Co-trimoxazole is an inexpensive, broad-spectrum antimicrobial drug that is widely used in developing countries. Before antiretroviral therapy (ART) scale-up, co-trimoxazole prophylaxis reduced morbidity and mortality in adults and children with HIV by preventing bacterial infections, diarrhoea, malaria, and Pneumocystis jirovecii pneumonia, despite high levels of microbial resistance. Co-trimoxazole prophylaxis reduces early mortality by 58% (95% CI 39–71) in adults starting ART. Co-trimoxazole provides ongoing protection against malaria and non-malaria infections after immune reconstitution in ART-treated individuals in sub-Saharan Africa, leading to a change in WHO guidelines, which now recommend long-term co-trimoxazole prophylaxis for adults and children in settings with a high prevalence of malaria or severe bacterial infections. Co-trimoxazole prophylaxis is recommended for HIV-exposed infants from age 4–6 weeks; however, the risks and benefits of co-trimoxazole during infancy are unclear. Co-trimoxazole prophylaxis reduces anaemia and improves growth in children with HIV, possibly by reducing inflammation, either through direct immunomodulatory activity or through effects on the intestinal microbiota leading to reduced microbial translocation. Ongoing trials are now assessing the ability of adjunctive co-trimoxazole to reduce mortality in children after severe anaemia or severe acute malnutrition. In this Review, we discuss the mechanisms of action, benefits and risks, and clinical trials of co-trimoxazole in developing countries.

Introduction
Since the 1960s, a fixed-dose combination of trimethoprim-sulfamethoxazole (co-trimoxazole) has been used as a broad-spectrum antibiotic in many settings.1 After its initial use as empirical therapy for urinary and lower respiratory tract infections, it was shown to also have useful prophylactic activity against Pneumocystis carinii (now P jirovecii) pneumonia in patients receiving chemotherapy.2,3 Use of co-trimoxazole increased greatly from the 1980s during the HIV epidemic, because it provided protection against various opportunistic infections, and reduced mortality and morbidity in the pre-antiretroviral therapy (ART) era.4,5 However, even with the advent of effective ART regimens, co-trimoxazole continues to provide important benefits for individuals with HIV, particularly in developing countries. Co-trimoxazole is a unique compound that combines broad antimicrobial activity with immunomodulatory properties. Furthermore, it is well tolerated, widely available, off-patent, and inexpensive, which makes it ideal for use in low-income settings. This Review focuses on historical evidence and clinical trials that emphasise the expanding role of co-trimoxazole in developing countries.

Antimicrobial properties of co-trimoxazole
Trimethoprim and sulfamethoxazole synergistically block microbial synthesis of folic acid, a vital cofactor in the manufacture of thymidine and purines. Sulfamethoxazole is a competitive inhibitor of dihydrofolic acid synthesis, and trimethoprim acts downstream of sulfamethoxazole to inhibit production of the physiologically active tetrahydrofolic acid. Although each agent alone is bacteriostatic, their blockade of two sequential enzymes results in bactericidal activity when combined.19 Furthermore, the combination of two antimicrobials was postulated to decrease the emergence of resistance, although in certain circumstances (such as the treatment of urinary tract infections), trimethoprim is the more active component.13 Although there is now high antimicrobial resistance in many settings, co-trimoxazole has intrinsic activity against a broad spectrum of bacterial, fungal, and protozoal pathogens11 (panel 1).

Co-trimoxazole in HIV infection
Co-trimoxazole in the pre-ART era
In developed countries, co-trimoxazole prophylaxis reduced mortality in adults and children with HIV in the pre-ART era, predominantly by preventing Pneumocystis jirovecii pneumonia.14,15 Before the widespread scale-up of ART, co-trimoxazole was similarly shown to reduce morbidity and mortality in adults and children with HIV in sub-Saharan Africa.6,16–18 ‘The benefits of co-trimoxazole in this setting were due to substantial reductions in several infections, not only P jirovecii pneumonia.16–20 Children with HIV Antibiotic Prophylaxis (CHAP) was a double-blind, randomised, controlled trial enrolling Zambian children aged more than 12 months to either co-trimoxazole or placebo, before the availability of ART. The hazard ratio (HR) for death after a median of 19 months was 0.57 (95% CI 0.43–0.77; p=0.0002) for co-trimoxazole versus placebo, and hospital admission rates were reduced by 23%, irrespective of age or baseline CD4 cell count (69% of children had CD4 cell count <15% of the total lymphocyte count). Most of the excess deaths in the placebo group were from pneumonia (mostly diagnosed presumpitively), and antibiotic prescription rates were significantly higher, which suggests that co-trimoxazole has protective benefits against bacterial infections in sub-Saharan Africa.20

Prophylaxis against P jirovecii pneumonia is important in developing countries, although initial reports showed that this infection was uncommon in sub-Saharan African adults.20–22 The risk of P jirovecii pneumonia is related to age, and infants with HIV are especially vulnerable, irrespective of CD4 cell count. In a Zambian descriptive necropsy study of 264 children in hospital who died of a respiratory illness before ART, 76 (51%)...
infants with HIV aged less than 6 months had evidence of *P jirovecii* pneumonia infection. Where diagnostic facilities were available, *P jirovecii* was found in nearly 10% of children who had not been treated with ART (median age 9 months; range 3–23) who were admitted to hospital with pneumonia in South Africa. In another South African study in which both nasopharyngeal aspirates and induced sputum were collected from 105 children aged less than 2 years who had not been treated with ART, and who were admitted to hospital for a respiratory disease, *P jirovecii* pneumonia was found in 48.6%, with higher rates in those not receiving adequate prophylaxis.

