Bacteremia, Causative Agents and Antimicrobial Susceptibility Among HIV-1–infected Children on Antiretroviral Therapy in Uganda and Zimbabwe

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Background: Bacteremia is common in HIV-infected children in Africa, including after start of antiretroviral therapy (ART), but there are limited data on causative pathogens and their antimicrobial sensitivity patterns in this population.

Methods: We analyzed data on blood cultures taken from HIV-infected children developing acute febrile illness after enrollment to the Antiretroviral Research for Watoto (ARROW) clinical trial in Uganda and Zimbabwe. Patterns of bacterial pathogens and their antimicrobial susceptibilities were determined and bacteremia rates calculated over time from ART initiation.

Results: A total of 848 blood cultures were obtained from 461 children, of which 123 (14.5%) from 105 children (median age 3.5 years, 51% girls) were culture positive, including 75 (8.8%) with clearly pathogenic organisms. The event rates for positive cultures with clearly pathogenic organisms after 0–1, 2–3, 4–11 and ≥12 months on ART were 13.3, 11.4, 2.1 and 0.3 per 1000 person-months of follow-up, respectively. The pathogens isolated (n; %) were *Streptococcus pneumoniae* (36; 28.3%), *Staphylococcus aureus* (11; 8.7%), *Klebsiella pneumoniae* (6; 4.7%), *Pseudomonas aeruginosa* (6; 4.7%), *Salmonella* spp (6; 4.7%), *Escherichia coli* (5; 3.9%), *Haemophilus influenzae* (1; 0.8%) and fungal spp (4; 3.1%). Other bacteria of doubtful pathogenicity (n = 52; 42%) were also isolated. Most isolates tested were highly (80–100%) susceptible to ceftriaxone, cefotaxime and ciprofloxacin; very few (~5%) were susceptible to cotrimoxazole; *S. pneumoniae* had high susceptibility to amoxicillin/ampicillin (80%).

Conclusions: Rates of proven bacteremia were >20-fold higher immediately after starting ART compared with 12 months later in African HIV-infected children. *S. pneumoniae* was most commonly isolated, suggesting need for pneumococcal vaccination and effective prophylactic antibiotics.

Key Words: bacteremia, HIV, children, Africa

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HIV-infected children are at increased risk of acquiring and dying from bacteremia.[1–8] Although antiretroviral therapy (ART) has reduced the incidence,[9–11] the burden of bacteremia remains high in HIV-infected compared with HIV-uninfected children, especially in the first 3 months on ART.[12,13] Studies in Kenya and South Africa reported that the most commonly isolated pathogens in HIV-infected children with bacteremia were *Streptococcus pneumoniae*, *Staphylococcus aureus* and Enterobacteriaceae.[12,14,15] In Uganda and Zimbabwe, the most common pathogens isolated from HIV-infected and uninfected hospitalized children (many with severe acute malnutrition) were *Salmonella* spp, *Escherichia coli*, *S. aureus* and *S. pneumoniae*.[1,5,16]

Bacterial isolates have been reported to have high rates of resistance to commonly used antibiotics in sub-Saharan Africa, such as cotrimoxazole, ampicillin, chloramphenicol and gentamicin. Furthermore, Enterobacteriaceae have been identified as extended-spectrum β-lactamase (ESBL) producers in several studies, implying that they would be resistant to third generation cephalosporins.[1,14,16] These data are worrying, given the limited range of antibiotic options in most sub-Saharan African countries.

Data from sub-Saharan Africa are very limited on both the pathogens and antimicrobial susceptibility patterns among ART-treated HIV-infected children with bacteremia. We therefore set out to investigate these factors in a large cohort of HIV-infected children who initiated ART in the Antiretroviral Research for Watoto (ARROW) trial in Uganda and Zimbabwe.

