


SHORT REPORT

Cotrimoxazole prophylaxis was associated with enteric commensal bacterial resistance among HIV-exposed infants in a randomized controlled trial, Botswana

Kathleen M Powis^{1,2,3*} , Sajini Souda^{4*}, Shahin Lockman^{2,3,5*}, Gbolahan Ajibola^{3*}, Kara Bennett^{6*}, Jean Leidner^{7*}, Michael D Hughes^{8*}, Sikhulile Moyo^{3*}, Erik van Widenfelt^{3*}, Haruna B Jibril⁹, Joseph Makhema^{3*}, Max Essex^{2,3*} and Roger L Shapiro^{2,3*}

Corresponding Author: Kathleen Powis, 125 Nashua Street, Office 8426, Boston, Massachusetts 02114. Tel: (617)643-2054. (kpowis@mgh.harvard.edu)

*These authors have contributed equally to this work.
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Abstract

Introduction: Despite declining risk of vertical HIV transmission, prophylactic cotrimoxazole (CTX) remains widely used to reduce morbidity and mortality in the event of HIV infection among exposed infants, with an inherent risk of conferring commensal antimicrobial resistance. Using data from a randomized, placebo-controlled trial of infant CTX prophylaxis, we sought to quantify emergence of antibiotic resistance.

Methods: HIV-exposed uninfected infants enrolled in the Botswana Mpepu study were randomized to prophylactic CTX or placebo between 14 and 34 days of life and continued through 15 months. Stool samples were collected from a subset of participating infants at randomization, three, and six months, and stored at -70°C prior to culture. Specimens that grew *Escherichia coli* (*E. coli*) or *Klebsiella* species (*Klebsiella* spp.) underwent antibiotic susceptibility testing by Kirby Bauer method using CTX (CTX 1.25/23.75 μg) and Amoxicillin (10 μg) in Mueller Hinton agar. Fisher's exact testing was used to compare prevalence of resistance by randomization arm (CTX/placebo).

Results and Discussion: A total of 381 stool samples from 220 infants were cultured: 118 at randomization, 151 at three months, and 112 at six-months. *E. coli* was isolated from 206 specimens and *Klebsiella* spp. from 138 specimens. Resistance to CTX was common in both *E. coli* and *Klebsiella* spp. at the randomization visit (52.2% and 37.7% respectively) and did not differ by study arm. *E. coli* isolates from CTX recipients at three and six months had 94.9% and 84.2% CTX resistance, as compared with 51.4% and 57.5% CTX resistance in isolates from placebo recipients ($p=0.01$). *Klebsiella* spp. isolates from CTX recipients had 79.0% and 68.8% CTX resistance at three and six months, as compared with 19.1% and 14.3% in isolates from placebo recipients ($p<0.01$).

Conclusions: HIV-exposed infants randomized to CTX prophylaxis had increased CTX-resistant commensal gastrointestinal bacteria compared with placebo recipients. Additional research is needed to determine the longer-term clinical, microbiologic, and public health consequences of antimicrobial resistance selected by infant CTX prophylaxis.

Keywords: HIV-exposed infants; cotrimoxazole prophylaxis; commensal organism resistance

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1 | INTRODUCTION

The World Health Organization (WHO) recommends cotrimoxazole (CTX) prophylaxis for HIV-exposed infants at risk of postnatal HIV transmission, continuing until six weeks after breastfeeding cessation (with confirmation of negative HIV-status) [1]. These guidelines were based upon evidence that CTX prophylaxis reduces mortality among HIV-infected infants and children in an era of high risk of mother-to-child HIV transmission (MTCT) and low availability of infant HIV DNA PCR testing [2–8]. However, increased maternal antiretroviral (ARV) access has reduced the annual number of new

paediatric HIV infections worldwide by 56% between 2010 and 2015 [9]. As early infant HIV testing programs are scaled-up and late transmission of HIV to breastfed infants is virtually eliminated with improved programmatic implementation of universal ARV treatment, the risk-benefit for CTX prophylaxis among HIV-exposed children requires reevaluation.