Before ART availability, co-trimoxazole prophylaxis was therefore a highly cost-effective intervention for both adults and children in developing countries, particularly when started early rather than late in disease progression.

**Co-trimoxazole in the ART era**
The role of co-trimoxazole in the ART era seems to differ between settings. In developed countries, where infectious disease prevalence is lower and immunisation rates are higher than in developing countries, co-trimoxazole is predominantly used to prevent opportunistic infections in patients with advanced disease. Data from observational studies and small trials suggest that co-trimoxazole can safely be stopped after immune reconstitution with ART. In a randomised trial of co-trimoxazole cessation in 60 Spanish adults taking ART, there were no episodes of *P jirovecii* pneumonia in those discontinuing co-trimoxazole. Similarly, in a European observational cohort of 325 adults stopping co-trimoxazole prophylaxis at a CD4 cell count of at least 350 cells per μL, there were no episodes of *P jirovecii* pneumonia over a median 13 months of follow-up, and in a further European study, there were no cases of toxoplasmosis after discontinuation of co-trimoxazole.

In a randomised trial of co-trimoxazole cessation in ART-treated Thai adults with virological suppression but incomplete immune recovery (CD4 cell count <200 cells per μL), only one of 37 patients discontinuing prophylaxis developed *P jirovecii* pneumonia, leading the investigators to conclude that even in this middle-income setting, discontinuation of co-trimoxazole was safe.

In children, the US-based PACTG 1008 trial assessed the discontinuation of co-trimoxazole prophylaxis after immune reconstitution (CD4 cell count ≥20% total lymphocyte count for age >6 years; or CD4 cell count ≥25% for age 2–6 years). There were no occurrences of *P jirovecii* pneumonia during 347 person-years of follow-up, and although a control group was not assessed, the background rate in children without HIV, and similar to the historic incidence in children with HIV, was 1.5%.

A retrospective analysis of cohorts from the Paediatric European Network for the Treatment of AIDS showed no episodes of *P jirovecii* pneumonia in 335 patient-years after discontinuation of co-trimoxazole in children with adequate immune reconstitution. Paediatric guidelines from the USA and Europe therefore recommend stopping of co-trimoxazole prophylaxis when an adequate

### Panel 1: Organisms susceptible to co-trimoxazole

**Gram-negative bacteria**
- *Escherichia coli*
- *Salmonella Typhi*
- *Salmonella Typhimurium*
- *Salmonella Paratyphi*
- *Non-typhi salmonella*
- *Shigella spp*
- *Haemophilus influenzae*
- *Neisseria meningitidis*
- *Vibrio cholerae*
- *Yersinia enterocolitica*
- *Klebsiella spp*
- *Achromobacter spp*
- *Acinetobacter spp*
- *Aeromonas hydraphila*
- *Alcaligenes spp*
- *Brucella spp*
- *Burkholderia spp*
- *Coxiella burnetii*
- *Proteus mirabilis*
- *Stenotrophomonas maltophilia*
- *Tropheryma whipplei*

**Gram-positive bacteria**
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*
- *Group B Streptococcus*
- *Staphylococcus aureus*
- *Meticillin-resistant staphylococcus aureus (MRSA)*
- *Staphylococcus epidermidis*
- *Listeria monocytogenes*
- *Actinomyces spp*
- *Nocardia spp*

**Mycobacteria**
- *Mycobacterium tuberculosis*
- *Mycobacterium fortuitum*
- *Mycobacterium marinum*
- *Mycobacterium kansasii*

**Protozoa**
- *Plasmodium falciparum*
- *Toxoplasma gondii*
- *Acanthamoeba spp*
- *Cyclospora spp*
- *Isospora belli*

**Fungi**
- *Pneumocystis jirovecii*
- *Paracoccidioides brasiliensis*
CD4 cell count is achieved after at least 6 months of ART.35,36 In developing countries, provision of ART has increased by 20 times since 2003; by the end of 2012, around 9·7 million people in these countries were receiving treatment.35 A meta-analysis of observational studies reported a 58% (95% CI 39–71%) reduction in early mortality in adults starting ART with co-trimoxazole prophylaxis at CD4 cell counts of 350 cells per μL or less.35,36 An analysis of expanding co-trimoxazole coverage in developing countries estimated it to be highly cost effective in the first 6 months of ART, at US$146–91 per death averted.39 Co-trimoxazole prophylaxis also has benefits in adults with less advanced immunodeficiency, reducing WHO stage 3 and 4 disease, severe bacterial infections, and admissions to hospital in those with CD4 cell counts of 350 cells per μL or more.35,36