MATERIALS AND METHODS

Study Subjects

This analysis used data from the ongoing ARROW clinical trial (ISCRTN 24791884; www.arrowtrial.org), a randomized factorial trial of 2 clinical monitoring strategies and 3 antiretroviral drug treatment strategies at 3 clinical sites in Uganda and 1 in Zimbabwe. HIV-1 infected, ART-naive (except for prior infant antiretroviral drug prophylaxis of mother-to-child transmission of HIV) children ages 3 months to 17 years were randomized into 3 ART regimen arms: abacavir, lamivudine (3TC) and a non-nucleoside reverse transcriptase inhibitor in the first arm; abacavir, 3TC, non-nucleoside reverse transcriptase inhibitor and zidovudine in arms 2 and 3, with zidovudine in arm 2 and the non-nucleoside reverse transcriptase inhibitor in arm 3 being dropped after 9 months, as per the trial protocol. Children were reviewed in the clinic every 4–6 weeks, and each had CD4 count/percentage, complete blood counts, liver and renal function tests done every 12 weeks. If a child developed any illness, the caregiver was encouraged to return to the study clinic for evaluation and management; all children had access to hospitalization if required. If treated in centers other than study clinics, caregivers were asked to bring treatment forms at the next scheduled visit.

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The large majority of children (1199/1206; 99.4%) were receiving cotrimoxazole prophylaxis at enrollment (started at screening in those newly diagnosed with HIV) and took it until at least 96 weeks after ART initiation; thereafter, 760 of 1002 (75.8%) children were randomized to continue or to stop cotrimoxazole a median of 108 (range 96, 144) weeks after ART initiation, as an additional randomization and as per the amended trial protocol. Children who developed febrile illnesses during follow-up had investigations that included blood culture and antibiotic sensitivity testing.

Blood Culture and Sensitivity Testing

Blood culture and sensitivity testing were done in 3 laboratories: 2 in Uganda (Joint Clinical Research Centre and Medical Research Council/Uganda Virus Research Institute [MRC/UVRI]) and 1 (UZ-CHS) in Zimbabwe. At the Joint Clinical Research Centre laboratory, bacterial isolation and typing as well as antimicrobial susceptibility testing were undertaken using the automated Becton Dickinson Bactec system (www.bd.com) with incubation of a targeted 1–3 mL of blood for 7 days.[17] Positive cultures were subcultured on blood agar, MacConkey agar and chocolate agar. After overnight incubation, isolates were identified and antimicrobial susceptibility (including the minimum inhibitory concentration) was determined using the Phoenix Becton Dickinson Microbiology Systems. In particular, ESBL-producing bacteria were identified on the Phoenix Becton Dickinson instrument following the manufacturer's recommendations.[18] Interpretation and reporting of all susceptibility testing results were in accordance with the Clinical Laboratory Standard Institute, United States (www.clsi.org) guidelines.[19] At the MRC/UVRI laboratory, bacterial isolation was undertaken using the Bactec 9120 blood system with incubation of a targeted 1–3 mL of blood for 7 days. Positive cultures were subcultured onto blood agar, chocolate agar and MacConkey agar plates. Isolated organisms were identified using serological and biochemical tests such as the Analytical Profile Index system,[20] the Staphylase test and the streptococcal grouping test. Antimicrobial susceptibility was undertaken using the Kirby–Bauer method.[21] Antibiotic-impregnated paper discs were used to test whether bacteria were susceptible to specific antibiotics. Zone diameters of inhibition around the antibiotic discs were measured in millimeters and interpreted according to British Society of Antimicrobial Chemotherapy (www.bsac.org.uk) guidelines.[22] At the UZ-CHS laboratory, bacterial isolation was done using manual methods. In this case, a targeted 1–3 mL blood was inoculated into pediatric blood culture bottles containing 10-mL tryptone soy broth and incubated for 10 days at 37°C, culturing at days 1, 5 and 10 onto blood, chocolate and MacConkey agars. Isolated organisms were identified using standard manual techniques including Gram stain, biochemical tests, Streptex and Analytical Profile Index systems. Susceptibility testing was done using the Clinical Laboratory Standard Institute[19] method for disc susceptibility testing using *E. coli* American Type Culture Collection 25922, *S. aureus* American Type Culture Collection 25923 and *Pseudomonas aeruginosa* American Type Culture Collection 27853 as controls.

Ethical Review

All caregivers of study participants gave written informed consent, and children of ages 13 years and above, all aware of their HIV status, gave written informed assent. The study protocol was approved by the ethics review committee/Institution Review Board of each participating center and by the International Ethics Committee of University College, London, United Kingdom.

