Antimicrobial resistance following azithromycin mass drug administration: surveillance strategies to assess public health impact

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Article's main points

Azithromycin Mass Drug Administration results in a sustained increase in antimicrobial resistance when implemented at a population level. Targeted risk-based metagenomics approaches complementing traditional microbiological methods are recommended for surveillance of emerging short- and long-term antimicrobial resistance.

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ABSTRACT

A reduction of childhood mortality has been noted in trials investigating azithromycin mass drug administration (MDA) for trachoma control, and has been confirmed by a recent largescale randomised controlled trial. There are concerns that population-level implementation of azithromycin MDA will lead to selection of multi-resistant pathogens. The available evidence suggests that repeated azithromycin MDA may result in a sustained increase in macrolide and other classes of resistance in gut and respiratory bacteria. Current evidence comes from standard microbiological techniques in studies focused on a time-limited intervention, while MDA implemented for mortality benefits would likely repeatedly expose the population over a prolonged period and may require a different surveillance approach. Targeted short-term and long-term surveillance of resistance emergence to WHO Access antibiotics is needed throughout any implementation of azithromycin MDA, using a combined phenotypic and genotypic approach to overcome the limitations of resistance surveillance in indicator bacteria from routine microbiological specimens.

DISCUSSION

Intermittent childhood azithromycin mass drug administration in sub-Saharan Africa: current indications and supporting evidence

The most frequent indication for azithromycin mass drug administration (AZM MDA) across Africa is endemic trachoma [1]. In 1997, the WHO established the Global Alliance for the Elimination of Blinding Trachoma by 2020 (GET 2020), and there is clear evidence that single-dose AZM MDA reduces the prevalence of active trachoma and ocular infection in treated communities [2]. An initially unexpected reduction in childhood mortality was observed in studies focusing on AZM MDA for trachoma in the sub-Saharan setting [3 , 4]. The MORDOR I study (Macrolides Oraux pour Réduire les Décès avec un Oeil sur la Résistance, clinicaltrials.gov # NCT02048007) [4] was specifically designed to investigate a potential mortality benefit. The study assigned communities in Malawi, Niger, and Tanzania to four twice-yearly MDA rounds of either 20 mg/kg per dose oral AZM or placebo. This cluster randomized controlled trial demonstrated a reduction of all-cause mortality in under-fives of approximately 14% in the treatment group [4]. Mortality reduction (18%) was observed most clearly among infants in Niger who were less than 6 months of age and who had the highest mortality rate at baseline. Extension for two more rounds during MORDOR II did not show statistically significant evidence for a waning effect of AZM MDA on childhood mortality [5]. In communities who received placebo originally, childhood mortality decreased after receipt of AZM [5].

Emergence of antibiotic resistance linked to antibiotic MDA could be a barrier to widespread distribution. There are concerns that AZM MDA will lead to selection of macrolide-resistant strains of *Chlamydia trachomatis*, and resistance macrolides and

other classes of antimicrobials in other pathogens. Here, we discuss how to address these concerns and propose a strategy to monitor emerging antimicrobial resistance (AMR) alongside the implementation of AZM MDA for prevention of childhood mortality in sub-Saharan Africa.

Anticipated antimicrobial resistance and microbiome changes associated with azithromycin use

Macrolides bind to the 23S rRNA of the 50S ribosomal subunit and inhibit protein synthesis. Interestingly, other antibiotic classes such as the lincosamides and streptogramins B share overlapping binding sites on the ribosome with the macrolides and this translates to co-resistance to all three antibiotic classes upon alteration of the ribosomal binding site by methylation or by mutation.

Resistance occurs by alteration of the target, active efflux, and antibiotic inactivation [6 , 7]. It can be selective for the 14- and 15-membered macrolides (erythromycin, clarithromycin, azithromycin; M phenotype) or be relevant for the 16-membered macrolides (spiramycin, josamycin), lincosamides (clindamycin), and streptogramin B as well (MLSB phenotype) [8]. M-type resistance is mediated by chromosomally (*mef*) or plasmid-encoded macrolide efflux genes (*msrA*) [9 , 10 , 11] and generally confers low-level resistance among streptococci, whereas MLSB resistance is caused by methylation of the 23S rRNA, which blocks the ribosomal binding site and commonly confers high-level resistance [8]. The methylase is encoded by *erm* (erythromycin ribosome methylase) genes. This phenotype can be constitutive (MLSB-C) or inducible (MLSB-I) [6 , 7 , 8]. Highly macrolide-resistant *Streptococcus pneumoniae* isolates that have both *erm* and *mef* resistance mechanisms are increasingly reported [8].