Among the few studies reporting antimicrobial drug resistance patterns among populations receiving CTX prophylaxis, most have focused on HIV-infected cohorts [6,10–12]. Where the population of interest has been HIV-exposed uninfected (HEU) infants or children, studies have focused predominantly on folate resistance mutations in *Plasmodium falciparum* [13,14]. Using data

from the Mpepu study, a Botswana-based trial in which HIV-exposed uninfected infants were randomized to CTX or placebo between 14 and 34 days of life through 15 months of life, we sought to quantify the emergence of antibiotic drug resistance in commensal enteric bacteria isolated from infant stool prior to and after dosing of CTX or placebo.

2 | METHODS

2.1 | Study population and monitoring

The Mpepu Study was a double blinded, randomized controlled trial investigating whether prophylactic CTX improved survival among HEU infants [15]. The governing Institutional Review Boards, Botswana Health Research and Development Committee and Harvard T. H. Chan School of Public Health, approved the study. Maternal participants provided written informed consent for their and their infants' participation.

HIV-exposed infants of HIV-infected women were eligible for enrolment from birth to 34 days-of-life. Mother-infant pairs were enrolled in Gaborone, Botswana's capital city, and in the village of Molepolole. Mothers received 3-drug ARV treatment, and infants received 30 days of ARV prophylaxis (per Botswana standard of care). Infants were randomized to receive CTX or placebo at 14 to 34 days of life through 15 months of age. Infants received 2.5 milliliters of CTX (sulfamethoxazole 100 mg/trimethoprim 20 mg) or placebo once daily from 14 to 34 days through six months of age, followed by five millilitres of CTX (sulfamethoxazole 200 mg/trimethoprim 40 mg) or placebo once daily until 15 months of age. CTX/placebo, packaged identically and with identical taste, was issued to the mother at each study visit. Mothers administered the liquid to their infants at home.

Study visits occurred at birth, randomization, 2, 3, 6, 9, 12, 15 and 18 months of life. At the enrolment and randomization visits, as well as any study visit where the infant was less than six weeks from breastfeeding cessation, infant HIV-1 testing was performed using qualitative polymerase-chain-reaction (PCR) DNA assay (Amplicor HIV-1, Roche Diagnostic Systems, New Jersey, USA). Prior to and at delivery, maternal CTX use was abstracted from maternal medical records. A consent modification, implemented on 8 May 2014, permitted collection of infant stool samples at the randomization (14 to 34 days of life), three- and six-month study visits.

2.2 | Processing of stool specimens

Mothers who consented for study participation after 7 May 2014 were contacted prior to the scheduled study visit and reminded to bring their infant's diaper containing stool to the study visit. Stool was preferentially collected in real-time if available or from a diaper if the infant produced stool within 24-hours prior to the study visit and the mother brought the diaper. For stool recovered from a diaper, the date and time of collection were obtained by maternal self-report and the home storage conditions (ambient air or refrigerated) were noted. Collected stool specimens were stored at -70°C .

To discern longitudinal patterns of resistance, all stool specimens obtained at three and six months of age were thawed and inoculated on MacConkey (MAST DM 140, without salt) agar plates for culture. If bacteria were cultured from three-

or six-month stool specimens and a specimen from the randomization visit was available, the randomization stool specimen, obtained prior to initiation of CTX or placebo, was also cultured. Lactose fermenting colonies were identified by biochemical tests and sub-cultured for purity. Antimicrobial susceptibility testing by Kirby Bauer disc diffusion method was done for CTX (25 μg) for the *Escherichia coli* (*E. coli*) and *Klebsiella* species (*Klebsiella* spp.) isolates and for Amoxicillin (10 μg) on *E. coli* isolates using Mueller Hinton (MAST) agar plates. Suspensions of *E. coli* or *Klebsiella* spp. isolates of 0.5 McFarland turbidity were spread on Mueller-Hinton Agar. Antibiotic discs were placed on Mueller-Hinton Agar and incubated at 37°C for 18 to 24 hours. Diameters of inhibition zones for cultured bacteria were interpreted according to Clinical Laboratory Standards Institute guidelines [16]. For CTX, a diameter of ≥ 16 mm was interpreted as susceptible, 11 to 15 mm as intermediate, and ≤ 10 mm as resistant. For Amoxicillin, a diameter ≥ 17 mm was interpreted as susceptible, 14 to 16 mm as intermediate, and ≤ 13 mm as resistant. Antibiograms were validated using standardized control strains of *E. coli* ATCC No. 25,922 (American Type Culture Collection). Microbiology laboratory staff members were blinded to infant randomized regimen.

