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The aetiology of diarrhoea, pneumonia and respiratory colonization of HIV-exposed infants randomized to breast- or formula-feeding

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Abstract

Background—Diarrhoea and pneumonia are common causes of childhood death in sub-Saharan Africa but there are few studies describing specific pathogens.

Objectives—The study aimed to describe the pathogens associated with diarrhoea, pneumonia and oropharyngeal colonization in children born to HIV-infected women (HIV-exposed infants).

Methods—The Mashai Study randomized 1200 HIV-infected women and their infants to breastfeed for 6 months with ZDV prophylaxis or formula-feed with 4 weeks of ZDV. Children were tested for HIV by PCR at 1, 4, 7, 9 and 12 months and by ELISA at 18 months. Pre-defined subsets of children were sampled during episodes of diarrhoea ($n = 300$) and pneumonia ($n = 85$).

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Stool was tested for bacterial pathogens, rotavirus and parasites. Children with pneumonia underwent bacterial blood culture, and testing of nasopharyngeal aspirates for viral pathogens by PCR. Oropharyngeal swabs were collected from a consecutive subset of 561 infants at the routine 3-month visit for bacterial culture.

Results—The median age (range) at sampling was 181 days for diarrhoea (0–730) and 140 days for pneumonia (2–551). Pathogens were identified in 55 (18%) children with diarrhoea and 32 (38%) with pneumonia. No differences in pathogens by child HIV status (HIV-infected *vs* HIV-uninfected) or feeding strategy were identified. *Campylobacter* was the most common diarrhoeal pathogen (7%). Adenovirus (22%) and other viruses (19%) were the primary pathogens isolated during pneumonias. More formula-fed infants had oropharyngeal colonization by pathogenic Gram-negative bacteria (16.8% *vs* 6.2%, $P = 0.003$), which was associated with a non-significant increased risk of pneumonia (OR 2.2, 95% CI 0.8–5.7).

Conclusion—A trend toward oropharyngeal bacterial colonization was observed in formula-fed infants. Although viruses were most commonly detected during pneumonia, respiratory colonization by Gram-negative bacteria may have contributed to pneumonia in formula-fed infants.

Introduction

In resource-limited settings, the risk of death in HIV-exposed infants is greater than for HIV-unexposed infants.^{1–3} Mortality in HIV-infected and HIV-exposed but uninfected (HEU) infants is primarily caused by respiratory and diarrhoeal diseases.^{4–9} However, the specific pathogens causing these infections are largely unknown because of barriers to diagnostic testing in resource-limited settings. Therefore, the contribution of the usual bacterial and viral pathogens to excess mortality in HEU is poorly described. Furthermore, formula-feeding by HIV-infected women [to reduce the risk of mother-to-child-transmission of HIV (MTCT)] may affect the rate and aetiology of pneumonia and diarrhoea, especially in the first few months of life.^{9–15} Therefore, this study, a clinical trial in Botswana, aimed to describe the infectious agents associated with diarrhoea and pneumonia, as well as colonization of bacterial respiratory pathogens in infants born to HIV-infected women randomised to breastfeed or formula-feed.

Subjects and Methods

The Mashi study methods and main study results have been described previously.⁴ In brief, Mashi was a randomized clinical trial that used a two-by-two factorial design to compare interventions for preventing MTCT [single-dose nevirapine *vs* placebo, breastfeeding with infant zidovudine (ZDV) for 6 months *vs* formula feeding with ZDV for 1 month]. The study enrolled 1200 HIV-infected women from March 2001 to October 2003 in four sites in Botswana (one city, one town and two villages). HIV-infected pregnant women received ZDV from 34 weeks' gestation through labour, and were randomized to receive either single-dose nevirapine or placebo peripartum; mother-infant pairs were also randomized to either formula-feed from birth (with 4 weeks of ZDV) or breastfeed for 6 months (with 6 months of daily infant ZDV prophylaxis). As previously reported, adherence to exclusive

formula feeding was 93% and adherence to exclusive breastfeeding at 1 month, 3 months and 5 months was 57%, 31% and 18% respectively.⁴

The Botswana government donated infant formula to participants up to 12 months of age. Mothers were taught safe formula preparation and were observed preparing formula and given vacuum flasks. Mothers in the breastfeeding arm were instructed to breastfeed exclusively and wean the infant at 5–6 months age, and were provided with free formula from 5 to 12 months of age. All infants were also provided with high-protein complementary food from 6 to 12 months of age.