Interestingly, pneumococcal lineages that harbour multiple antibiotic resistance determinants also show a higher degree of mosaicism in housekeeping genes [12]. This facilitates horizontal gene transfer from genetically related organisms, such as viridans streptococci, and efficient colonization with in turn increased exposure to co-colonizing resistant bacteria. The final result may be more interstrain homologous-recombination events with the incorporation of resistance determinants for β-lactams, fluoroquinolones, and co-trimoxazole in the core genome or on integrative transposable elements for macrolides, lincosamides, tetracycline, and chloramphenicol. These data highlight the importance of the commensal oral flora as a reservoir of macrolide resistance determinants, urging holistic metagenomic studies [13].

Macrolides are also expected to affect Gram-negative *Enterobacteriaceae*, which are known to harbour various mobile genetic elements (MGE) [14] and serve as a reservoir for antibiotic resistance genes in the gut [15]. The acquisition of novel genes by plasmids through MGE such as transposons or insertion sequences, and their ability to replicate in a wide range of bacterial hosts, makes them perfect vectors for the spread of antimicrobial resistance [16]. Unrelated to macrolide use, such resistance evolution is best described in Gram-negatives where extended-spectrum beta-lactamases (ESBL) are frequently associated with co-resistance to aminoglycosides and fluoroquinolones [17]. Selection of these isolates may be driven by a single agent resulting in resistance to multiple unrelated agents. Azithromycin is considered a potent potential driver in the selection of such co-resistance because of its pharmacological properties: very long elimination half-life >50 hours, high intracellular and prolonged tissue concentration, prolonged rate of dissociation from the ribosomal target with a prolonged postantibiotic effect, large volume of distribution

resulting in possible long-term effects in various body compartments, and better activity against common Gram-negative bacteria compared with other macrolides [8 , 15 , 18]. While evidence linking AZM use to emergence of resistance in Gram-negatives is sparse, there is a clear need for active surveillance in the context of AZM MDA.

Co-resistance and co-selection processes driving rising AMR may additionally be compounded by microbiome impacts if alterations in the microbiome result in a predominance of resistance gene-carrying organisms. The gut as a reservoir for antibiotic resistance genes can be disturbed by antibiotics in its composition and function as well as by selecting for antibiotic-resistant microbes [19]. Several studies have evaluated the effects of antibiotic exposure on the paediatric gut microbiome diversity, showing variable results [20 , 21 , 22 , 23 , 24]. In general, these studies find reductions in observed richness and Shannon diversity during or shortly after AZM exposure. Once antibiotic treatment is stopped, microbiota may display a certain degree of resilience, being capable of reverting to near their pre-exposure composition after many months [24]. However, complete recovery to the initial state may not occur or be age-dependent, particularly in the context of repeated antibiotic insults during vulnerable time-periods of age [23 , 25]. Overall, AZM may cause important changes in the human gut microbiome, but the effects on antimicrobial resistance of these shifts remain unclear.

Evidence summary on antimicrobial resistance following azithromycin MDA in Sub-Saharan Africa

A recent systematic review [26] of antimicrobial resistance following AZM MDA for trachoma identified that this approach selects for macrolide resistance in some

potentially pathogenic organisms, with a possible population-level dose-response causing increased resistance selection as the number of distribution cycles increases (**Table 1**). Antibacterial resistance emergence has also been seen in the MORDOR I trial (12.3% vs. 2.9% of children carried macrolide-resistant pneumococci in communities receiving AZM vs. placebo) [19]. When antibiotic selection pressure is removed, the prevalence of resistance may return to baseline levels over time, though most studies followed populations for 6 months or less, and results were mixed in studies with shorter follow-up periods [26]. About half of studies evaluating AMR after AZM MDA did not measure the baseline antibiotic resistance in the target pathogens, making it difficult to prove that AZM MDA caused any changes. *Streptococcus pneumoniae* in nasopharyngeal samples was the main target organism of most studies with a much lesser focus on other organisms, such as *Escherichia coli* (stool samples) and *Staphylococcus aureus* (nasopharyngeal samples). The majority of the studies came from the African continent, were published between 1997 and 2019 with the reported resistance data collected between 1995 and 2017. In terms of the strength of the included studies, most trials were longitudinal cohort studies or (repeated) cross-sectional studies except Skalet [27] and Keenan [28], which were RCTs.