2.3 | Statistical methods

SAS, version 9.3 (SAS Institute, Cary, North Carolina, USA) was used for statistical analyses. Maternal and infant characteristics were compared by maternal-infant pairs consented after 7 May 2014 based on provision of an infant stool sample or not and by randomization arm for infants with cultured stool samples. Wilcoxon Rank Sum Test was employed for comparison of continuous variables. Fisher's exact testing was used for comparison of non-continuous variables and to assess for significant differences in proportions of *E. coli* and *Klebsiella* spp. resistance by randomized arm. A sensitivity analysis was performed to assess for prevalence of antimicrobial drug resistance among the subset of infants who were not prescribed antibiotics in the first six months of life for acute illnesses. All testing used a significance level of ≤ 0.05 , with two-sided hypothesis testing.

3 | RESULTS

After stool collection was initiated on 7 May 2014, a total of 648 infants were enrolled in the Mpepu study. Almost all mothers of enrolled infants received 3-drug ARV treatment in pregnancy (96.7%), and none had documentation of CTX use during pregnancy.

Of these 648 infants, 220 (34%) provided at least one stool sample at the three- or six-month study visit. Median duration of infant ARV prophylaxis was slightly longer among infants who provided a stool sample compared with those who did not (31 days vs. 28 days; $p < 0.001$). In all other respects, infant characteristics were similar between those who provided a sample and those who did not. Among infants who provided a stool sample, 105 (47.7%) received CTX and 115 (52.3%) received placebo. Baseline characteristics of mothers and infants who provided a specimen did not differ by CTX/placebo arm (Table 1). Prescribing of antibiotics by

Table 1. Comparison of Maternal and Infant Characteristics by Infant Randomization Arm (CTX vs. Placebo) for Infants Providing a Stool Sample

Maternal/infant characteristics	Mothers of infants receiving CTX (n=105)	Mothers of infants receiving placebo (n=113) ^a	p-value
Median maternal age (years) [IQR]	32.5 [25.9 to 35.9]	32.5 [28.9 to 35.9]	0.67
Gravida including current pregnancy (#, %)			
One	21 (20.0%)	18 (15.9%)	0.64
Two	16 (15.2%)	20 (17.7%)	
Three	31 (29.5%)	28 (24.8%)	
Four or more	37 (35.3%)	47 (41.6%)	
Median enrolment CD4+ count (cells/ μ l) [IQR]	526 [383 to 667]	526 [350 to 675]	0.85
CD4+ count <200 cells/ μ l (#, %)	9 (9.1%)	9 (8.2%)	1.0
Maternal ARV regimen			
Triple ARVs initiated before delivery	60 (57.7%)	67 (59.8%)	0.93
Triple ARVs initiated in pregnancy	42 (40.4%)	42 (37.5%)	
No ARVs	2 (1.9%)	3 (2.7%)	
Enrolment site (#, %)			
Molepolole (village)	40 (38.1%)	45 (39.8%)	0.89
Gaborone (city)	65 (61.9%)	68 (60.2%)	
Marital status (#, %)			
Single	87 (82.9%)	98 (86.7%)	0.39
Married/cohabitating	18 (17.1%)	14 (12.4%)	
Widowed/divorced	0 (NA)	1 (0.9%)	
Education (#, %)			
None or primary	16 (15.2%)	11 (9.7%)	0.40
Secondary	78 (74.3%)	92 (81.4%)	
University	11 (10.5%)	10 (8.9%)	
Electricity in household (#, %)	66 (62.9%)	79 (69.9%)	0.32
Piped water in household (#, %)	16 (15.2%)	14 (12.4%)	0.56
Flush toilet in household (#, %)	19 (18.1%)	17 (15.0%)	0.59
Infant characteristics	CTX randomized infants (n=105)	Placebo randomized infants (n=115)	
Infant sex (#, %)			
Male	51 (48.6%)	54 (47.0%)	0.89
Female	54 (51.4%)	61 (53.0%)	
Median gestational age at delivery [IQR]	39 [37 to 40]	39 [37 to 40]	0.93
Mean anthropometric measures			
Birthweight (kg) [95% CI]			
Male infants	3.10 [2.79 to 3.30]	2.98 [2.52 to 3.30]	0.14
Female infants	2.89 [2.66 to 3.27]	2.93 [2.57 to 3.20]	0.68
Birth length (cm) [95% CI]			
Male infants	51 [49 to 52]	50 [49 to 52]	0.30
Female infants	50 [49 to 52]	49 [47 to 52]	0.22
Median # of days of ARV prophylaxis [IQR]	29 [27 to 31]	29 [27 to 30]	0.88
Breastfed (#, %)	21 (20.0%)	27 (23.5%)	0.62
Median age in days at 1st stool collection [IQR]	33 [15 to 97]	28 [14 to 96]	0.34

