Clinical Infectious Diseases Editorial Commentary

Cotrimoxazole prophylaxis selects for antimicrobial resistance in HIV-exposed uninfected infants

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Rates of mother-to-child transmission of HIV are falling globally with an estimated 200,000 child infections averted in 2018 thanks to implementation of prevention of mother-to-child-transmission (PMTCT) interventions[1]. As a result, fewer children are living with HIV and a growing number of children born to mothers living with HIV remain uninfected. However,

children who are HIV-exposed but uninfected (HEU) continue to have higher all-cause mortality than HIV-unexposed infants[2], primarily due to greater risk and severity of common childhood infections[3]. As an expanding, clinically distinct group of children, a greater understanding of why HEU fail to survive and thrive to the extent of HIV-unexposed children is warranted[3-5]. Optimal interventions, particularly those to reduce infectious morbidity and mortality, may well deviate from those developed for children living with HIV or for those who are HIV-unexposed[5].

Antimicrobial prophylaxis guidelines for HIV-exposed infants were developed prior to the wide availability of antiretroviral therapy (ART), when mother-to-child transmission rates, and subsequent infant infectious mortality, were high [6, 7]. In this context, treating HEU in the same way as HIV-positive infants was justified because ongoing exposure to HIV *via* breastfeeding and the challenges of infant HIV testing posed a risk that initially uninfected infants who subsequently acquired HIV would miss out on life-saving treatment[13]. The World Health Organization (WHO) recommends that all HIV-exposed infants receive prophylactic cotrimoxazole (CTX) from 4-6 weeks' of age[7]; CTX is continued long-term for HIV-positive children living in regions with high prevalence of severe bacterial infections and malaria, and only discontinued for HEU upon cessation of breastfeeding and conclusive determination of their HIV-negative status[7]. CTX is a broad-spectrum antibiotic made up of two folate synthesis inhibitors (trimethoprim and sulfamethoxazole) with activity against *Pneumocystis jirovecii* and a range of other bacterial, fungal and *Plasmodium* species[8]. There is strong evidence that CTX significantly reduces infectious morbidity and mortality[8], and our group[9] and others[10] have recently shown that long-term continuation of CTX reduces systemic and intestinal levels of inflammatory mediators associated with poor clinical outcomes for children and adults living with HIV. The impact of CTX on the health of HEU is far less well supported. Although morbidity and mortality reductions have been observed among HEU infants living in malaria-endemic regions[11], two recent randomised

controlled trials in non-malarial regions show that CTX did not improve 18-month survival[12], 12-month survival[13] or incidence of severe pneumonia or diarrhoea[13] among HEU.

Without strong evidence for a clinical benefit, recommendations to provide CTX prophylaxis to all HEU have been called into question[14, 15], particularly in light of substantial improvements in PMTCT coverage and early infant diagnosis of HIV since treatment guidelines were developed. There are rising concerns that CTX may select for antimicrobial resistance (AMR)[12, 16-18], which is already very high among CTX-targeted pathogens in many LMIC[8]. Furthermore, there is a growing understanding of how resident microbial populations (the microbiome) contribute to child development and long-term health[19], which may be disrupted by early exposure to antibiotics. Exposure to maternal HIV is associated with a distinct gut microbiome amongst HEU relative to HIV-unexposed infants[20, 21], but little is known about how this contributes to their clinical phenotype. Contrary to expectations that CTX might reduce diversity of the gut microbiome through its broad antimicrobial activity, recent studies have not identified global difference in the gut microbiome after long-term CTX treatment of HIV-positive ART-treated children[9] or after short-term treatment of a mixed population of HIV-positive and HIV-negative children[22]. At a species level, our group has shown that CTX specifically suppressed gut-resident *Streptococci* associated with intestinal inflammation among HIV-positive children who continued treatment for 84 weeks, but this was not evident for global taxa[9]. The impact of CTX on HEU microbiomes has not been previously assessed.

In this issue of *Clinical Infectious Diseases*, D'Souza and colleagues provide the first evaluation of how CTX affects the gut microbiomes and AMR gene carriage of HEU during their first year of life. The sub-study is nested within a recently completed randomised

controlled trial among HEU in South Africa, which showed that no CTX (N=609) was not inferior to CTX prophylaxis administered according to WHO guidelines (N=611) for the prevention of severe childhood illnesses or death in an urban setting without endemic malaria transmission, low incidence of pneumonia and diarrhoea, and good coverage of PMTCT and infant HIV testing[13]. Using stool samples from a small subset of trial participants, this sub-study identifies a convincing signature of AMR selection over time amongst children who received CTX (N=34) relative to untreated controls (N=29), informing on a pertinent concern around the use of prophylactic antibiotics in LMIC. Despite global stability in the variety of genes associated with microbial taxa and their functions within individuals (α-diversity) in both CTX-treated and untreated groups of HEU, the abundance and α-diversity of trimethoprim- and sulphonamide-resistance genes increased with time only in the CTX-treated group. In parallel, variation between individuals in genes associated with microbial taxa, function and AMR (β-diversity) decreased amongst children receiving CTX, suggesting that prophylaxis exerted an ongoing selection pressure on the microbiome with continued use. These more detailed assessments are consistent with observations from the trial of CTX among HEU in Botswana, which reported a higher proportion of CTX-resistant *Escherichia coli* isolates cultured from stool in CTX-treated versus untreated HEU post-randomisation[12].