Statistical Analyses

Characteristics of children with or without isolation of pathogens were compared using *t* tests and chi-square tests. The event rate of positive cultures was calculated using person-time on ART. Descriptive statistics were used to demonstrate the pathogens identified and their antibiotic susceptibilities. All analyses were done using Stata v12.0. (StataCorp LP, College Station, TX).

RESULTS

A total of 1206 children were enrolled in the ARROW trial between March 2007 and November 2008 and continued follow-up to March 2012, but with bacteremia data evaluated up to July 2011. Their median age was 6 years, 31% were under 3 years and there were approximately equal numbers of boys and girls (Table 1). Most participants had advanced HIV disease at enrolment, 71% had World Health Organization (WHO) stage 3 or 4 disease and 65% had CD4 cell percentage below 15%.

Investigation of febrile illness included blood cultures in 461 children, of median age 4.4 years; 52% were female (Table 1). The children who had blood cultures performed were significantly younger than other trial participants (median age 4.4 versus 6.9 years, $P < 0.001$) and more likely to have WHO stage 4 conditions (19% versus 11%, $P = 0.002$). However, there were no significant differences in CD4 cell count or percentage or in weight-for-age or height-for-age Z scores at ART initiation (Table 1). Each child experienced a median of 4 episodes of fever, and a total of 848 blood cultures were performed during 6704 febrile episodes. Pathogens were isolated in 123 cultures (14.5%), from 105 children. Four samples had 2 different pathogens isolated, resulting in a total of 127 isolates. The 105 children with bacterial isolates had a median age of 3.5 years; 76% had WHO stage 3 or 4 and 65% had CD4% <15%; their mean weight-for-age and height-for-age Z scores were –2.73 and –2.64, respectively, at enrollment; they did not significantly differ from children without bacterial isolates, as shown in Table 1.

Incidence of Bacteremia

The incidence rate of all positive cultures (both pathogenic and those of doubtful pathogenicity) was highest in the first 3 months after ART initiation (Table 2). In the first month on ART, there were 23 positive cultures in 1202 person-months of observation, an event rate of 19.1 per 1000 person-months. With more time on ART, fewer positive cultures were observed, with the event rate dropping to 13.5, 3.62 and 0.74 per 1000 person-months at 1–<3, 3–<12 and ≥12 months post-ART initiation, respectively (Table 2). When only clearly pathogenic isolates were considered (ie, excluding pathogens of doubtful pathogenicity), the respective event rates dropped to 13.3, 11.4, 2.1 and 0.3 per 1000 person-months at 0–<1, 1–<3, 3–<12 and ≥12 months post-ART initiation (Table 2). Of 123 bacteremia episodes, 60 (49%) were hospitalized and 9 (7%) resulted in death within 28 days of taking the blood sample.

The culture positivity rate in Harare, Zimbabwe (31/109; 28%), was significantly higher than at the Ugandan sites (MRC Entebbe 10/64, 16%; Joint Clinical Research Center Kampala 46/408, 11%; Mulago Paediatric Infectious Diseases Clinic 36/267, 13%), $P < 0.0001$.

Bacterial Pathogens Isolated

Pathogenic Bacteria

S. pneumoniae was the most commonly isolated pathogen (n = 36, 28%). Other frequently isolated pathogenic organisms included: *S. aureus* (n = 11, 9%), *Klebsiella pneumoniae* (n = 6, 5%), *P. aeruginosa* (n = 6, 5%), *Salmonella* spp (n = 6, 5%) and *E. coli* (n = 5, 4%) (Fig. 1A). Of note, there was only 1 isolate of *Haemophilus influenzae*; typing was not undertaken. Fungal spp were isolated in 4 cases (3%).