Children were seen and examined and their medical records reviewed at birth, monthly until 7 months, at 9 months, and then every 3 months until 24 months of age. Infant blood was obtained at birth, age 4 weeks, and at 4, 7, 9 and 12 months for HIV testing by DNA PCR, and at age 18 months by ELISA. Infants were considered HIV-infected if they had a positive PCR or ELISA and a subsequent confirmatory test (or died or were lost to follow up before a confirmatory test could be performed). The term ‘HIV-exposed’ refers to all infants whose mothers are HIV-infected, regardless of the infant’s HIV status. Routine immunizations (BCG, measles, DPT, polio, hepatitis B), general outpatient medical care and ART (when indicated) were provided by trained study staff. HIV-infected infants received cotrimoxazole prophylaxis. Until 24 months of age, detailed information on the occurrence of diarrhoeal disease or pneumonia was collected at each visit.

Laboratory methods

A convenience sample (limited by the availability of funding) included the first 300 consecutive HIV-exposed infants who presented with acute diarrhoeal illness in a diarrhoeal disease sub-study. Diarrhoea was defined as three or more loose stools within the preceding 24 hours which also represented a change in the child’s normal stool pattern. Additional specimens were collected from these infants if subsequent diarrhoeal episodes occurred, defined as a return of diarrhoea after at least 3 diarrhoea-free days. Whole stool specimens were obtained from infants with acute diarrhoea, although rectal swabs were acceptable if no stool was produced within 30 minutes of the attempt at collection. All swabs were placed in Cary Blair transport media, kept cold and sent to Gaborone Private Hospital (GPH) Microbiology Laboratory for same-day processing. Stools were plated on xylose lysine deoxycholate (XLD) and MacConkey media with crystal violet, inoculated in to selenite broth and incubated at 35–37°C. After 24 hours, broth was also plated on to XLD and MacConkey agar. Non-lactose fermenting and H₂S-producing colonies were then sub-cultured and Gram-stained. Isolates were typed with antisera for *Shigella* spp. and *Salmonella* serotype typhi. All specimens were also inoculated in to *Campylobacter* media and kept at 42° for 48 hours, after which colonies were Gram-stained. Antibiotic resistance was tested by disc diffusion. Whole stool was tested with acid-fast stain for cryptosporidia, isospora and cyclospora, and wet prep was performed for giardia and entamoeba. A subset of 120 consecutive samples was tested for rotavirus using the Rotaclone² kit.

Children enrolled in the Mashhi study who met the WHO definition for pneumonia or who were diagnosed with pneumonia by a health-care provider were further worked up for aetiology of pneumonia when seen at the time of illness. This work-up consisted of

nasopharyngeal aspirate (NPA) and blood culture. NPA specimens were stored at -70°C and then shipped on dry ice to the Children's Hospital in Boston where all testing, except for adenovirus, was performed at the end of the study. NPA samples were tested using real-time PCR for influenza A, influenza B and RSV by the ProFlu-1 Real Time Assay (Prodesse), human metapneumovirus (hMPV) using the Pro hMPV Real Time Assay (Prodesse), bocavirus, rhinovirus, enterovirus and parainfluenza type 3 using assays developed by the hospital's laboratory for research purposes. *Mycoplasma pneumoniae* and *Bordetella pertussis* were detected by real-time PCR using the *M. pneumoniae* and *B. pertussis* analyte specific reagent (ASR) produced by Cepheid. Adenoviruses were tested for at the Children's Hospital of Philadelphia using a laboratory-developed real-time TagMan PCR assay.¹⁶ Bactec Peds Plus blood culture bottles were kept at room temperature after injection and sent to the GPH Microbiology Laboratory or the National Health Laboratory at Princess Marina Hospital within 6 hours. Blood culture bottles were incubated and sub-cultured on to both chocolate and blood agar every day for 5 days. Disk diffusion sensitivity testing was used for the bacterial species isolated and an optochin disc to identify pneumococcal isolates.

To assess for colonization by bacterial pathogens, an oropharyngeal (OP) swab was obtained from a convenience sample of the first 561 consecutive infants enrolled in the Mashu study who presented for a routine scheduled 3-month study follow-up visit. A cotton swab (wooden stick) was passed across the back of the infant's oropharynx, placed into a bacterial culture transport medium, and kept at room temperature. The swabs were then sent for bacterial culture to the GPH microbiology laboratory.

