{24}\) The significance of the isolation of CONS in blood culture, however, is more difficult to assess, and in many studies CONS have been discounted as skin contaminants. \(^\text{22}\) Even when great care is taken in skin preparation, commensals can be detected in 2 to 5\% of blood cultures. \(^\text{8}\) The absence of clinical or hematologic abnormalities may help in identifying these as contamination rather than infection. Care is necessary with specimens from neonates who have been intubated or who received intravenous fluids \(^\text{25}\) or who are
immunocompromised, given that these children appear to be particularly susceptible to CONS infection. In this study blood cultures were taken before intravenous catheters had been introduced; thus this route of entry could be discounted, but because of the high prevalence of HIV infection the isolation of a CONS from blood was given special attention. In view of the apparent relationship between rapid growth in the blood culture medium and clinical indications of infection, it may be prudent in pediatric patients who are at high risk of HIV infection to regard detection of CONS in blood within 48 h of culture incubation as a significant finding requiring antimicrobial therapy.

A number of other organisms, normally considered to be of low virulence, may also be isolated from blood cultures of immunocompromised children. There were, for example, two infections with R. equi, both in HIV-infected children with symptoms of pneumonia, and both of these children died despite empiric therapy with a penicillin and an aminoglycoside. Although infections with R. equi have been detected in HIV-positive adults, this appears to be the first record of infection in HIV-positive children in Africa. Multiple drug resistance is characteristic of this organism, but most are susceptible to erythromycin and clofazimine.

The high mortality in bacteremic HIV-infected children in this study, emphasizes the importance of early and effective antimicrobial treatment. The choice of a antimicrobial agent to be used in the setting of a developing African country must be made on the basis of many factors: potential efficacy; safety in young children; availability; and cost. Resistance to trimethoprim-sulfamethoxazole was common among Gram-positive and Gram-negative isolates; thus this combination would appear to be of little value, despite its wide availability and low cost. Beta-lactams were effective against a number of pathogens, notably most pneumococci and the single isolate of H. influenzae type b, but since pneumococci with decreased susceptibility to penicillin occur frequently in South Africa, this situation must be carefully monitored. The clinical efficacy of other antimicrobial agents that may be of benefit in severely ill children needs to be assessed, so that appropriate recommendations on treatment policy can be given.

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**REFERENCES**


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Key words: Bacteremia; blood culture; human immunodeficiency virus; sepsis