One important benefit for people living in developing countries is the antimalarial protection provided by co-trimoxazole. In two prospective cohorts of adults with HIV in Uganda, co-trimoxazole prophylaxis, ART, and bednets were sequentially introduced over a 4-year period.32 Malaria incidence substantially reduced after the introduction of co-trimoxazole alone (from 50·8 to 9·0 episodes per 100 person-years); the subsequent addition of ART and bednets further reduced the incidence of malaria to 3·5 and 2·1 episodes per 100 person-years, respectively. In an observational substudy of the DART trial,40 co-trimoxazole prophylaxis was associated with a 26% reduction of new malarial episodes in current users, which was maintained throughout follow-up (median 4·9 years). In adults with HIV with high CD4 cell counts (≥350 cells per μL), co-trimoxazole prophylaxis has similarly been associated with reductions in malaria in Côte d’Ivoire and Uganda.35,36

Whether co-trimoxazole prophylaxis can safely be stopped in adults receiving ART has been investigated in several studies. A randomised trial of co-trimoxazole cessation in 836 ART-treated Ugandan adults with CD4 cell counts of more than 200 cells per μL was discontinued after only 4 months because of a significantly increased incidence of diarrhoea (incidence rate ratio [IRR] 1·8, 95% CI 1·3–2·4; p<0·001) and malaria (IRR 32·5, 95% CI 8·6–127·5; p<0·001) in those who stopped treatment; a post-hoc analysis also showed increased respiratory tract infections in the group who stopped treatment.35 The early study termination after these results precluded finding out the long-term effects of stopping co-trimoxazole, and raised the possibility that increased infections might have been transient rebound effects.35 The duration of protection provided by co-trimoxazole after ART initiation was also difficult to establish in the meta-analysis of adult studies, because most had less than 1 year of follow-up.35 However the DART trial,40 which had the longest follow-up, showed a survival benefit of co-trimoxazole for the first 72 weeks of ART in a non-randomised analysis, with no effect on mortality thereafter; the benefits of co-trimoxazole were noted across a range of CD4 cell counts.40 A trial of co-trimoxazole cessation in a malaria-endemic region of western Kenya,41 in 500 adults with HIV receiving ART for a median of 4·5 years, showed increased morbidity driven entirely by malaria, but no significant differences in pneumonia, diarrhoea, or mortality, between those stopping versus those continuing co-trimoxazole. An ongoing study in Uganda (COSTOP; ISRCTN44723643) is also investigating co-trimoxazole cessation in adults with HIV receiving ART. Therefore, although the duration of protection provided by co-trimoxazole is uncertain, co-trimoxazole seems to provide ongoing benefits to adults receiving long-term ART, despite immune reconstitution, in settings with a high incidence of malaria and bacterial infections.

Children in developing countries receiving long-term ART also benefit from co-trimoxazole prophylaxis. In the open-label Antiretroviral Research for Watoto (ARROW) trial,758 Ugandan and Zimbabwean children aged more than 3 years, with good CD4 cell counts after a median 2·1 years receiving ART, were randomly assigned to stop or continue co-trimoxazole. The rates of admission to hospital or death were significantly higher in the stop versus continue group (hazard ratio [HR] 1·64, 95% CI 1·14–2·37; p=0·007), because of malaria and other infections (predominantly pneumonia, sepsis, and meningitis), in both Uganda (malaria endemic) and Zimbabwe (not malaria endemic). This effect occurred irrespective of age or CD4 cell count, which emphasises the protection that co-trimoxazole provides against severe bacterial infections even at high CD4 cell counts, by contrast with developed countries.44 Children in ARROW had sustained protection provided by co-trimoxazole for more than 2 years of follow-up, with no evidence of attenuation over time. Co-trimoxazole also lowered costs, mainly because of reduced hospital admissions.8

Tolerability of co-trimoxazole

In the DART trial,8 during 8128 person-years receiving co-trimoxazole, only 3% of serious adverse events were potentially attributed to this drug. Additionally, prophylaxis with co-trimoxazole was well tolerated in children not receiving ART in the CHAP trial; although neutrophil counts declined in those taking co-trimoxazole, the rate of grade 3 or 4 adverse events (including neutropenia) was similar and very low between the treatment group and the placebo group. Only one child (in the placebo group) developed grade 3 rash necessitating drug discontinuation in CHAP. In the ARROW trial, although there was a significant increase in both the white cell count and neutrophils in the stop versus continue group, only one child (in the stop group) had a grade 3 or 4 adverse event that was judged to be potentially related to co-trimoxazole, and definitely related to zidovudine.40 However, neutropenia was reported in adults initiating ART in Côte d’Ivoire. 80% of whom were receiving co-trimoxazole. 118 of 498 adults developed grade 3 or 4 neutropenia, of whom 86 stopped
Review

co-trimoxazole because of persistent neutropenia. Co-trimoxazole has overlapping side-effects with other drugs that are commonly prescribed for individuals with HIV. For example, bone marrow suppression can be caused by antiretroviral drugs such as zidovudine; skin rash (and even Stevens-Johnson syndrome) can occur with non-nucleoside reverse transcriptase inhibitors such as nevirapine; and hepatotoxic effects can occur with antituberculous drugs such as rifampicin and isoniazid. Even within a clinical trial, it can be difficult to ascertain which drug is causative, and a staged approach to drug cessation is often needed.