Statistical methods

Descriptive analyses are reported by infant HIV-status and feeding strategy. Differences between groups were calculated using Pearson's χ^2 test or Fisher's Exact test for categorical variables and the *t*-test or Mann-Whitney test for continuous variables. Statistical analyses were performed using SAS, version 9.3 (SAS institute, Cary, NC). All reported *P*-values are based on a two-sided test.

Ethics

All women gave written, informed consent for their and their child's participation in the Mashu study and sub-studies, and the study/sub-studies were approved by the Botswana Health Research Development Committee and the Harvard School of Public Health Human Subjects Committee.

Results

Diarrhoea

Of 1179 live infants born to HIV-infected mothers, 691 (59%) had at least one episode of diarrhoea within 24 months. A total of 456 stool samples (most representing a distinct episode) were collected from 300 infants (148 in the breastfeeding arm and 152 in the formula-feeding arm); the seasonality of diarrhoeal disease and stool sample collection are shown in Fig. 1a, and the overall characteristics of the study population are presented in

Table 1. Assigned treatment and feeding arms, sex, maternal baseline CD4 count, infant HIV status and the severity of diarrhea were similar in infants who did and did not have stool sent for testing during an episode of diarrhoea (data not shown). The median age at first stool sampling was 181 days (IQR 98–243), and was significantly lower in the formula-feeding than in the breastfeeding arm (153 vs 206 days, $P = 0.002$).

Bacterial pathogens were identified in 45 (15%) of the 300 infants from whom one or more diarrhoeal samples were collected (Table 2). An additional eight (7%) of 120 samples tested were positive for rotavirus by latex agglutination, and cryptosporidium was identified in two (1%) of 138 whole stool samples examined. There were no clinical or sociodemographic differences between those in whom pathogens were identified and those in whom they were not. A bacterial pathogen was identified in only two (7%) of 28 HIV-infected infants sampled. *Campylobacter* species were the most common bacterial pathogens ($n = 22$, 49%), followed by *Salmonella* non-typhi ($n = 17$, 38%), *Shigella* ($n = 3$, 7%) and *Salmonella* serotype typhi ($n = 3$, 7%). All three isolates of *S. typhi* were sensitive to ceftriaxone, ciprofloxacin and naladixic acid. Cotrimoxazole resistance was common: 5/7 *Campylobacter* spp., 9/20 *Salmonella* spp. and 3/4 *Shigella* isolates tested were resistant. One isolate was resistant to ceftriax-one and one to ciprofloxacin (both resistant isolates were *Campylobacter*).

Median age at detection by bacterial pathogen was as follows: *S. typhi* 3.7 months, *Campylobacter* 6.3 months, *Salmonella* non-typhi 7.1 months and *Shigella* 11.8 months; however, these differences were not statistically significant.

Pneumonia

The characteristics of infants who were tested during episodes of pneumonia are shown in Table 1, and the seasonality of pneumonia diagnosis and sampling is shown in Fig. 1B. The tested infants were similar to those who were not tested (data not shown), except that fewer tested infants had electricity in the home (14% vs 27%, $P = 0.02$). In the first 2 years of life there were 319 episodes of pneumonia among 219 infants, at a median age of 140 days (IQR 73–224). Of these, 58% were hospitalized, 69% had a chest radiograph, and 49% had severe pneumonia by WHO criteria. There was a work-up for pathogens in 78 infants with 85 episodes of pneumonia: 27 (32%) had NPA only, 35 (41%) had blood culture only and 23 (27%) had both blood culture and NPA.

At least one pathogen was detected by NPA in 29 (58%) children, and more than one pathogens were detected in nine (18%). A pathogen was detected by blood culture in six (10%) children (Table 3). There was no difference in pathogen detection by infant HIV status ($P = 0.42$) or feeding status ($P = 0.26$).

The most common pathogen recovered from NPA was adenovirus ($n = 19$, 50%), followed by rhinovirus ($n = 7$, 18%), *Bordetella pertussis* ($n = 3$, 8%), para-influenza 3 (PI3) ($n = 3$, 8%), human metapneumovirus (hMPV) ($n = 3$, 8%) and RSV ($n = 3$, 8%). The combination of adenovirus and rhinovirus was responsible for 66% of NPAs containing more than one organism. Of six positive blood cultures, there was one *Staphylococcus aureus*, one *Streptococcus pneumoniae*, one *Streptococcus* (non-Groups A, B and D), one *Proteus* spp.,

one Acinetobacter and one isolate that could not be identified by our methods. There was no difference in the type of pathogen by infant HIV status or randomized feeding strategy.