Impact of different techniques determining AMR

The majority of studies above determined antimicrobial resistance by phenotypic susceptibility testing using for example Etest or disk diffusion [26]. Only in three studies were molecular methods applied (such as multilocus sequencing [29], targeted PCR [28] or DNA microarray [30] for detection of e.g. *mef* or *erm* genes). Most of the data generated so far are presented in the form of proportions, meaning the percentage of isolates of a given organism collected that are resistant to a given

antibiotic. Such data are readily available and easily interpreted, however analysis of proportions may not be the optimal method by which to measure changes in resistance from the public-health perspective, and in particular it may be misleading when examining changes brought about by antibiotic use [31]. When evaluating the burden of resistance, the density of resistant isolates as expressed by rates should be assessed, i.e. the absolute number of resistant isolates in an at-risk population over time [31].

On-going clinical studies/trials

There are currently 20 actively recruiting or about to recruit randomised controlled trials investigating AZM treatment in the target population registered in ClinicalTrials.gov (**Table 2**). In 3 cases the trialled AZM treatment course includes more than a single dose. Five studies are associated with the MORDOR trial [4]. Six trials specify that resistance will be assessed for respiratory or gut bacteria with a variety of microbiological techniques being used. An additional 7 trials intend to investigate impacts on the nasopharyngeal or gut microbiome without specific assessments of antibiotic resistance. Finally, 6 trials are not planning to evaluate antimicrobial resistance or are limited to the target pathogen for the intervention (*Chlamydia trachomatis* or *Treponema pallidum ssp. pertenue*).

Surveillance strategies for antimicrobial resistance during continuous azithromycin MDA

Genotypic versus phenotypic testing of AMR

Although phenotypic methods remain the cornerstone of clinical antimicrobial susceptibility testing, molecular characterization of AMR determinants are being considered for local, national, or even global surveillance of AMR [32]. In 2015, WHO

launched the Global Antimicrobial Resistance Surveillance System (GLASS) in order to standardize the collection of data on AMR for global planning, prevention and intervention programs [33]. Reports to GLASS currently rely on detection of phenotypic resistance, however in the future GLASS may incorporate the results of molecular testing for AMR detection by appropriate methods. Molecular diagnostic methods can be used with phenotypic testing to yield additional information, however selection of the most appropriate molecular AMR test by setting, including availability in clinical and reference laboratories, is crucial. During recent years there has been a dramatic reduction in cost and an increase in the quality and availability of whole-genome sequencing (WGS), making this technology gradually more accessible for routine scientific use but also for clinical diagnostics and surveillance. In the following section, we discuss advantages and disadvantages of genotypic versus phenotypic surveillance of antimicrobial resistance (**Table 3**).

Whole metagenome sequencing (WMGS) to detect AMR genetic determinants: Opportunities and challenges

Traditional microbiology relies upon clonal cultures that select for dominant bacterial species/strains and largely ignore non-pathogenic bacterial species, and this approach has also been used for AMR surveillance. In routine clinical care, culturing of more than a few selected “indicator” organisms is generally difficult for logistical reasons (especially in clinical specimens with a high bacterial load such as stool samples) and may not be helpful in the optimization of patient care. Early sequencing examined specific genes such as the 16S rRNA gene and revealed the microbial biodiversity that had been missed by culture-based methods. Non-pathogenic “commensal” bacteria serve as an antibiotic resistance reservoir and must be addressed since these microorganisms may gain, maintain and deliver genes to

other microorganisms [15]. Indeed, many of the clinically relevant resistant bacteria are believed to originate from the environment, together constituting a large and almost unexplored resistance reservoir [34]. As an example, Devirgiliis et al. [35] report an update on AMR in foodborne *Lactobacillus* and *Lactococcus* species, two genera of Lactic Acid bacteria that often represent the dominant bacterial population in breast-fed infants. Different *Lactobacillus* species were shown to transfer erythromycin and tetracycline resistance genes to *Enterococcus faecalis*, indicating a potential risk of using Lactic Acid Bacteria starters that have not been tested for the absence of AMR genes.