**Co-trimoxazole in women with HIV and exposed infants**

Co-trimoxazole prophylaxis has important benefits for pregnant and breastfeeding women with HIV, and their infants. Trimethoprim crosses the placenta and enters breast milk at a concentration equal to the concentration in maternal plasma. Sulfamethoxazole partly crosses into these compartments, meaning that fetuses and infants are substantially exposed to co-trimoxazole during pregnancy and breastfeeding. Although there have been concerns about the risks of folate antagonists to a developing fetus, a systematic review undertaken by WHO did not find evidence of increased teratogenicity in the children of pregnant women taking co-trimoxazole, although most studies were of low quality and further prospective surveillance is needed.

Antenatal co-trimoxazole in HIV-infected pregnant women with low CD4 cell counts (<200 cells per μL) was associated with less preterm delivery, reduced neonatal mortality, and a trend towards improved birthweight compared with pregnant women with HIV not taking co-trimoxazole. In a double-blind, placebo-controlled trial, postpartum co-trimoxazole prophylaxis for breastfeeding Zambian women with HIV had no effect on maternal mortality or hospital admissions, although interpretation was limited by a high rate of loss-to follow-up; however, exploratory analyses showed a trend towards reduced infant mortality in the maternal intervention group (death rate 19.5 with co-trimoxazole [95% CI 12.9–29.3] versus 34.5 with placebo [95% CI 24.4–48.8] per 100 child-years; p=0.1).  

In geographical areas with moderate-to-high malaria transmission, WHO recommends intermittent, preventive treatment with antimalarial drugs in pregnancy (IPTp). HIV infection almost doubles the risk of placental malaria, but daily co-trimoxazole prophylaxis reduces this risk and can be used instead of IPTp in pregnant women with HIV. Several randomised trials in western and southern Africa and an observational study in Malawi have shown that co-trimoxazole is non-inferior to IPTp in terms of birth outcomes in women with HIV.

Since 2000, WHO has recommended co-trimoxazole prophylaxis for all HIV-exposed infants because of frequent delays in early infant diagnosis and the high risk of *P jirovecii* pneumonia in infected infants. Co-trimoxazole should be started at age 4–6 weeks and continued until HIV infection has been excluded and the child is no longer at risk of infection. Because of an increase in prevention of mother-to-child transmission (PMTCT) programmes, the number of HIV-exposed uninfected (HEU) infants is increasing. HEU infants have increased morbidity and mortality compared with unexposed infants, including susceptibility to *P jirovecii* pneumonia. To investigate the possible protective effects of co-trimoxazole, 185 Ugandan HEU infants, enrolled aged between 6 weeks and 9 months, were randomly assigned upon cessation of breastfeeding to either stop or continue co-trimoxazole until age 2 years. Although rates of diarrhoea, pneumonia, hospital admission, and mortality were unaffected, the incidence of malaria was reduced by 39% in the continue co-trimoxazole group (IRR 0.61, 95% CI 0.46–0.81; p=0.001), despite a high prevalence of antifolate resistance. An observational analysis of infants before and after co-trimoxazole introduction in the BAN trial also showed reduced malaria but no effect on severe illness, death, weight-for-age, or anaemia.

Some researchers argue that co-trimoxazole for HEU infants might be detrimental. An observational South African study showed no protection against pneumonia, and a possible increase in diarrhoea in HEU infants receiving co-trimoxazole prophylaxis. Co-trimoxazole can cause skin rashes, gastrointestinal disturbances, and neutropenia, particularly if infants are also taking ART. However, a study of 1705 HEU infants in Botswana reported a low frequency of severe anaemia and neutropenia between ages 1 to 6 months, providing reassurance that prolonged co-trimoxazole is generally not associated with severe haematological toxicity. Coutsoudis and colleagues have, however, argued that the risks of co-trimoxazole prophylaxis might outweigh the benefits for HEU infants, particularly because HIV transmission rates within well functioning prevention of mother-to-child transmission (PMTCT) programmes are low, and evidence for benefit of co-trimoxazole in HIV-uninfected children is weak. A laboratory study of infants in the BAN trial proposed that co-trimoxazole could actually increase the risk of mother-to-child HIV transmission, although reasons for this are unclear. HIV-exposed infants receiving co-trimoxazole had higher concentrations of plasma lipopolysaccharide than those not receiving co-trimoxazole (p=0.004), leading the investigators to speculate that changes to the intestinal microbiota after long-term antibiotic use led to gut damage (with associated microbial translocation) and increased risk of HIV transmission. However, co-trimoxazole was not randomised in this trial, which enrolled infants before and after introduction of prophylaxis, and there was no direct assessment of intestinal damage or microbiota composition, so further studies are needed to better understand this finding.

Widespread co-trimoxazole prophylaxis is also associated with increased community antimicrobial resistance. A longitudinal cohort study of Zambian infants showed an initial decrease in nasopharyngeal pneumococcal colonisation in HEU infants taking co-trimoxazole; however, within 6 weeks there was increased colonisation with co-trimoxazole-resistant pneumococci compared with HIV-unexposed infants (relative risk 3.2, 95% CI 1.3–7.8). In a substudy of the CHAP trial, children with HIV randomly assigned to co-trimoxazole paradoxically had higher rates of pneumococcal carriage and higher rates of co-trimoxazole resistance among Streptococcus pneumoniae and Haemophilus influenzae isolates than with placebo; but despite increased resistance, co-trimoxazole reduced morbidity and mortality from invasive bacterial disease.