ARVs, antiretrovirals; CI, confidence interval; IQR, interquartile range.

^aTwo women gave birth to twins.

government health facilities was common, but did not differ by randomization arm; 26 (25%) of CTX recipients and 30 (26%) of placebo recipients ($p=0.88$) received an additional prescribed antibiotic in the first six months of life. The three most frequently prescribed antibiotics were amoxicillin, metronidazole, and erythromycin.

In total, 220 infants provided 381 stool samples, including 118 specimens from the randomization visit (prior to CTX or placebo initiation), 151 from the three-month visit, and 112 from the six-month visit. A total of 446 isolates were cultured from these 381 specimens, including 134 isolates from the randomization visit, 173 from the three-month visit, and 139 from the six-month visit.

There were 206 isolates of *E. coli* and 138 of *Klebsiella* spp. Culture yields of *E. coli* and *Klebsiella* spp. did not differ significantly at the three- and six-month study visits between infants receiving CTX or placebo ($p>0.35$) (Table 2). Among all infants with *E. coli* isolates, CTX resistance was common prior to randomization (between 2 and 4 weeks of life); 64.7% among CTX-randomized infants, and 60.7% among placebo-randomized infants ($p=1.0$) (Table 3A). By the three-month study visit, 94.9% of *E. coli* isolates from the CTX arm versus 51.4% from the placebo arm had CTX resistance ($p<0.0001$). At the six-month visit, 84.2% of *E. coli* isolates from the CTX arm versus 57.5% from the placebo arm had CTX resistance ($p=0.01$). Findings were similar among the subset of 164 (74.5%) infants who were not prescribed other antibiotics in the first six months of life. *E. coli* resistance to CTX was significantly higher in isolates of CTX recipients at three- and six-months, 93.9% and 84.0% respectively, versus 50.0% and 53.6% respectively among isolates of placebo recipients ($p=0.0004$ and 0.02 respectively) (Table 3B).

Escherichia coli sensitivity to amoxicillin was also evaluated. Prior to randomization, 58.8% of *E. coli* isolates from infants subsequently randomized to CTX were resistant to amoxicillin, compared with 71.4% from placebo recipients ($p=0.52$). Among *E. coli* isolates from the three-month visit, 79.5% of isolates in CTX recipients had *E. coli* resistance to amoxicillin compared with 51.4% among placebo recipients ($p=0.02$). At the six-month visit, amoxicillin resistance in *E. coli* isolates was 73.7% in the CTX arm versus 53.2% in the placebo arm ($p=0.07$).

Klebsiella spp. isolate resistance to CTX was 37.7% prior to randomization overall, and 40.6% among infants subsequently randomized to CTX versus 34.5% among placebo recipients ($p=0.79$). By three months, 79% of *Klebsiella* spp. isolates from CTX recipients were CTX resistant compared with 19.1% among placebo recipients ($p=0.0003$; Table 3A). This pattern persisted at six-months, with 68.8% of *Klebsiella* spp. isolates from the CTX arm being CTX resistant versus 14.3% from placebo recipients ($p=0.002$).