Oropharyngeal colonization of infants at the 3-month visit

The characteristics of infants who were tested for oropharyngeal colonization at the 3-month visit are shown in Table 1. Tested infants were similar to those who were not tested, except that mothers of tested infants were younger (mean 26 vs 28 yrs, $P = 0.0001$), more likely to report no income (64% vs 56%, $P = 0.05$), and had higher log₁₀ baseline viral loads (median 4.4 vs 4.3, $P = 0.03$). Oropharyngeal swabs were performed on 561 infants at a median age of 92 days (IQR 91–93). Among tested infants, 287 (51%) were in the breastfeeding arm and 274 (49%) were in the formula-feeding arm; 49 (8%) were HIV-infected and 512 (92%) were HIV-uninfected.

Colonizing organisms were identified in 162 (26%) samples (Table 4). Of all organisms identified, 40% were Gram-negative bacteria, 41% were Gram-positive bacteria, and 19% were yeast. The most common Gram-negative organisms were *Klebsiella* (55%); *Haemophilus influenza*, not type B (20%), and *Escherichia coli* (14%). Fifteen of the Gram-positive organisms were *S. aureus* and the remainder were *Streptococcus* spp.: 57% group A streptococcus, 7% *Streptococcus pneumoniae*, 4% Group B *Streptococcus*, 1% Group D *Streptococcus* and 15% *Streptococcus*, not group A, B or D. *Candida* spp. accounted for all but one of the fungal organisms (the other organism was not identified).

A non-significant trend towards a higher prevalence of oropharyngeal colonization by a potential pathogen was observed in HIV-infected infants compared with HIV negative infants (35% vs 25%, $P = 0.16$), particularly with Gram-negative organisms (although infant HIV status was not significantly associated with the type of organism identified).

Also, a trend toward higher colonization rates was observed among formula-fed infants compared with breastfed infants (29% vs 23%, $P = 0.13$). Gram-negative organisms were found significantly more frequently in infants randomized to formula feeding (16.8% vs 6.2%, $P = 0.003$).

Infants with any organisms identified on OP swab had a slightly (non-significant) higher risk of pneumonia in the first 6 months of life than infants without organisms (13.6% vs 9.7%, $P = 0.19$). In infants in whom organisms were identified, there was a trend towards a higher rate of pneumonia in those colonized by Gram-negative rods than in infants with other organisms (19% vs 10%, $P = 0.12$), but this difference also did not reach statistical significance.

Discussion

This study provides the first detailed description of pathogens associated with diarrhoea, pneumonia and respiratory colonization in infants born to HIV-infected women in Botswana. Pathogens associated with diarrhoea and pneumonia did not differ significantly by randomized feeding strategy or infant HIV status.

Acute diarrhoeal illness was very common, with 59% of infants having at least one episode in the first 2 years of life. Episodes of diarrhoea occurred earlier in formula-fed infants (median 153 days) than in breastfed infants (median 206 days). As most breastfed infants were weaned at 6 months of age, this suggests that there is an increased risk of diarrhoeal illness shortly after weaning.

The finding that *Campylobacter*, *Salmonella* and rotavirus were the most common pathogens isolated in infants with diarrhoea is similar to previous studies,^{17–19} but differs somewhat from a recent large prospective study in seven Asian and African countries which found that most cases of moderate-to-severe diarrhoea were caused by four pathogens: rotavirus, cryptosporidium, enterotoxigenic *E. coli* and *Shigella*.²⁰ These differences may be owing to geographic variability in the frequency of pathogens, as well as differences in techniques used for pathogen detection. In this study, there was no evaluation for enterotoxigenic *E. coli*, and acid-fast staining for parasite detection (which is less sensitive than immuno-assays used in the multi-national study)²¹ was used. The relatively small proportion of diarrhoea caused by rotavirus in our study could also be owing to fewer samples being tested over the winter months in Botswana (May–August) when rotavirus is most common. Our overall yield was lower than expected for all diarrhoeal pathogens (and lower than reported in a recent study of diarrhoeal disease in children hospitalized with diarrhoeal disease in Botswana),²² but similar to a study in Kenya.²³ Additional testing for enterotoxigenic, entero-haemorrhagic and entero-aggregative *E. coli*, as well as immunological assays and PCR detection methods for parasites and viruses, are required to completely evaluate the spectrum of diarrhoeal disease in future studies.^{24–26} This study was undertaken before a large outbreak of diarrhoeal disease in infants in Botswana which coincided with heavy rains and found that cryptosporidium and entero-pathogenic *E. coli* were common, and formula-feeding was strongly associated with infant mortality.²⁷