To assess the benefits and risks of co-trimoxazole prophylaxis for HIV-exposed uninfected infants, a trial in Botswana is randomly assigning more than 3000 HEU infants to co-trimoxazole or placebo until age 15 months (ClinicalTrials identifier NCT01229761). The primary outcome is survival at age 18 months; secondary endpoints include morbidity and mortality at 18 months, and safety of co-trimoxazole. A second trial of co-trimoxazole prophylaxis in breastfed HEU infants, with primary outcomes of morbidity (diarrhoea and pneumonia) and all-cause mortality up to age 54 weeks, is recruiting in Durban, South Africa (ClinicalTrials identifier PACTR201311000621110); neither trial is taking place in a malaria-endemic area.

Guidelines for co-trimoxazole for people living with HIV

Guidelines have generally shifted over time, from use of co-trimoxazole as a pre-ART intervention for people with advanced HIV disease to universal, adjunctive, co-trimoxazole prophylaxis at ART initiation as part of a package of HIV care. Even in those not yet eligible for ART, provision of free co-trimoxazole improves retention in care. WHO guidelines were updated in 2014 to recommend long-term co-trimoxazole prophylaxis for adults (including pregnant women), irrespective of disease stage or CD4 cell count in geographical areas with a high prevalence of malaria or severe bacterial infections, or both, although initiation and discontinuation criteria are still provided for other settings. Co-trimoxazole should be initiated for all infants, children, and adolescents, irrespective of disease stage or use of ART; similar to adults, prophylaxis should continue long term in settings of high malaria or severe bacterial infections, or both.

Implementation of these guidelines is challenging because co-trimoxazole coverage is low. Of 4050 HIV-infected adults attending 19 clinics in the Asia-Pacific region between 2003 and 2009, around a third were not taking co-trimoxazole; these patients had more than ten times higher mortality than those receiving prophylaxis.

Globally, WHO estimated 71% co-trimoxazole coverage in 2010, but acknowledged that these data were probably inaccurate. Only 23% of HEU infants received co-trimoxazole in 2010, because of difficulties in follow-up and challenges with drug forecasting, procurement, and supply. Co-trimoxazole scale-up therefore provides a good example of how research evidence does not necessarily translate into programmatic changes, which might be highly dependent on the political and economic situation in a country. Effective implementation of new guidelines will need strengthening of drug supply management systems to prevent stock-outs, linkage with other programmes, better community awareness, sensitisation and training of health-care workers, and free provision of co-trimoxazole. Expansion of co-trimoxazole provision should also be accompanied by strategic surveillance for toxic effects and antimicrobial resistance.

### Antimicrobial benefits despite high-level resistance

Bacterial resistance to co-trimoxazole is predominantly mediated by efflux pumps and mutated target enzymes. The mutated enzymes are transferable via plasmid-encoded changes in dihydrofolate reductase. Antimicrobial resistance started to emerge after greater empirical use of co-trimoxazole. A longitudinal cohort study of Zambian children showed an initial decrease in nasopharyngeal pneumococcal colonisation in HEU infants taking co-trimoxazole; however, within 6 weeks there was increased colonisation with co-trimoxazole-resistant pneumococci compared with HIV-unexposed infants (relative risk 3.2, 95% CI 1.3–7.8). In a substudy of the CHAP trial, children with HIV randomly assigned to co-trimoxazole paradoxically had higher rates of pneumococcal carriage and higher rates of co-trimoxazole resistance among Streptococcus pneumoniae and Haemophilus influenzae isolates than with placebo; but despite increased resistance, co-trimoxazole reduced morbidity and mortality from invasive bacterial disease.

<table>
<thead>
<tr>
<th>Bacterial Species</th>
<th>Resistance Prevalence</th>
<th>Country or Region</th>
<th>HIV-Positive Participants</th>
<th>Co-trimoxazole Prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>56–91%</td>
<td>Africa, Côte d’Ivoire, Kenya, South Africa, Uganda</td>
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<td>Mixed</td>
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<td><em>E coli</em></td>
<td>64%</td>
<td>Laos</td>
<td>Unknown (&lt;1%)</td>
<td>None</td>
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<td><em>Group B streptococcus</em></td>
<td>35%</td>
<td>Sub-Saharan Africa and Asia</td>
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<td>Not stated</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>33–98%</td>
<td>Africa, South Africa, Kenya, Uganda</td>
<td>Mixed</td>
<td>Mixed</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>62%</td>
<td>Sub-Saharan Africa and Asia</td>
<td>Not stated</td>
<td>Not stated</td>
</tr>
<tr>
<td><em>Non-Typhi salmonella</em></td>
<td>0–85%</td>
<td>Africa, Côte d’Ivoire, Kenya, Malawi, Uganda</td>
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<td>Mixed</td>
</tr>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>13–100%</td>
<td>Africa, Kenya, Uganda, Mali</td>
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<td><em>Salmonella Typhi</em></td>
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<td>Africa</td>
<td>Mixed</td>
<td>Unknown</td>
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<td><em>Shigella spp</em></td>
<td>84–100%</td>
<td>Africa, Uganda, Côte d’Ivoire</td>
<td>Mixed</td>
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<td><em>S aureus</em></td>
<td>25–100%</td>
<td>Africa, Côte d’Ivoire, South Africa, Uganda</td>
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<td><em>S aureus</em></td>
<td>22%</td>
<td>Laos</td>
<td>Unknown (&lt;1%)</td>
<td>None</td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
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<td>Mixed</td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>80%</td>
<td>Laos</td>
<td>Unknown (&lt;1%)</td>
<td>None</td>
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</table>

Table: Co-trimoxazole resistance in common bacterial isolates from developing countries
co-trimoxazole in the 1970s. With more widespread use during the HIV epidemic, further increases in microbial co-trimoxazole resistance were noted, and rates are now extremely high in many settings (table and appendix).