4 | DISCUSSION

Randomization to prophylactic CTX among HIV-exposed uninfected infants in Botswana was associated with early emergence and persistence of CTX-resistant *E. coli* and *Klebsiella* spp, and receipt of CTX was also associated with *E. coli* resistance to amoxicillin.

Our study was the first to evaluate emergence of commensal organism resistance among HIV-exposed uninfected infants receiving CTX prophylaxis. The high rate of baseline resistance to CTX among infants in the study was not surprising, as other studies have reported similar findings [7,17,18]. In a cohort of HIV-infected children in South Africa, antibiotic sensitivities of nasopharyngeal bacteria was assessed prior to randomization to once daily versus three times per week CTX prophylaxis [19]. Baseline CTX resistance was common, with CTX resistance found among 88% of the isolates, with over 80% of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Staphylococcus aureus* isolates demonstrating CTX resistance [19]. Similarly, the emergence of resistance following CTX receipt was high in our cohort. This finding is consistent with other studies from Africa involving HIV-infected individuals. In a Uganda-based CTX prophylaxis efficacy study, 76% of commensal bacterial isolates cultured from stool of HIV-infected children and adults had CTX resistance [20]. In a Zambian-based study of HIV-infected children and adults with chronic diarrhea, 75%, 97% and 100% of isolates of nontyphoidal salmonella, *Shigella flexneri* and *Shigella dysenteriae* were CTX resistant [21]. Finally, we observed increased resistance to Amoxicillin among CTX-exposed infants. This finding may indicate plasmid-mediated resistance to multiple antimicrobial agents [22–24], and raises concern that clinical implications from CTX exposure may extend to other antimicrobial agents as well.

Strengths of this analysis included the randomized design of the parent study, longitudinal follow-up, and data for other infant antibiotic use. We recognize that the study also has limitations. CTX/placebo was administered at home. While self-reported adherence to dosing was equally high in both arms, even if this reporting reflects desirability bias, poor adherence would have biased the results toward the null, which we did not observe. It was difficult to collect stool from infants, particularly at later visits, due to stooling frequency patterns. However, there was no significant difference in the proportion of infants who provided stool samples by randomization arm at the three- and six-months visits. Other researchers have described the progression of the gut microbiome in the first two years of life in cohorts of healthy children [25–27]. While the progression of microbiome may evolve differently among HEU children, or by feeding patterns, the randomized design of the Mpepu study ensured that differences between the infant CTX and placebo arm were unlikely. In our cohort, 25% of all infants

Table 2. Yield of *Escherichia coli* and *Klebsiella* species by infant randomization arm and visit from 446 isolates

Randomization arm	Pre-randomization	p-value ^a	Six months	p-value ^a	Six months	p-value ^a
<i>E. coli</i>						
CTX	17/60 (28%)	0.27	39/81 (48%)	0.36	38/60 (63%)	0.73
Placebo	28/74 (38%)		37/92 (40%)		47/79 (59%)	
<i>Klebsiella</i> spp.						
CTX	32/60 (53%)	0.12	19/81 (23%)	1.0	16/60 (27%)	1.0
Placebo	29/74 (39%)		21/92 (23%)		21/79 (27%)	

CTX, cotrimoxazole.

^ap-value from Fisher's exact test.

Table 3. *Escherichia coli* and *Klebsiella* species resistance prevalence by randomization arm and visit for (A) all infants and (B) for infants without other antibiotic use