As far as we know, this is the first study to use molecular techniques to test for multiple viral and atypical pathogens and compare the organisms associated with pneumonia by both infant HIV infection and randomized feeding status. No significant differences among viral and atypical organisms by infant HIV-status or feeding strategy were found, although our study was designed to be descriptive and may not have had the power to detect differences. However, there was no testing for *Pneumocystis jirovecii* or *Mycobacterium tuberculosis*, which would be expected to occur more frequently in HIV-infected children.^{28–30}

There are recent studies that also used molecular techniques to identify viral pathogens in lower respiratory infections (LRTI) in infants in sub-Saharan Africa, although they do not specifically report by infant HIV-infection and feeding strategy.^{31–38} Several studies found that RSV was the most frequent pathogen, particularly in infants under 1 year.^{35,36} Our study, and one from Mozambique,³⁴ found that adenovirus and rhinovirus were the two most common viral potential pathogens and were commonly found together in infants with pneumonia. Differences in this study might be explained by regional differences in the viral milieu, sensitivity of testing or, possibly, seasonality of testing as fewer NPAs were obtained during the winter months when RSV is most common.

A non-significant trend towards more frequent oropharyngeal colonization by potentially pathogenic organisms (Gram-negative organisms in particular) was found in HIV-infected infants (with Gram-negative organisms tending to be detected more frequently in formula-fed than in breastfed infants). While previous studies have also shown that Gram-negative organisms are more commonly found colonizing the respiratory tract of formula-fed infants than breastfed infants,^{39–41} these studies were not undertaken in HIV-exposed infants. Furthermore, while prior studies have shown that colonization by Gram-negative organisms can lead to an increased risk of otitis media and other upper respiratory tract infections in healthy infants,^{42–44} our results suggest that it could also increase the risk of lower respiratory tract infection outside intensive care units and in non-cystic fibrosis patients. Despite the association between Gram-negative colonization and increased pneumonia, this study did not find Gram-negatives to be a common cause of pneumonia by blood culture (though all Gram-negative pneumonias occurred in formula-fed infants).

The study was designed to describe respiratory colonization but not to detect differences in the rates of pneumonia by respiratory colonization, but the possible association between pathogenic Gram-negative colonization, formula-feeding and HIV-infection is intriguing because of the potential to explain the increased incidence of pneumonia in formula-fed infants. It has been hypothesized that the difference in colonization by infant feeding strategy could be owing to factors in breast-milk (such as IgA) which inhibit Gram-negative bacteria from adhering to mucosal surfaces,⁴⁵ although this is not well studied.

The strength of this study was that it was nested in a randomized trial of breast- vs formula-feeding and therefore confounding socio-economic differences which would lead to different feeding choices are fewer than in an observational study. This strength is particularly relevant for comparison of prevalence of colonization by bacterial pathogens at 3 months of age, given the larger sample size for that analysis by feeding method. The study also used a community rather than hospitalized population and thus reduced bias related to severity of disease.

There are several limitations to the study. Firstly, infants with the most severe disease may not have been tested if they died before reaching a health care facility. Also, some infants with reported pneumonia or diarrhoea were not tested because they were not seen by study staff during the period of illness, particularly during winter months. Secondly, detection of a pathogen in stool or NPA does not necessarily mean that it was the causative agent for diarrhoea or pneumonia;^{20,46} conversely, blood culture is not very sensitive for diagnosing the aetiology of pneumonia. Thirdly, both diarrhoea and pneumonia are difficult to diagnose accurately in infants. Although strict criteria were applied to define diarrhoea, infant stooling patterns can change for non-infectious reasons such as maternal diet or may be a symptom of a systemic infection that does not primarily occur in the gut. Similarly, difficulty in breathing may be the result of a non-pulmonary illness and chest radiograph findings can represent an inflammatory reaction in the lungs rather than infection. Some episodes of diarrhoea and pneumonia might therefore have been misclassified. Fourthly, mixed feeding was common among mothers randomized to breast-feeding⁴ and it is therefore possible that the true differences in pathogens between feeding strategies was underestimated. Finally,

testing for *P. jirovecii* or *Mycobacterium tuberculosis*, which are common opportunistic pathogens causing pneumonia in Bots wana, was not included.