Despite high resistance rates, co-trimoxazole continues to reduce morbidity and mortality from bacterial infections, for reasons that are unclear. In Zambia, background resistance was 61% among *S pneumoniae* isolates, yet the predominant effect of co-trimoxazole in the CHAP trial was reduction in pneumonia. In Uganda, despite a rise in diarrhoea-pathogen resistance to 83% after co-trimoxazole introduction, significant reductions in diarrhoea were seen (HR 0·65, 95% CI 0·53–0·81; *p*<0·0001). Despite high resistance rates, co-trimoxazole could still have sufficient antimicrobial activity to prevent severe disease, particularly because it is concentrated intracellularly. Furthermore, its activity against non-bacterial pathogens could have been underestimated.

**Mechanism of action of co-trimoxazole: beyond antimicrobial properties**

Co-trimoxazole has been known for several decades as a unique compound with important properties beyond its antimicrobial effects, and these mechanisms could be crucial to understand some of its clinical benefits (figure 1). The immunomodulatory properties of co-trimoxazole were first suggested in a study by Gilchik and colleagues in 1970. They noted that trimethoprim has a pyrimidine ring that is structurally similar to the immunosuppressive agent azathioprine, and they showed that giving intramuscular trimethoprim prolonged the survival of skin grafts transplanted from brown to white mice, with graft survival times comparable to those achieved using azathioprine. In the presence of a folinate analogue, the effect was no longer sustained, leading the investigators to make two inferences: first, that the immunosuppressive action of trimethoprim was derived from the same antifolate properties that confer antimicrobial activity; and second, that this occurs specifically through the inhibition of T cells, which are needed for allograft rejection. Soon after this discovery, other researchers confirmed that co-trimoxazole can directly modulate lymphocyte function. Lymphocytes from 25 healthy adult volunteers were cultured with phytohaemagglutinin (a T-cell mitogen) in the presence of trimethoprim, sulfamethoxazole, or co-trimoxazole. Decreased thymidine uptake was noted in cells from 15 (60%) of the volunteers, with the greatest suppression of proliferation in lymphocytes incubated with co-trimoxazole, compared with either of its constituent drugs. In a study of 40 individuals vaccinated against tetanus, half received 4 days of co-trimoxazole (480 mg twice a day) immediately after vaccination, and the other half received no antibiotics. Antitetanus antibody titres 4 and 5 weeks after vaccination were significantly lower in the treated participants compared with the untreated participants. The results from these studies suggest that co-trimoxazole is able to prevent lymphocyte proliferation, perhaps through inhibition of the human folate reductase enzyme.

Co-trimoxazole could conversely enhance innate immune function by increasing the bactericidal activity of neutrophils. An in-vitro study of alveolar macrophage function in a few adults with advanced HIV infection showed enhanced phagocytosis and killing of *Staphylococcus aureus* in those taking co-trimoxazole, compared with untreated individuals. However, there is some inconsistency between studies. For example, inhibition of both lymphocyte and neutrophil activity in one clinical study was evident only at high drug concentrations in vitro and was not found in cells taken from three participants after oral dosing (960 mg twice a day) of co-trimoxazole. Moreover, children treated with co-trimoxazole for urinary tract infections had no differences in antibacterial cellular or humoral immune responses compared with children treated with other antibiotics, and an investigation of the immunoregulatory properties of co-trimoxazole in guinea pigs showed no effect on either interleukin 1 or tumour necrosis factor α production from alveolar macrophages.

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**Figure 1:** Mechanisms of action of co-trimoxazole

Co-trimoxazole, a fixed-dose combination of trimethoprim and sulfamethoxazole, has both immunomodulatory and antimicrobial properties. Some in-vitro evidence suggests that co-trimoxazole can modulate both innate and adaptive immune cells, with increased bactericidal activity and chemotaxis of neutrophils, enhanced phagocytosis and intracellular killing by macrophages, and reduced proliferation of lymphocytes. Antimicrobial activity of co-trimoxazole occurs through the sequential blockade of two enzymes in the folate biosynthetic pathway: inhibition of dihydropteroate synthetase by sulfamethoxazole, and inhibition of dihydrofolate reductase by trimethoprim. The compound therefore has broad-spectrum bactericidal activity, which might be enhanced by high intracellular concentrations of co-trimoxazole.
Several clinical studies of rheumatological and autoimmune conditions support the postulation of immunomodulatory and anti-inflammatory effects of co-trimoxazole.100,101 Two small, non-randomised studies that assessed adjunctive therapy in rheumatoid arthritis showed improved clinical function and reduced inflammation in patients treated with co-trimoxazole or its component sulfamethoxazole.100,101 Co-trimoxazole has also been used to prevent relapses in Wegener’s granulomatosis.101,102 Although this effect has been partly attributed to its antibacterial properties, because S aureus can trigger relapses,103 co-trimoxazole could also modulate T-cell activity or reduce free-radical production through its anti-inflammatory properties.101,102 Co-trimoxazole has also been used with variable effects in the management of inflammatory bowel disease and temporal arteritis.116,117 Therefore, there is reasonable evidence that co-trimoxazole has mechanisms of action beyond its antimicrobial effects, but its immunomodulatory properties require better in-vitro characterisation and further clinical assessment in randomised trials of chronic inflammatory diseases.