Timing of stool specimen collection	<i>E. coli</i> resistance to CTX			<i>Klebsiella</i> spp. resistance to CTX		
	CTX recipients	Placebo recipients	p-value	CTX recipients	Placebo recipients	p-value
(A) All infants						
Randomization (14 to 34 days of life)	11/17 (64.7%)	17/28 (60.7%)	1.0	13/32 (40.6%)	10/29 (34.5%)	0.79
Three months	37/39 (94.9%)	19/37 (51.4%)	<0.0001	15/19 (79.0%)	4/21 (19.1%)	0.0003
Six months	32/38 (84.2%)	27/47 (57.5%)	0.01	11/16 (68.8%)	3/21 (14.3%)	0.002
(B) For infants without other antibiotic use						
Randomization (14 to 34 days of life)	10/16 (62.5%)	16/27 (59.3%)	1.0	12/31 (38.7%)	10/27 (37.0%)	1.0
Three months	26/28 (92.9%)	15/30 (50.0%)	0.0004	12/16 (75.0%)	3/16 (18.8%)	0.004
Six months	21/25 (84.0%)	15/28 (53.6%)	0.02	9/12 (75.0%)	2/14 (14.3%)	0.004

were prescribed at least one antibiotic in the first six month of life and this may have contributed to antimicrobial resistance. However, CTX prophylaxis remained significantly associated with resistance in the remaining 75% of infants who received no other prescribed antibiotics. While maternal use of antibiotics other than CTX during pregnancy and breastfeeding could contribute to the commensal bacterial resistance in an infant, we did not collect data on maternal antibiotic use, other than CTX use during pregnancy. However, in a sub-analysis of infants exclusively formula fed from birth, *E. coli* and *Klebsiella* spp, resistance to CTX remained significantly associated receipt of CTX (data not shown).

Additional concerns included the possibility that CTX might alter commensal bacterial load, affecting yield from the culture results. However, we did not find evidence of this, and it is noteworthy that the culture yield of *E. coli* and *Klebsiella* spp. did not vary significantly at the three timepoints when comparing randomization arms. We cannot exclude the possibility that CTX may have had a different impact on more pathogenic isolates, or among non-commensal pathogens. The risk for commensal organism resistance being transferred to pathogenic organisms, and the effectiveness of current antimicrobial treatment options for severe infections, were not assessed by this study but are of importance. Finally, while we did not find any association between commensal organism resistance and infant mortality (data not shown), this analysis was not powered to detect significant mortality differences by arm. Mortality in the Mpepu study was significantly lower than other studies involving HIV-exposed infants in Africa [28–31], including studies in Botswana [32].

Our resistance data provide additional information for policymakers when weighing the pros and cons of CTX prophylaxis for HIV-exposed infants with low MTCT risk. As countries successfully scale-up WHO programmatic recommendations and achieve MTCT reductions, fewer HIV-exposed infants will acquire HIV. Therefore, policies promoting CTX prophylaxis for HIV-exposed infants with continued HIV acquisition risk can likely be limited to settings where maternal ARV access is low; where infant follow-up testing is not routinely and frequently performed; and possibly to regions experiencing the combined burden of a generalized HIV epidemic

and high malarial transmission, given data suggesting morbidity and mortality reduction with infant CTX prophylaxis in such settings [14,20].

5 | CONCLUSIONS

HIV-exposed infants receiving CTX prophylaxis had higher rates of CTX drug resistance in the commensal bacterial of their stool at three and six months of life. In settings with well-functioning MTCT prevention programming and infant HIV testing, antimicrobial resistance considerations at the population level, and the potential for increased resistance occurring in pathogenic bacteria, should be factored into public health decisions related to the risks and benefits of infant CTX prophylaxis.

AUTHORS' AFFILIATIONS

¹Massachusetts General Hospital, Departments of Medicine and Pediatrics, Boston, MA, United States; ²Department of Immunology and Infectious Diseases, Harvard T. H. Chan School of Public Health, Boston, MA, United States; ³Botswana-Harvard AIDS Institute Partnership, Gaborone, Botswana; ⁴Faculty of Medicine, University of Botswana, Gaborone, Botswana; ⁵Brigham and Women's Hospital, Infectious Disease Division, Boston, MA, United States; ⁶Bennett Statistical Consulting, Inc., Ballston Lake, NY, United States; ⁷Goodtables Data Consulting, Norman, OK, United States; ⁸Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, MA, United States; ⁹Ministry of Health, Gaborone, Botswana

COMPETING INTERESTS

The authors have no competing interests to report.

AUTHORS' CONTRIBUTIONS

KMP and GA managed the Mpepu study. SS performed stool cultures and resistance analysis. JL and EvW provided data support and KB and MDH provided statistical support. KMP created the initial manuscript with support from SL and RLS. All authors reviewed and provided edits to the manuscript.