TABLE 1. Participant Characteristics

		Specimen Cultured			Pathogen Isolated*		
		All	No	Yes	No	Yes	
		n (%)	n (%)	n (%)	n (%)	n (%)	*P*
Total		1206 (100%)	745 (62%)	461 (38%) (461 = 356 + 105)	356 (77%)	105 (23%)	
Sex	Male	596 (49%)	375 (50%)	221 (48%)	170 (48%)	51 (49%)	0.88
	Female	610 (51%)	370 (49%)	240 (52%)	186 (52%)	54 (51%)	
Age	Yr†	6.0 (2.4, 9.3)	6.9 (2.9, 9.7)	4.4 (1.8, 8.6)	4.7 (1.8, 8.8)	3.5 (1.9, 7.9)	0.29
	<3 yr	370 (31%)	191 (26%)	179 (39%)	134 (38%)	45 (43%)	
WHO stage	1/2	354 (30%)	218 (29%)	136 (30%)	121 (31%)	25 (24%)	0.48
	3	682 (57%)	443 (59%)	239 (51%)	181 (51%)	58 (55%)	
	4	170 (14%)	84 (11%)	86 (19%)	64 (18%)	22 (21%)	
CD4%	Median	12	13	12	11	13	0.17
	IQR	7, 17	8, 18	7, 16	7, 16	8, 17	
	Range	0, 49	0, 49	0, 46	0, 46	0, 37	
	≥15%	424 (35%)	276 (37%)	148 (32%)	112 (31%)	36 (34%)	0.44
	≥10%, <15%	336 (28%)	205 (28%)	131 (28%)	97 (27%)	34 (32%)	
	≥5%, <10%	244 (20%)	151 (20%)	93 (20%)	77 (22%)	16 (15%)	
	<5%	202 (17%)	113 (15%)	89 (19%)	70 (20%)	19 (18%)	
$CD4^{\beta}$ count	Median	355	343	392	389	394	0.48
	IQR	175, 685	183, 657	159, 746	146, 750	188, 746	
	Range	1, 4458	1, 4458	1, 4237	1, 3543	3, 4237	
Weight-for-age	Z score‡	–2.43 (1.62)	–2.30 (1.52)	–2.63 (1.76)	–2.60 (1.66)	–2.73 (2.07)	0.53
Height-for-age		–2.39 (1.42)	–2.34 (1.35)	–2.47 (1.52)	–2.42 (1.47)	–2.64 (1.67)	0.19

*All those with specimen cultured.
†Median (IQR).
‡Mean (standard deviation), using UK 1990 reference data.
IQR indicates interquartile range.

Bacteria of Doubtful Pathogenicity

Other isolated organisms (n = 52, 41%), shown in Figure 1B, which were classified as being of doubtful pathogenicity, included: staphylococcal spp other than *S. aureus* (including *Staphylococcus haemolyticus* and *Staphylococcus hominis*; n = 12, 9%); streptococcal spp other than *S. pneumoniae* (including *Streptococcus viridans* and *Streptococcus mitis*; n = 10, 8%); and other bacteria including *Enterococcus* spp (n = 4, 3%), *Corynebacteria* spp (n = 3, 2%), *Moraxella* spp (n = 2, 1.5%) and *Providentia* spp (n = 2, 1.5%).

Antimicrobial Susceptibility Patterns

We considered susceptibility rates of <50% as low, those 50–79% as moderate and those ≥80% as high. Among *S. pneumoniae* isolates, there were low rates of susceptibility to cotrimoxazole (5%), moderate levels to penicillin G (58%) but high levels to amoxicillin/ampicillin (80%) and erythromycin (89%) (Table 3). All the *S. pneumoniae* isolates were susceptible to ceftriaxone, cefotaxime, chloramphenicol and cefuroxime. Of note, the 4 isolates that were resistant to amoxicillin/ampicillin

TABLE 2. Bacteremia Event Rates Among Children Who Developed Febrile Illnesses in Follow-up