Expanding roles for co-trimoxazole in developing countries?

The anti-inflammatory potential of co-trimoxazole might be especially useful in developing countries, where several diseases, such as HIV infection and malnutrition, are characterised by recurrent infections and chronic inflammation.118,119 Some of the benefits of co-trimoxazole in individuals with HIV could therefore arise from reductions in immune system activation.100,102 In the ARROW trial, children who were randomly assigned to discontinue co-trimoxazole had more anaemia (including potentially life-threatening anaemia) than children continuing co-trimoxazole—this effect was not simply due to increased malaria.8 Children who were not receiving ART who were randomly assigned to co-trimoxazole in the CHAP trial had a four times greater increase in haemoglobin than those taking placebo and a slower decrease in weight and height.120 Similarly, there was a trend towards greater weight (but not height) in children in ARROW who continued co-trimoxazole.4 Although growth failure and anaemia in children with HIV have multifactorial causes, immune activation is probably a major contributor and co-trimoxazole could partly work through a reduction in chronic inflammation. Consistent with this concept, children who were randomly assigned to discontinue co-trimoxazole in ARROW showed a transient rise in several inflammatory biomarkers (interleukin 6, soluble CD14, and tumour necrosis factor α), and a sustained rise in C-reactive protein which persisted for more than 2 years after stopping co-trimoxazole.121

More mechanistic studies are needed to investigate whether co-trimoxazole has anti-inflammatory benefits in individuals with HIV; however, plausibly these could occur either directly, through effects on innate and adaptive immune cells, or indirectly by attenuating microbial translocation across an impaired intestinal barrier. Simian immunodeficiency virus-infected sooty mangabeys had reduced plasma concentrations of lipopolysaccharide (a marker of microbial translocation) when treated with a bowel-sterilising antibiotic regimen, and co-trimoxazole has been used previously in the treatment of small intestinal bacterial overgrowth with metronidazole.122,123 In the DART trial,4 reduced microbial translocation could have contributed to the lower mortality seen in adults with HIV receiving co-trimoxazole.

Whether co-trimoxazole might similarly reduce mortality and improve growth in children without HIV is unknown, but seems plausible. A systematic review126 of pooled data from ten randomised trials of various antibiotics in prepubertal children in developing countries showed significant increases in both weight and height, with effects postulated to be due to treatment of subclinical infections and modulation of intestinal microbial populations.124 Co-trimoxazole has also been shown to improve anthropometric indicators (body-mass index, arm muscle circumference, and percentage of fat mass) in malnourished adults with HIV in Côte d’Ivoire.125 However, the greatest benefits are likely to be in children aged less than 2 years for whom a window of opportunity exists to improve linear growth and long-term neurodevelopmental outcomes during the first 1000 days.

Children living in impoverished conditions frequently have compromised intestinal barrier function.126 Enteropathy can have many causes in this setting, including environmental enteric dysfunction, persistent diarrhoea, micronutrient deficiencies, and severe acute malnutrition, which overlap and interact to drive morbidity and mortality in young children.127 Interest is increasing in the potential for antibiotics such as co-trimoxazole to affect the interplay between enteropathy, malnutrition, and morbidity.128 In a double-blind, randomised, placebo-controlled trial in Bangladesh, children with persistent diarrhoea had reduced stool output and higher recovery rates within 7 days of starting co-trimoxazole.128 Children admitted to hospital with severe acute malnutrition frequently have invasive bacterial infections.129 However, WHO guidelines recommend broad-spectrum antimicrobials because several studies of children with complicated severe acute malnutrition have shown that bacteraemia frequently occurs with organisms that are resistant to co-trimoxazole.128,129 A community-based trial in Malawian children with uncomplicated, severe acute malnutrition showed lower mortality in those randomly assigned to antibiotics (either amoxicillin or cefdinir), compared with placebo, despite no clinical signs of infection.130 In view of the emerging role of the microbiota in the pathogenesis of malnutrition, antibiotics could plausibly
modulate intestinal microbial populations and confer benefits beyond a simple reduction in invasive bacterial infections. A large clinical trial in Kenya (NCT00934492) is investigating the effect of co-trimoxazole after the stabilisation of children admitted to hospital for severe acute malnutrition. Children without HIV were randomly assigned to receive co-trimoxazole or placebo for 6 months after their discharge from hospital, with a primary endpoint of survival at 1 year. Secondary endpoints in this trial include growth, microbial resistance, and markers of inflammation and immune function. Another large African trial, Transfusion and Treatment of Severe Anaemia in African Children (TRACT, ISRCTN84086586), includes co-trimoxazole as an adjunctive treatment after blood transfusion in nearly 4000 children with life-threatening anaemia. Children have an ongoing high risk of mortality and hospital readmission after treatment for severe anaemia, and as part of a factorial design, interventions include a 3 month course of co-trimoxazole as a strategy to reduce infectious morbidity and mortality in this high-risk group.