However, despite the limitations, we believe that this is an important addition to the limited literature on the infectious agents found in pneumonia and diarrhoea in HIV-exposed infants in resource-limited settings. Further research is needed to explore the relationship between infant feeding status, oropharyngeal colonization and pneumonia as targeted interventions could decrease morbidity and mortality in HIV-exposed infants.

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Figure 1.
A Diarrhoea by calendar month
B Pneumonia and pathogen data by calendar month

Table 1

Characteristics of the study population

Baseline characteristics	Sub-set with samples tested			
	Total cohort n = 1200 (%)	Diarrhoea n = 300 (%)	Pneumonia n = 85 (%)	Oropharyngeal colonization n = 561 (%)
Assigned feeding arm	602 (50)	152 (51)	48 (56)	274 (49)
	Formula-feeding			
	Breastfeeding	148 (49)	37 (44)	287 (51)
Maternal age, median, yrs	26.8	26.8	26.8	26.0
Clinic site				
	Gaborone (urban)	43 (14)	8 (9)	78 (14)
	Lobatse (town)	103 (34)	28 (33)	122 (22)
	Mochudi (rural)	61 (20)	19 (22)	189 (34)
	Molepolole (rural)	93 (31)	30 (35)	172 (31)
Electricity in the home	349 (29)	93 (31)	12 (14)	118 (21)
	280 (24)*	52 (17)		
No maternal income	717 (61) ^f	201 (67)	55 (65)	358 (64)
HIV-infected infants	87 (7) ^z	28 (9)	18 (21)	49 (8)
Maternal baseline log10 viral load	Median	4.4	4.5	4.4
Maternal baseline CD4	Median	366	374	369
Birthweight, g	Median	3100	3100	3100
Infant age at sampling, days	Median	181	140	92

Owing to missing values:

* 19 missing,

^f 25 missing,

^z 21 missing.

Table 2
Diarrhoeal pathogens identified in HIV-exposed infants, by HIV status and feeding strategy

	Total <i>n</i> (%)	Infant HIV status		Randomized feeding strategy	
		HIV infected <i>n</i> = 28 (%)	HIV uninfected <i>n</i> = 272 (%)	BF ZDV* <i>n</i> = 148 (%)	FF ZDV* <i>n</i> = 152
Bacterial pathogen (<i>n</i> = 300)					
Campylobacter spp.	22 (7.3)	0	22 (8.1)	11 (7.4)	11 (7.2)
Salmonella, non-typhi	17 (5.7)	2 (7.1)	15 (5.5)	9 (6.1)	8 (5.3)
Salmonella typhi	3 (1)	0	3 (1.1)	1 (0.7)	2 (1.3)
Shigella	3 (1)	0	3 (1.1)	1 (0.7)	2 (1.3)
Rotavirus (<i>n</i> = 120)	8 (6.6)	0	8 (2.9)	7 (4.7)	1 (0.7)
Parasites (<i>n</i> = 138)	2 (1.4)	1 (3.6)	1 (0.4)	0	2 (1.3)

BF, breastfeeding; FF, formula feeding; ZDV, zidovudine.

* Breastfeeding infants were given ZDV prophylaxis for 6 months and formula-feeding infants for 1 month.