Recent studies, especially in Africa, have predominantly used 16S metagenomics to determine taxonomic profiling and describe community composition (diversity and abundance) [24]. Alternatively, a shotgun metagenomics approach can be used to directly detect antibiotic resistance genes in samples of interest, potentially indicating the impact of an exposure like AZM MDA on the microbial resistance landscape. Arguably this would be highly relevant for public health as an 'early warning' system compared with the slower expected AMR changes in indicator pathogens from routine microbiology samples.

Extracting the relevant information to detect genetic determinants related to AMR from WMGS data encounters two main challenges: (1) access to comprehensive databases containing the relevant DNA or protein sequence targets and (2) application of appropriate bioinformatic methodologies to accurately extract the relevant information from WMGS data based on these target databases [32]. This is further complicated by the fact that many genetic mechanisms can be accountable for an AMR phenotype and that there are no easy decision rules for prediction of their corresponding resistance phenotype. As a consequence, many of the bioinformatic

tools to detect AMR genetic determinants rely on target databases containing well-defined genes or specific single point mutations, where a strong correlation between the genetic determinant and a given phenotype exists and can be extracted from either published peer-reviewed articles or from pre-existing archives such as the Antibiotic Resistance Gene Database (ARDB) [32 , 36 , 37]. These databases are based on *a priori* data and are therefore not suitable for detecting completely new gene families, genes, or point mutations and have to be updated frequently. Such databases do not support the analysis of the large-scale, ecological sequence datasets required for AMR surveillance. Specifically tailored databases such as MEGARes (https://megares.meglab.org) could facilitate the characterization of AMR determinants in the context of large metagenomics studies [36].

Time points and target population of AMR testing

One important limitation of many of the available studies on antimicrobial resistance after AZM MDA is the lack of baseline antibiotic resistance data in the target population. However, clearly a high prevalence of resistant pathogens before exposure to AZM MDA is a major additional risk factor for increasing antimicrobial resistance. One can imagine that this baseline or “trough” prevalence of resistance might build up over several years and progressively increase before each MDA round. Hence, the key sampling time point for AMR is this “trough”, and the key population are infants prior to the age of receipt of AZM MDA (as well as under-fives who are the target population for ongoing MDAs). To appropriately target AMR surveillance in the context of AZM MDA, an active surveillance programme targeting infants should be instituted. For older children, sampling could be done in a smaller, more targeted way just before MDA is administered, or in communities who are not (yet) exposed to MDA depending on the implementation approach.

Summary: Potential strategies for AMR surveillance

- Strengthening surveillance of invasive or clinical isolates of key pathogenic bacteria is desirable but is limited by local capacity, difficult to quality assure, and crucially expected to result in a small number of isolates showing the impact of AMR after a long lag time.
- Young age is most relevant for invasive disease and, for example, pneumococcal carriage. Active surveillance should focus on infants and young children, and could be linked to health services contact, e.g. for immunisations [38], or incorporated into the study design/implementation approach.
- Pre-MDA round 'trough' prevalence of resistance in non-exposed populations is a key indicator, can be linked to the MDA, providing targeted assessment of a broader age range.
- Alongside investments in routine microbiological capacity in regions for which AZM MDA for mortality benefits is relevant, capacity building for local sequencing-based active surveillance is desirable.

CONCLUSIONS

Azithromycin provides undisputed beneficial effects for treatment of various infectious diseases, however sparse evidence suggests that widespread and long-term exposure of children during MDA will promote macrolide and other antimicrobial resistance. For future studies or where AZM MDA is rolled out, capacity building including financial support should be guaranteed to actively monitor resistance by both standard susceptibility testing and genotypic methods. On the one hand, this will mean strengthening microbiological capacity for appropriate evaluation of patients in the clinical setting resulting in data on the clinical impact of AZM MDA. On the other hand, the use of genotypic methods should be supported to investigate antimicrobial resistance at a population level. This will answer questions about changes in resistance in clinically relevant and non-pathogenic colonizing bacteria, and provide insights into increasing or changing AMR in exposed populations. Impacts on clinical isolates would be expected to be observed in the more distant future when the impact of AZM MDA may no longer be modifiable. Sampling of a baseline (“trough”) prevalence of AMR is desirable, and can be linked to health care visits such as vaccination programmes or occur prior to MDA within studies or implementation programmes. This will enable early consideration of steps to mitigate against changing resistance patterns while harnessing AZM MDA benefits for childhood mortality.