Months on ART	0–<1	1–<3	3–<12	≥12	Total
Children in follow-up, n (%)	1206(100%)	1194(100%)	1179(100%)	1156(100%)	1206(100%)
Person-months follow-up	1202	2371	10,498	40,633	54,704
Febrile episodes, n	651	738	1659	3656	6704
Number of cultures					
Total cultures, n	143	152	291	262	848
Children with culture, n (%)	130(11%)	128(11%)	209(18%)	193(17%)	461(38%)
Total positive cultures, n	23	32	38	30	123
Children with positive culture, n (%)	23(1.9%)	28(2.3%)	35(3.0%)	29(2.5%)	105(8.7%)
Positive culture incidence rate per 1000 mo					
All isolates, rate (n)	19.13 (23)	13.50 (32)	3.62 (38)	0.74 (30)	2.25 (123)
Pathogenic isolates*	13.31 (16)	11.39 (27)	2.09 (22)	0.25 (10)	1.37 (75)
Streptococcus pneumoniae	6.66(8)	5.48(13)	1.24 (13)	0.05(2)	0.07(36)
Staphylococcus aureus	2.50(3)	2.53(6)	0.10(1)	0.02(1)	0.02(11)
Salmonella spp, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*	4.16(5)	2.53(6)	0.57(6)	0.15(6)	0.04(23)
Fungal spp	0.0(0)	0.42(1)	0.19(2)	0.02(1)	0.01(4)
CD4 cell %†					
Children with CD4%	1164(100%)	1137(100%)	1170(100%)	1155(100%)	1206(100%)
CD4 % < 15%, n (%)	723(62%)	320(28%)	262(22%)	162(14%)	782(65%)
CD4 cell count per μL†					
Children with CD4	1164(100%)	1137(100%)	1170(100%)	1155(100%)	1206(100%)
CD4 count < 200, n (%)	351(30%)	172(15%)	142(12%)	137(12%)	426(35%)

*All isolates, excluding other streptococcal spp, other staphylococcal spp and other bacteria.
†CD4 tests were done routinely every 12 weeks: those in the 0–<1 month were taken at enrolment; 1–3 months at week 12; in the subsequent time periods several were taken at 12 weekly intervals.

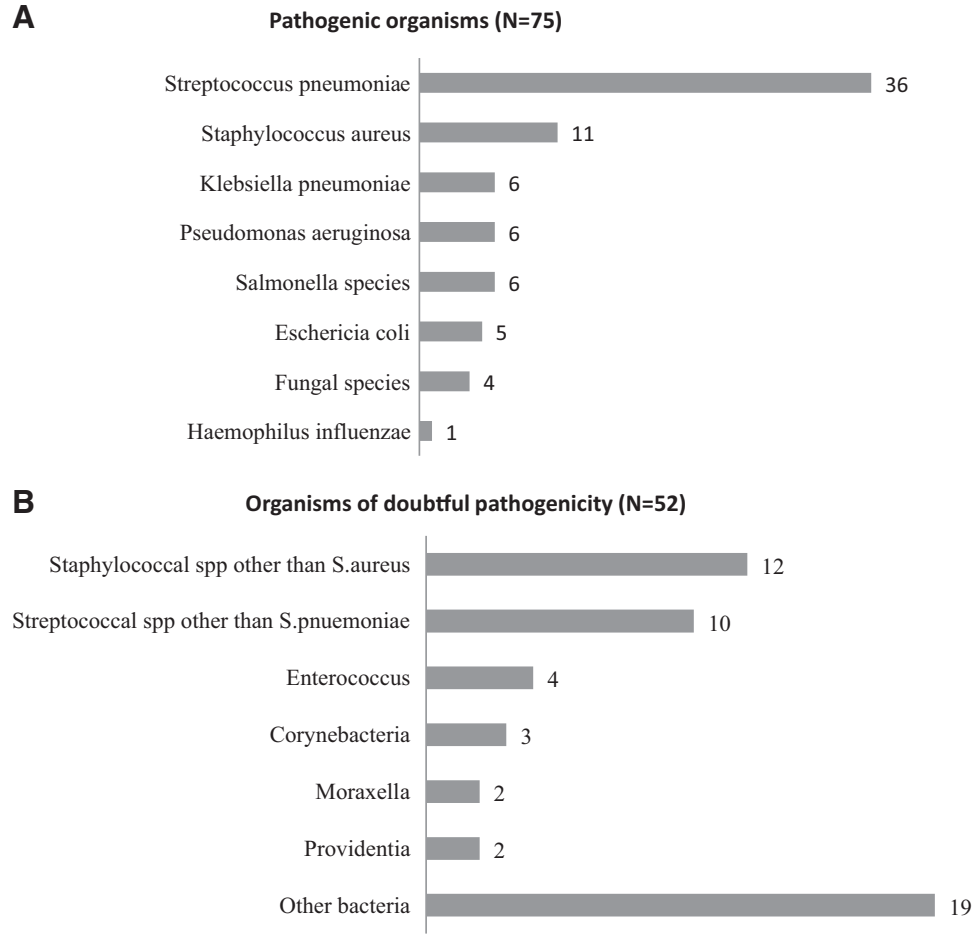

FIGURE 1. Relative frequency of organisms isolated from HIV-infected children with bacteraemia (N = 127).

were susceptible to ceftriaxone, cefotaxime, vancomycin and erythromycin.