Therefore, co-trimoxazole could operate through several interlinked pathways: direct reduction of opportunistic infections; changes in nasopharyngeal colonisation; modulation of the intestinal microbiota, leading to amelioration of enteropathy and reduction of microbial translocation; direct enhancement of immune responses; and a reduction in systemic inflammation (figure 2). These multiple pathways could partly explain the broad benefits of co-trimoxazole in patients in developing countries, despite high rates of microbial resistance, and they suggest other plausible benefits of co-trimoxazole that have not yet been investigated, such as improvements in oral vaccine responses.

In both developed and developing countries, co-trimoxazole use has resurged for a wide range of infections, particularly for those usually deemed difficult to treat, such as melioidosis, brucellosis (in younger children), Stenotrophomonas maltophilia, Burkholderia cepacia in immunocompromised patients, and Q fever (although the evidence for long-term treatment during pregnancy is conflicting). Co-trimoxazole is also a recognised treatment for paracoccidioidomycosis. Moreover, in-vitro studies have suggested that co-trimoxazole has activity against Mycobacterium tuberculosis, particularly the sulphonamide component of the drug. This suggestion is supported by a recent report of adults with HIV followed up for 20 years within the Swiss HIV Cohort, for whom cumulative co-trimoxazole exposure reduced the risk of incident tuberculosis, particularly in individuals not receiving ART. However, not all studies have shown a protective effect of co-trimoxazole against tuberculosis.

Importantly, the use of co-trimoxazole could extend to the treatment of multidrug-resistant tuberculosis and meticillin-resistant Staphylococcus aureus, with susceptibility seeming to differ by geographical region.

Conclusions

Co-trimoxazole provides important benefits for adults and children in developing countries, reducing morbidity and mortality particularly in the setting of HIV infection, despite high levels of pathogen resistance. Recent data emphasise the long-term benefits of continuation of co-trimoxazole in patients receiving ART. There is both optimism and scepticism about expanding the use of co-trimoxazole in populations without HIV in developing countries. Its unique antimicrobial and immunomodulatory properties could provide benefits beyond a simple reduction in infections, and therefore interest is increasing in adjunctive use of co-trimoxazole for susceptible populations, such as children recovering from malnutrition or severe anaemia. Whether co-trimoxazole has a role in modulating the microbiota, reducing
inflammation, or promoting growth during the first 1000 days of a child’s life in developing countries warrants further exploration (panel 2), but more widespread use of antibiotics generates concerns. The number of HIV-exposed children who qualify for co-trimoxazole has increased hugely, because effective PMTCT regimens reduce vertical HIV transmission. Additionally, the risks of side-effects, increasing antimicrobial resistance, and increased costs need to be balanced against the potential benefits of wider use of co-trimoxazole, and evidence from ongoing trials is awaited. Further studies are needed to better understand the mechanisms through which co-trimoxazole reduces morbidity and mortality so that we can facilitate judicious expansion of co-trimoxazole to populations who are likely to benefit from this well tolerated and affordable intervention.

Panel 2: Future research directions for co-trimoxazole

- Investigate how co-trimoxazole reduces morbidity and mortality in settings of high-microbial resistance
- Explore the immunomodulatory properties of co-trimoxazole with contemporary laboratory methods
- Investigate the effect of co-trimoxazole on the intestinal microbiota, gut barrier function, and microbial translocation
- Assess the role of co-trimoxazole in the reduction of morbidity and mortality and improvement of growth in HIV-exposed children without infection and HIV-unexposed children in developing countries
- Explore the effect of antenatal co-trimoxazole in the reduction of adverse birth outcomes and improvement of fetal growth in pregnant women with or without HIV in developing countries
- With the expansion of co-trimoxazole prophylaxis for adults and children with HIV, assess the duration of protection and tolerability of long-term co-trimoxazole and undertake surveillance for toxic effects and microbial resistance
- Assess the benefits of long-term co-trimoxazole for individuals with HIV receiving antiretroviral therapy in developed countries—eg, a reduction in bacterial infections, immune activation, and non-AIDS morbidity and mortality
- Define the role of co-trimoxazole in the prevention and treatment of tuberculosis, including multidrug-resistant tuberculosis

Search strategy and selection criteria

We searched PubMed using the terms “trimethoprim-sulfamethoxazole combination”, “trimethoprim-sulfamethoxazole”, “CTX”, “septrin”, “cotrimoxazole”, and “co-trimoxazole” for English-language papers published between Jan 1, 1969, and Jun 26, 2014. We screened titles and abstracts of papers, and reviewed the reference lists of selected articles and relevant conference proceedings.

Contributors

AJP, ASW, and DMG had the original idea for the review. JAC undertook the literature search. JAC, FF, and AJP wrote the first draft of the manuscript (JAC and FF contributed equally to this manuscript). ASW and DMG helped with data interpretation and critically revised the manuscript with AJP.

Declaration of interests

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