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Abbreviations

AMR, antimicrobial resistance; AZM, azithromycin; CAR, Central African Republic; CEF, ceftriaxone; CLI, clindamycin; CLSI, Clinical & Laboratory Standards Institute; COT, cotrimoxazole; cRCT, cluster randomised controlled trial; DOXY, doxycycline; EONS, early onset neonatal sepsis; ERY, erythromycin; GLASS, Global Antimicrobial Resistance Surveillance System; LEVO, levofloxacin; LIN, linezolid; LONS, late onset neonatal sepsis; OTU, operational taxonomic unit; MDA, mass drug administration; MERO, meropenem; MIC, minimum inhibitory concentration; MLSB, Macrolide-Lincosamide-Streptogramin B resistance; MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction; PEN, penicillin; RCT, randomised controlled trial; TET, tetracycline; VANCO, vancomycin; W(M)GS, whole (meta-)genome sequencing.

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Table 1. Evidence summary of systematic literature review

		Studies	Baseline measure-ments	Single MDA	Multiple MDA	Outcome Macrolide	Outcome Non-Macrolides
Gut bacteria	**Treated children**	Seidman JC, Int J epidemiol, 2014[39]	Yes	X		Increase in AZM-resistant *E. coli* from 21% (bl) to 61% (1m), 42% (3m), 23% (6m) and higher at each time point c/w controls.	No data.
		Doan T, NEJM, 2019[19]	No		X	Determinants of MLSB-resistance more prevalent in the communities that received azithromycin (68%) than in those that did not (46%).	No evidence of a significant between-group difference.
	Household contacts	Bloch EM, Am J Trop Med Hyg, 2017[40]	No		X	Moderate rates of AZM-resistant *E. coli* (17%) among children born after the last MDA.	No data.
Naso-pharyngeal bacteria	**Treated children**	Leach AJ, Clin Inf Dis, 1997[41]	Yes	X		Increase in AZM-resistant *S. pneumoniae* from 1.9% (bl) to 55% (2-3w), 35% (2m), and 5.9% (6m).	No data.
		Coles CL, Clin Inf Dis, 2013[42]	Yes	X		Increase in AZM-resistant *S. pneumoniae* from 36% (bl) to 82% (6m).	No consistent pattern for *S. pneumoniae* resistance to COT or PEN.
		Gaynor BD, BJO, 2003[43]	No	X		No AZM-resistant *S. pneumoniae* .	No data.

	Batt SL, Antimicr agents Chemother, 2003[44]	Yes	X		No increase in ERY-resistant *S. pneumoniae* .	No consistent pattern for *S. pneumoniae* resistance to COT or PEN.
	Fry AM, Clin Inf Dis, 2002[45]	Yes	X		Increase in AZM-resistant *S. pneumoniae* from 0% (10d) to 4.5% (6m)	No consistent pattern for *S. pneumoniae* resistance to PEN, CLI, SULF or CHLOR
	Burr SE, WHO Bulletin, 2014[46]	No	X	X	No relevant increase in AZM-resistant *S. pneumoniae* until 6m after MDA (0% to 0.9%). No difference of AZM-resistant *S. pneumoniae* after 3 versus 1 MDA round (0.9% vs. 0.3%, 6m vs. 30m).	No data.
	Doan T, NEJM, 2019[19]	No		X	Increase in ERY-resistant *S. pneumoniae* in treatment (12%) vs. placebo (3%) group (6m)	No difference in PEN, COT or DOXY-resistant *S. pneumoniae* in the treatment vs. placebo group. No MERO, LEVO, CEF, VANCO, LIN-resistant pneumococcus in either treatment group.
	Skalet AH, PLoS med, 2010[27]	Yes		X	Increase in AZM-resistant *S. pneumoniae* from 3.6% (bl) to 47% (12m), vs. 9% (12m) in the untreated group.	Increase in *S. pneumoniae* resistant to CLI and TET both in the treatment and non-treatment group (without significant difference). No PEN-resistant pneumococcus in both groups.