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REFERENCES

1. World Health Organization. Guidelines on co-trimoxazole prophylaxis for HIV-related infections among children, adolescents and adults - recommendations for a public health approach. 2006 [cited 2016 October 21]. Available from: <http://www.who.int/hiv/pub/guidelines/ctxguidelines.pdf?ua=1>.
2. World Health Organization, UNICEF. Towards Universal Access: Scaling priority HIV/AIDS interventions in the health sector. 2008 [cited 2016 October 21]. Available from: http://www.who.int/hiv/pub/towards_universal_access_report_2008.pdf?ua=1.
3. UNAIDS. Report on the Global HIV/AIDS Epidemic. 2008 [cited 2016 October 23]. Available from: http://www.unaids.org/sites/default/files/media_asset/jc1510_2008globalreport_en_0.pdf.
4. Baggaley R, Hensen B, Ajose O, Grabbe KL, Wong VJ, Schilsky A, et al. From caution to urgency: the evolution of HIV testing and counselling in Africa. *Bull World Health Organ*. 2012;90(9):652–658B.
5. I. Children and AIDS - Third Stocktaking Report, 2008. 2008 [cited 2016 October 23]. Available from: http://www.unicef.org/publications/files/CATS_R_EN_11202008.pdf.
6. Mulenga V, Ford D, Walker AS, Mwenya D, Mwansa J, Sinyinza F, et al. Effect of cotrimoxazole on causes of death, hospital admissions and antibiotic use in HIV-infected children. *AIDS*. 2007;21(1):77–84.
7. Chintu C, Bhat GJ, Walker AS, Mulenga V, Sinyinza F, Lishimpi K, et al. Cotrimoxazole as prophylaxis against opportunistic infections in HIV-infected Zambian children (CHAP): a double-blind randomised placebo-controlled trial. *Lancet*. 2004;364(9448):1865–71.
8. Crook AM, Turkova A, Musiime V, Bwakura-Dangarembizi M, Bakeera-Kitaka S, Nahirya-Ntege P, et al. Tuberculosis incidence is high in HIV-infected African children but is reduced by co-trimoxazole and time on antiretroviral therapy. *BMC Med*. 2016;14:50.
9. UNAIDS. Prevention Gap Report. 2016. Available from: http://www.unaids.org/sites/default/files/media_asset/2016-prevention-gap-report_en.pdf.
10. Munier AL, de Lastours V, Varon E, Donay JL, Porcher R, Molina JM, et al. Invasive pneumococcal disease in HIV-infected adults in France from 2000 to 2011: antimicrobial susceptibility and implication of serotypes for vaccination. *Infection*. 2013;41(3):663–8.
11. Anglaret X, Chêne G, Attia A, Toure S, Lafont S, Combe P, et al. Early chemoprophylaxis with trimethoprim-sulphamethoxazole for HIV-1-infected adults in Abidjan, Cote d'Ivoire: a randomised trial. *Cotrimo-CI Study Group*. *Lancet*. 1999;353(9163):1463–8.
12. Nunn AJ, Mwaba P, Chintu C, Mwinga A, Darbyshire JH, Zumla A, et al. Role of co-trimoxazole prophylaxis in reducing mortality in HIV infected adults being treated for tuberculosis: randomised clinical trial. *BMJ*. 2008;337: a257.
13. Davis NL, Barnett EJ, Miller WC, Dow A, Chasela CS, et al. Impact of daily cotrimoxazole on clinical malaria and asymptomatic parasitemias in HIV-exposed, uninfected infants. *Clin Infect Dis*. 2015;61(3):368–74.
14. Mbeye NM, ter Kuile FO, Davies MA, Phiri KS, Egger M, et al. Cotrimoxazole prophylactic treatment prevents malaria in children in sub-Saharan Africa: systematic review and meta-analysis. *Trop Med Int Health*. 2014;19(9):1057–67.
15. Shapiro RL, Hughes M, Powis K, Ajibola G, Bennett K, Moyo S, et al. Similar mortality with cotrimoxazole vs placebo in HIV-exposed uninfected children. In: 2016 Conference on Retroviruses and Opportunistic Infections. Boston, MA; 2016.