For other organisms, the numbers tested were small. However, low susceptibility rates to cotrimoxazole were observed among nearly all isolates. *S. aureus*, *E. coli* and *Salmonella* spp were also largely resistant to ampicillin/ amoxicillin (Table 3) but were highly susceptible to ceftriaxone, ciprofloxacin and meropenem. Of note, *K. pneumoniae* isolates, 60% of which were ESBL producers, were all resistant to ceftriaxone, cefotaxime, gentamicin and ampicillin/amoxicillin, but susceptible to amikacin, ciprofloxacin and meropenem. Of the 6 *K. pneumoniae* isolates, only 2 (33%) children had been hospitalized within a 7-day window either side of the date of the positive sample.

DISCUSSION

HIV-infected African children have high rates of bacteremia, even on ART, and especially in the first 3 months after ART initiation.[12,13] Among children who developed febrile illnesses while on ART in our study, 14.5% of blood cultures were positive for bacterial pathogens, with *S. pneumoniae* observed in nearly one-third of the isolates. Positive cultures were around 20-fold more likely to occur in the first 3 months on ART compared with after 12 months on ART. The rate of conducting blood cultures per febrile episode was relatively constant across the observation periods in the first year on ART in our study, at 22%, 21%, 18% and 19% during the 0–<1 month, 1–<3 months, 3–<12 months and overall, respectively; subsequently the rate dropped to 7%. Although this may have contributed to the lower rate of bacteremia after the first year on ART, higher rates of bacteremia during the first 3 months on ART were similarly observed in South Africa.[12] This pattern of high rates of bacterial infections contributed substantially to the high early morbidity and often mortality observed both in children in ARROW and adults in the Development of Antiretroviral Therapy in Africa trial.[23] In both trials, the mortality risk increased from enrolment to a maximum between days 30 and 50, then declined rapidly to day 180 before declining further but more slowly throughout the rest of the first year on ART.[24] The mortality risk was higher at lower CD4 counts/percentages, and the majority of deaths in both children and adults were deemed by independent review to be due to bacterial infections (mainly septicemia/meningitis). The bacteremia episodes in our study resulted in a 49% hospitalization rate and a 28-day mortality of 7%. This mortality is slightly higher than the 4.7% mortality associated with gram-negative bacteremia in Singapore,[25] but much lower than the 53% and 24% mortality rates observed with hospital-acquired and community-acquired bacteremia, respectively, in Kenya.[26]

S. pneumoniae has always been the most common reported isolate among HIV-infected children with bacteremia.[12,15] *S. pneumoniae* infections in HIV-infected children tend to be severe and are associated with high mortality.[2,6,8] From studies in both resource-limited and well-resourced countries, the incidence of invasive pneumococcal disease among HIV-infected adults and