		Keenan JD, J Infect Dis, 2015[29]	Yes		X	Increase in AZM-resistant *S. pneumoniae* from 5% (bl) to 15% (3m).	No increase in the proportion of TET or PEN resistant *S. pneumoniae*.
		Maher MC, PloS one, 2012[47]	Yes		X	Increase in AZM-resistant *S. pneumoniae* from 1% (bl) to 77% (directly after 6th MDA) and 21% (24m)	No data.
		Keenan JD, Pediatr Infect Dis J, 2016[48]	No		X	58% of AZM-resistant *S. pneumoniae* (3m)	35% TET-resistant *S. pneumoniae*, no PEN resistance.
		Haug S, Clin Inf Dis, 2010[49]	No		X	Increase in AZM-resistant *S. pneumoniae* to 77% (6m), 31% (12m), and 21% (24m). No resistance in control group isolates except one single resistant isolate (24m).	Increase in CLI-resistant *S. pneumoniae* from 35% (6m) to 60% (24m, not significant). No consistent change of TET, PEN-, and COT-resistant *S. pneumoniae* during study course in treated or untreated group.
	Household contacts	Bloch EM, Am J Trop Med Hyg, 2017[40]	No		X	14% AZM-resistant *S. pneumoniae*.	No data.

Footnotes:

Abbreviations: bl, baseline; 1m, 1 month; CEF, ceftriaxone; CHLOR, chloramphenicol; CLI, clindamycin; COT, cotrimoxazole; DOXY, doxycycline; LEVO, levofloxacine; LIN, linezolid; MERO, meropenem; PEN, penicillin; SULF, sulfamethoxazole; VANCO, vancomycin

Table 2. On-going studies addressing research questions of antimicrobial resistance after macrolide mass treatment

NCT Number	Country	Target disease	Part of MORDOR	Type of study	Target age group	Number of AZM doses/ course	Number of AZM courses	Microbiology endpoints
NCT03683667	Bangladesh	Malnutrition/ Stunting	No	cRCT	6-12m olds	1	2 (6 and 9m)	Enteropathogen burden (7x at age 6-18m), Gut microbiota composition (as above), AMR of *E. coli and S. pneumoniae* at 6, 9, 12, 15 and 18m of age in participating children.
NCT03682653	Burkina Faso	Mortality	Yes	RCT	8-27d olds	1	1 (during newborn period)	Intestinal and nasopharyngeal microbial diversity at 6m of age
NCT03676764	Burkina Faso	Mortality	Yes	cRCT	1-60m olds and those receiving first DTP vaccine (5-8w olds)	1	1 and 2x/year for older children	Carriage of *S. pneumoniae* and nasopharyngeal macrolide resistance at 36m post exposure, proportion of *E. coli* resistant to macrolides and other key antibiotics at 36m post exposure, microbial diversity in the nasopharyngeal and intestinal microbiome at 36m post exposure
NCT03676751	Burkina Faso	Growth & Development	Yes	RCT	8 day to 59m olds	1	1	Intestinal microbial diversity at 6m post exposure
NCT03676140	Papua New Guinea	Trachoma/ NTD	No	cRCT	Persons older than 5y of age in randomized communities	1	1	None specified
NCT03570814	Ethiopia	Trachoma/ NTD	No	cRCT	Persons older than 5y of age in randomized	1	1	None specified

					communities			
NCT03568643	Niger	Malnutrition/ Stunting	No	RCT	6-59m olds	1	1	None specified
NCT03564652	Pakistan	Malnutrition/ Stunting	No	RCT	(pregnant women),infants 42d of age	1	1	Enteropathogen burden at 40-42 and 56d of age
NCT03523156	Ethiopia	Trachoma	No	cRCT	6m to 9 year olds	1	1 (MDA annual) or 3 (MDA annual plus 2x targeted)	None specified beyond chlamydial infections (not AMR)
NCT03490123	Papua New Guinea	Yaws	No	cRCT	older than 6m	1	3	Macrolide resistance in *T.p.pertenue*
NCT03474276	MadagascarNiger, CAR, Senegal	Malnutrition/ Stunting	No	RCT	6 to 24m olds	3	1	Comparison of OTU composition of stool according to nutritional status (at baseline, 3 and 6m post exposure)
NCT03338244	Original MORDOR sites	Mortality	Yes	cRCT	1 to 60m of age	1	2x/y	Macrolide resistance 18m post exposure in nasopharyngeal and rectal swabs, microbial composition of stool at 18m, enteropathogen burden at 18m
NCT03335072	Ethiopia	Trachoma	No	cRCT	All persons in randomized communities eligible for MDA according to WHO	1	4x/y	None specified beyond chlamydial infections (not AMR)