Table 3

Respiratory pathogens identified during episodes of pneumonia

Pathogen	Total n = 85 (%)	Infant HIV status		Randomized feeding strategy	
		HIV-infected n = 18 (%)	HIV-uninfected n = 67 (%)	FF ZDV ^f n = 48 (%)	BF ZDV ^f n = 37 (%)
Bacteria:	12 (14.1)	2 (11.1)	10 (14.9)	8 (16.7)	4 (10.8)
Gram-positive *	6 (7.1)	1 (5.6)	5 (7.5)	2 (4.2)	4 (10.8)
Gram-negative *	2 (2.4)	0	2 (3.0)	2 (4.2)	0
<i>B. pertussis</i> ^f	3 (3.5)	1 (5.6)	2 (2.9)	3 (6.1)	0
Other *	1 (1.2%)		1 (1.5)	1 (2.1)	0
Viral:	35 (41.2)	8 (44.4)	27 (40.3)	24 (50.0)	11 (29.7)
Adenovirus ^f	19 (22.1)	4 (22.2)	15 (22.1)	14 (28.6)	5 (13.5)
Rhinovirus ^f	7 (8.1)	2 (11.1)	5 (7.4)	4 (8.2)	3 (8.1)
Para-influenza 3 ^f	3 (3.5)	0	3 (4.4)	3 (6.1)	0
hMPV ^f	3 (3.5)	1 (5.6)	2 (2.9)	1 (2.0)	2 (5.4)
RSV ^f	3 (3.5)	1 (5.6)	2 (2.9)	2 (4.1)	1 (2.7)
Enterovirus ^f	0	0	0	0	0
Influenza A or B ^f	0	0	0	0	0
Bocavirus ^f	0	0	0	0	0
More than 1 pathogen ^z	9 (10.6)	3 (16.7)	7 (10.3)	6 (12.2)	4 (10.8)
No pathogen	49 (57.0)	11 (61.0)	38 (55.9)	19 (38.8)	25 (67.6)

* Identified by blood culture, in total 6/61 blood cultures were positive;

^f identified by NPA;^z samples with multiple pathogens included 6 adenovirus/rhinovirus, 1 *B. pertussis*/para-influenza 3, 1 *B. pertussis*/adenovirus, 1 adenovirus/hMPV, hMPV, human metapneumovirus; RSV, respiratory syncytial virus.^f Breastfeeding infants were given ZDV prophylaxis for 6 months and formula-feeding infants for 1 month

Oropharyngeal colonization of infants attending the 3-month visit, by HIV and feeding status

Table 4

Organism	Infant HIV status			Randomized feeding strategy		
	Total <i>n</i> = 561 (%)	HIV-infected <i>n</i> = 49 (%)	HIV-uninfected <i>n</i> = 512 (%)	BF <i>n</i> = 287 (%)	ZDV <i>n</i> = 274 (%)	FF <i>n</i> = 274 (%)
Gram-negative bacteria:	65 (11.3)	9 (18.4)	56 (10.9)	19 (6.2)	46 (16.8)	
<i>Klebsiella</i> spp	36 (6.4)	5 (10.2)	31 (6.1)	8 (2.8)	28 (10.2)	
<i>H. influenza</i> not type B	13 (2.3)	1 (2.0)	12 (2.3)	8 (2.8)	5 (1.8)	
<i>E. coli</i>	9 (1.6)	1 (2.0)	8 (1.6)	1 (0.3)	8 (2.9)	
Enterobacter spp.	4 (0.7)	2 (4.1)	2 (0.4)	1 (0.3)	3 (1.1)	
<i>H. influenza</i> (non-typeable)	2 (0.4)	0	2 (0.4)	1 (0.3)	1 (0.4)	
<i>Pseudomonas</i> (non-aeruginosa)	1 (0.2)	0	1 (0.2)	0	1 (0.4)	
Gram-positive bacteria:	67 (11.6)	8 (16.3)	59 (11.5)	36 (12.5)	31 (11.3)	
Strep., Group A	38 (6.8)	3 (6.1)	35 (6.8)	20 (7.0)	18 (6.6)	
<i>S. aureus</i>	10 (1.8)	2 (4.1)	8 (1.6)	6 (2.1)	4 (1.5)	
Strep., non-Group A, B or D	10 (1.8)	1 (2.0)	9 (1.8)	4 (1.4)	6 (2.2)	
Strep. pneumoniae	5 (0.9)	2 (4.1)	3 (0.6)	3 (1.0)	2 (0.7)	
Strep., Group B	3 (0.5)	0	3 (0.6)	2 (0.7)	1 (0.4)	
Strep., Group D	1 (0.2)	0	1 (0.2)	1 (0.3)	0	
Yeast/fungi	30 (5.2)	3 (6.1)	27 (5.3)	19 (6.2)	11 (4.0)	
<i>Candida</i> spp.	29 (5.2)	3 (6.1)	26 (5.1)	19 (6.6)	10 (3.6)	
Other fungal	1 (0.2)	0	1 (0.2)	0	1 (0.4)	
No pathogen	414 (73.8)	32 (65.3)	382 (74.6)	220 (76.7)	194 (70.8)	