A strength of the study is its randomised design and longitudinal follow-up (stool was sampled at 6 weeks, 4 months and 6 months), which allowed for a causal role of CTX to be disaggregated from potential confounding factors known to shape microbiome diversity. Few studies have been able to assess the early effects of CTX on the microbiome of HEU or infants living with HIV since CTX is standard of care for both groups[7] and microbial diversity changes with age, particularly in young infants in whom colonisation is ongoing[19]. An additional advantage of this study over previous investigations of the gut microbiome of HEU[20, 21], is the use of shotgun metagenomics rather than targeted 16S sequencing or

culture-based antimicrobial resistance testing. This metagenomics approach sequences all microbial genomes present in a sample rather than a subset of pre-selected species or marker genes[23], allowing for a less biased and more accurate assessment of microbial taxa, function and AMR gene carriage[23]. Whilst sequencing read-depth in this study did not allow for confident resolution of potential CTX-driven changes at a species- or strain-level, the lack of global differences in α-diversity identified at a taxa-level goes some way to allay concerns that CTX drives dysbiosis among HEU.

This sub-study excluded infants who did not provide stool samples at all three timepoints and 15% of infants were lost to follow-up in the wider trial[13], meaning that it likely focuses on children with more favourable clinical outcomes. The study is also insufficiently powered to draw conclusions on the clinical importance of AMR selection by CTX. Thus, selection for AMR genes is insufficient evidence in isolation to change current guidelines on CTX use. Indeed, CTX is already known to select for AMR in HIV-positive cohorts[8] and, due to its broad-spectrum activity, appears to do so more rapidly than more restricted-spectrum antibiotics[24]. However, the beneficial effects of CTX prophylaxis on morbidity, mortality and inflammation persist with long-term use among children and adults living with HIV despite high resistance rates[6, 9, 25, 26]. Concerns around AMR become more critical in the context of the wider trial in which this sub-study was nested, which found similar clinical outcomes amongst those randomised to take versus not take CTX prophylaxis[13]. Therefore, the risk of driving AMR carriage is not being off-set by a clinical benefit for HEU in this context. However, calls to change treatment guidelines for HEU on the basis of the evidence that CTX selects for AMR are dependent on two further as yet untested assumptions: (1) AMR genes will persist long-term after discontinuation of CTX; and, (2) AMR gene carriage will compromise clinical management of subsequent infections. Both concerns require validation in future studies to provide proof of harm. Another important consideration is that maternal CTX use may have shaped infant microbiomes prior to

randomisation *via* vertical transmission of AMR genes and/or direct exposure of infants to CTX in breastmilk. D'Souza and colleagues do not report on maternal CTX treatment and it remains plausible that the most profound effects of CTX on the infant microbiome and resistome occur in response to initial CTX exposure from their mothers; were this to be the case, changing treatment guidelines for HEU infants would not avert AMR risk.

Current guidelines recommending CTX prophylaxis for HIV-exposed infants are almost exclusively due to the clinical benefits for HIV-positive children with limited evidence for a benefit for children who remain uninfected in settings where malaria is non-endemic[7]. Minimised vertical transmission and early and ongoing infant HIV testing reflects an ideal scenario for the clinical care of HIV-exposed infants, but cannot be considered the norm in many LMIC[7]. Therefore, a context-sensitive review of the relative risks and benefits of existing treatment guidelines for HEU is timely. D'Souza and colleagues provide the first direct evidence for AMR selection by CTX treatment of HEU in infancy using unbiased metagenomics, adding empirical evidence for why global antibiotic prophylaxis should be avoided where more targeted interventions are possible. Population-level AMR is a particularly critical consideration in the six countries where >10% of all children are HEU (eSwatini [32.4%], Botswana [27.4%], South Africa [21.6%], Lesotho [21.1%], Namibia [16.4%] and Zimbabwe [13.6%])[27]. Earlier and ongoing testing for HIV during infancy are already recommended[7]; wider implementation of such guidelines would enable CTX prophylaxis to be provided in a more targeted way, maintaining the essential protection against infections necessary for children living with HIV whilst avoiding unnecessarily selecting for AMR among HEU.

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