					guideline			
NCT03268902	Tanzania	Malnutrition/ Stunting	No	RCT	up to 14 days old	1	6, 9, 12 and 15 months	Enteropathogen burden (5x between 6 and 18m), intestinal microbiota composition (4x between 6 and 18m)
NCT03199547	The Gambia and Burkina Faso	Neonatal sepsis	No	RCT	Women in labour	1	1	EONS (culture confirmed) and LONS (culture confirmed)
NCT03187834	Burkina Faso	Growth & Development	No	cRCT (house holds)	6 to 59m olds	5	1	Nasopharyngeal and intestinal microbiome (day 9 post exposure)
NCT03032042	Ethiopia	Helminthic infection	No	RCT	1 to 60m of age	1	1	Microbial diversity in intestinal microbiome 7d post exposure
NCT02754583	Ethiopia	Trachoma	No	RCT	All persons in randomised communities	1 (MDA, annual), 1 (targeted)	1 (MDA, annual), 4 (quarterly)	Nasopharyngeal pneumococcal macrolide resistance (12, 24, 36m post exposure), intestinal microbiome at 12 months post exposure (sub study)
NCT02414399	Kenya	Mortality	No	RCT	1 to 59m of age	5	1	Prevalence of enteric pathogen and pneumococcal carriage (6m post exposure), proportion of beta-lactam or macrolide resistance or both (6m post exposure)
NCT02048007	Malawi, Niger, and Tanzania	Mortality	Yes	cRCT	1 to 60m of age	1	2x/y	Pneumococcal macrolide resistance at 24 and 48m, macrolide resistance (genetic) in stool and nasopharynx at 24 and 48m, carriage of resistant pneumococcus at 6 to 24m, proportion of rectal/stool isolates and *E. coli* isolates resistant to macrolides and other antibiotics at 6 to 24m,

	MRSA (NP) at 24m, carriage of *S. aureus* resistant to macrolides and other antibiotics at 6 to 24m, various deep sequencing endpoints.

Footnotes:

Abbreviations: 1d, 1 day; 1w, 1 week; 1m, 1 month; 1y, 1 year; AMR, antimicrobial resistance; CAR, Central African Republic; EONS, early-onset neonatal sepsis, LONS, late-onset neonatal sepsis; OTU, Operational Taxonomic Unit; RCT, randomized controlled trial; WHO, world health organization

Table 3. Advantages and disadvantages of genotypic vs phenotypic surveillance of antimicrobial resistance

Phenotypic/susceptibility testing methods¤		**Genotypic methods***	
Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Easy access globally (?)	Select for indicator bacterial organisms and largely ignore non-pathogenic bacterial species	Yield data about any resistance gene or mutation present	Insufficient knowledge about all genetic variation may complicate accurate prediction of resistance[50]
Low costs	Rely on bacterial growth, i.e. time-consuming	Can be performed directly on clinical specimens not relying on bacterial growth, i.e. faster turnaround times	Quality controls essential to assess whether WGS data have reached a suitable standard, while there are currently no international standards for QC thresholds to use for assessing quality[50]
Guidelines available to apply and teach interpretation of results (capacity building)	Screening of a limited number of (known) resistance genes	Meta-transcriptomic analysis can determine the expression of resistance genes at the moment of sampling	Need for a standardized comprehensive databases containing the relevant DNA or protein sequence targets known to be associated with AMR[32, 50]
	Limit possible conclusions about co-transmission of resistance genes and relatedness of identified isolates to reconstruct transmission networks		Appropriate bioinformatic methodologies needed to accurately extract relevant information from WMGS data based on target databases[32]
	Limited opportunities to compare genotype with phenotype		High costs (mainly related to the complex bioinformatics infrastructure)

Footnotes:

¤Phenotypic methods: agar and broth microdilution (the latter being the reference standard) or disc diffusion, followed by interpretation according to agreed guidelines.

*Genotypic methods: metagenomics; PCR assays are not included as they provide valid information on AMR determinants known to be associated with the identified pathogen, but they are not suitable for detecting completely new genes families, novel genes, or new point mutations.

Abbreviations: AMR, antimicrobial resistance; QC, quality control; WGS, whole-genome sequencing