16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. Wayne, PA: Clinical and Laboratory Standards Institute; 2015. CLSI document M100-S25 (ISBN 1-56238-989-0 [Print]; ISBN 1-56238-990-4 [Electronic]).
17. Shapiro RL, Kumar L, Phillips-Howard P, Wells JG, Adcock P, et al. Antimicrobial-resistant bacterial diarrhea in rural western Kenya. *J Infect Dis*. 2001;183(11):1701–4.
18. Hamel MJ, Greene C, Chiller T, Ouma P, Polyak C, et al. Does cotrimoxazole prophylaxis for the prevention of HIV-associated opportunistic infections select for resistant pathogens in Kenyan adults? *Am J Trop Med Hyg*. 2008;79(3):320–30.
19. Cotton MF, Wasserman E, Smit J, Whitelaw A, Zar HJ. High incidence of antimicrobial resistant organisms including extended spectrum beta-lactamase producing Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* in nasopharyngeal and blood isolates of HIV-infected children from Cape Town, South Africa. *BMC Infect Dis*. 2008;8:40.
20. Mermin J, Lule J, Ekwaru JP, Malamba S, Downing R, Ransom R, et al. Effect of co-trimoxazole prophylaxis on morbidity, mortality, CD4-cell count, and viral load in HIV infection in rural Uganda. *Lancet*. 2004;364(9443):1428–34.
21. Mwansa J, Mutela K, Zulu I, Amadi B, Kelly P. Antimicrobial sensitivity in enterobacteria from AIDS patients, Zambia. *Emerg Infect Dis*. 2002;8(1):92–3.
22. Uma B, Prabhakar K, Rajendran S, Kavitha K, Sarayu YL. Antibiotic sensitivity and plasmid profiles of *Escherichia coli* isolated from pediatric diarrhea. *J Glob Infect Dis*. 2009;1(2):107–10.
23. Akingbade O, Balogun S, Ojo D, Akinduti P, Okerentugba PO, Nwanze JC, et al. Resistant plasmid profile analysis of multidrug resistant *Escherichia coli* isolated from urinary tract infections in Abeokuta, Nigeria. *Afr Health Sci*. 2014;14(4):821–8.
24. Osterblad M, Hakanen A, Manninen R, Leisteuvo T, Peltonen R, et al. A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. *Antimicrob Agents Chemother*. 2000;44(6):1479–84.
25. Walker WA. Initial intestinal colonization in the human infant and immune homeostasis. *Ann Nutr Metab*. 2013;63 Suppl 2:8–15.
26. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science*. 2016;352(6285):539–44.
27. Sommer F, Backhed F. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol*. 2013;11(4):227–38.
28. Brahmabhatt H, Kigozi G, Wabwire-Mangen F, Serwadda D, Lutalo T, Nalugoda F, et al. Mortality in HIV-infected and uninfected children of HIV-infected and uninfected mothers in rural Uganda. *J Acquir Immune Defic Syndr*. 2006;41(4):504–8.
29. Chilongozi D, Wang L, Brown L, Taha T, Valentine M, Emel L, et al. Morbidity and mortality among a cohort of human immunodeficiency virus type 1-infected and uninfected pregnant women and their infants from Malawi, Zambia, and Tanzania. *Pediatr Infect Dis J*. 2008;27(9):808–14.
30. Marinda E, Humphrey JH, Iliff PJ, Mutasa K, Nathoo KJ, et al. Child mortality according to maternal and infant HIV status in Zimbabwe. *Pediatr Infect Dis J*. 2007;26(6):519–26.
31. Newell ML, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F, et al. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet*. 2004;364(9441):1236–43.
32. Shapiro RL, Lockman S, Kim S, Smeaton L, Rahkola JT, et al. Infant morbidity, mortality, and breast milk immunologic profiles among breast-feeding HIV-infected and HIV-uninfected women in Botswana. *J Infect Dis*. 2007;196(4):562–9.