TABLE 3. Antimicrobial Susceptibility of Isolated Bacteria

Antibiotic*	Antibiotic Susceptibility of Isolated PathogensNumber Susceptible/Number Tested (%)					
	Streptococcus pneumoniae	*Staphylococcus aureus*	*Salmonella* spp	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*
Ceftriaxone	26/26 (100%)	4/5 (80%)	5/5 (100%)	2/2 (100%)	1/2 (50%)	0/2 (0%)
Cefotaxime	22/22 (100%)	3/4 (75%)	2/2 (100%)	2/2 (100%)	—	0/1 (0%)
Vancomycin	22/22 (100%)	4/4 (100%)	—	—	—	—
Clindamycin	7/7 (100%)	7/7 (100%)	—	—	—	—
Cefuroxime	2/2 (100%)	—	—	—	—	—
Meropenem/ Imipenem	—	—	2/2 (100%)	3/3 (100%)	2/2 (100%)	2/2 (100%)
Ciprofloxacin	—	3/3 (100%)	3/3 (100%)	—	2/2 (100%)	1/1 (100%)
Amikacin	—	—	—	2/2 (100%)	1/1 (100%)	1/1 (100%)
Chloramphenicol	7/7 (100%)	1/1 (100%)	0/1 (0%)	—	0/1 (0%)	—
Erythromycin	17/19 (89%)	5/8 (63%)	—	—	—	—
Amoxicillin/ Ampicillin	16/20 (80%)	3/8 (38%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/2 (0%)
Oxacillin	14/22 (64%)	0/1 (0%)	—	—	—	—
Gentamicin	—	3/5 (60%)	3/4 (75%)	1/2 (50%)	2/3 (67%)	0/2 (0%)
Penicillin G	11/19 (58%)	0/2 (0%)	—	—	—	—
Cotrimoxazole	1/22 (5%)	0/8 (0%)	1/3 (33%)	0/2 (0%)	0/1 (0%)	0/1 (0%)

*Minimum inhibitory concentrations in μg/mL used for classifying *S. pneumoniae* as susceptible or resistant were as follows: cefotaxime – 0.5; vancomycin – 0.5; meropenem – 0.13; chloramphenicol – 2; erythromycin – 0.06; amoxicillin – 0.25; gentamicin – 250; penicillin G – 0.03 and cotrimoxazole – 2/38.

children has significantly declined in the ART era.[13,27] However, in a study from South Africa, the risk of invasive pneumococcal disease still remained 42-fold greater in HIV-infected compared with HIV-uninfected children.[13] This is probably because of the incomplete immune reconstitution after initiation of ART[27] together with an irreversible loss of IgD+ memory B cells.[28] Therefore, it is important to prevent pneumococcal bacteremia if morbidity and mortality among HIV-infected children are to be reduced. Strategies that have been shown to be effective in achieving this goal include chemoprophylaxis with drugs such as penicillin and vaccination.[29,30]

We found only 1 isolate of *H. influenzae*, likely due to routine HiB vaccination in Uganda and Zimbabwe; by contrast, the high rate of *S. pneumoniae* isolation was probably because pneumococcal vaccination is not currently routine in either country.[31,32] In a randomized double-blind trial using the 9-valent pneumococcal conjugate vaccine among HIV-infected and HIV-uninfected children in South Africa, the incidence of invasive pneumococcal disease was significantly reduced among both groups of children receiving the vaccine.[30] However, the qualitative immune responses to 9-valent pneumococcal conjugate vaccine were poorer among the HIV-infected children.[33] Furthermore, whereas the long-term immunogenicity and efficacy of the vaccine persisted in the HIV uninfected, it greatly declined in the HIV-infected children.[34] This is probably because HIV-infected children lose anamnestic responses to pneumococcal vaccines and have early loss of memory B cell populations that are important for the generation of pneumococcal-specific responses.[35] However, it should be noted that majority of the children in these South African studies were not on ART; only 22.5% of the children received ART in the long term. Another South African study including ART-treated HIV-infected children (over 60% on ART) as well as HIV-uninfected infants[36] showed that the timing of ART initiation did not affect quantitative antibody responses between the HIV-infected children receiving ART and the HIV-infected children not on ART, but the children who were not receiving ART had more functionally impaired antibody responses measured by opsonophagocytic activity. Furthermore, results from a recent adult study[37] suggest that serological response to the vaccine could be better on ART. It is therefore expected that ART initiation would improve the response of HIV-infected children to the pneumococcal vaccine, although further research is warranted into the optimal timing and frequency of booster doses of the vaccine in children.

Among HIV-infected children, the only current WHO-recommended antibiotic that is widely used for chemoprophylaxis is cotrimoxazole.[38] In our study, we found high rates of resistance of *S. pneumoniae* to cotrimoxazole and moderately high resistance to penicillin G. It is possible that cotrimoxazole prevented occurrence of some infections and that those we observed in this substudy were caused by drug-resistant strains. However, given that the morbidity caused by these resistant strains was high, there is need for more effective antibiotic choices in HIV-infected children, in order to increase prophylaxis against *S. pneumoniae* and widen protection against other pathogens frequently isolated from children with bacteremia. Azithromycin, a semisynthetic macrolide, is active against gram-positive organisms such as *S. pneumoniae*, *S. aureus*, *Streptococcus pyogenes* and *S. viridans*, and several gram-negative organisms including *Salmonella* spp.[39] It may therefore be an appropriate antibiotic for use in HIV-infected children in sub-Saharan Africa, given the bacterial isolates found by several investigators.[12,14,15] Considering that azithromycin is administered orally and once daily, and also has activity against mycobacterium avium complex disease, it could be suitable for use on a large scale. However, given that this would certainly raise concerns about development of resistance,[40,41] its use in prophylaxis against bacterial and other infections would be best evaluated in the most highly vulnerable HIV-infected individuals with very low CD4 counts/percentages at ART initiation and should be given for a limited period only. The Reduction of EArly mortaLITY in HIV infected adults and children starting antiretroviral therapy (REALITY) trial (ISRCTN43622374), which is exploring short-term interventions at ART initiation to reduce early mortality in very immunosuppressed HIV-infected adults and children, will include azithromycin in its evaluation of a package of anti-infective interventions. Considering the role of cotrimoxazole in reducing morbidity and mortality among HIV-infected individuals even in places with high antibacterial resistance to the drug,[42,43] any prophylactic agent against bacteremia should be in addition to cotrimoxazole.

The start of ART in a South African trial[12] was associated with an increase in the incidence of bacteremia among HIV-infected

children 8 months to 12 years, when compared with children not yet on ART; the incidence dropped significantly (by 74%) once children were established on ART. Our study showed a more dramatic 20-fold decrease in positive culture rates after 12 months on ART when compared with the first 3 months on ART, paralleling the improvement in CD4 count and percentage. Starting ART at higher CD4 counts/percentages therefore would also be likely to reduce the risk of children developing bacteremia after ART initiation. As expected, we also found that children with febrile illness and possible bacteremia were younger than other trial participants, suggesting that any interventions against bacteremia should also target younger HIV-infected children.

We isolated ESBL-producing *K. pneumoniae* from several children in ARROW. As had been observed in South Africa, isolation was not associated with prior hospitalization,[14] implying that they were likely to be community acquired, which is a worrying trend.

We report isolation of *S. haemolyticus* and *S. hominis* that are known to be normal skin flora, and it is possible they were contaminants. However, these organisms together with coagulase-negative *Staphylococcus epidermidis* have been reported to cause bacteremia and sepsis among immunocompromised patients,[44,45] such as those participating in this study. We also report the isolation of streptococcal spp other than *S. pneumoniae*, as well as other bacteria of doubtful pathogenicity. Given that the children had clinical symptoms, further research into their pathogenicity among HIV-infected children is warranted. Although children from whom blood cultures were taken presented with varied provisional diagnoses (including urinary tract infection, otitis media, malnutrition, respiratory tract infections and bacteremia), all had 1 common feature—fever. This implies that the rates of bacteremia and pattern of the isolated pathogens refer to febrile illnesses in general rather than specific syndromes.

The culture positivity rates were higher in Zimbabwe than at the Ugandan sites, probably because of the differential contribution of malaria to febrile illnesses across the sites. Whereas the whole of Uganda is endemic for malaria[46]; Harare has no malaria.[47] This implies that among febrile illnesses, bacteremia was more likely in Harare than at the Ugandan sites because of the absence of malaria that contributed to the febrile episodes at the Ugandan sites. Although there was a difference in the methods of isolation, this is an unlikely explanation for the differential isolation rates, and the pattern of isolated pathogens was similar across sites. A limitation of this study is that we were not able to test the susceptibility profiles of all the isolates to the same antimicrobial agents, and in several cases, the numbers tested for certain antibiotics against particular isolates were small.

In summary, we observed high rates of proven bacteremia in HIV-infected children in the first 3 months on ART, with *S. pneumoniae* most commonly isolated. This reaffirms the need to incorporate pneumococcal vaccination in national immunization programs. The low susceptibility rates observed among isolated pathogens to cotrimoxazole suggest that alternative prophylactic antibiotic strategies need evaluation, including which populations to target and when. The results of our study suggest that the period immediately after ART initiation should be a period where targeted prophylaxis could be of benefit.

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