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Drug Resistance Surveillance in Resource-Poor Settings: Current Methods and Considerations for TB, HIV, and Malaria

Bethany L. Hedt,* Miriam K. Laufer, and Ted Cohen

Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; Department of Global Health and Social Medicine, Harvard Medical School, Boston, Massachusetts; Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland; Division of Global Health Equity, Brigham and Women's Hospital, Boston, Massachusetts; Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts

Abstract. In resource-constrained environments, monitoring the occurrence of tuberculosis (TB), human immunodeficiency virus (HIV), or malaria resistant to the limited number of available drugs is essential for national treatment program success. Countries with limited resources and technical capacity rely on survey designs and methods that are simple and easily integrated into routine clinical activities to minimize the impact on overburdened clinics. This paper reviews the most commonly used methods for drug-resistance surveillance of TB, HIV, and malaria and discusses the strengths and limitations of these different strategies.

INTRODUCTION

Over the past decade, increased resources have been dedicated for limiting the global impact of infectious diseases of public health importance, especially tuberculosis (TB), human immunodeficiency virus (HIV), and malaria.[1–5] There are immense challenges associated with the scale-up of disease control and mitigation efforts, including the appearance and spread of drug-resistant forms of these pathogens. Because curative treatment (for TB and malaria) and suppressive therapy (for HIV) depend on a limited number of agents, ensuring that these drugs remain effective is of tremendous importance.

Monitoring resistance to drugs used to treat these diseases is a necessary element of public health surveillance. In most resource-constrained settings where TB, HIV, and malaria are concentrated, treatment strategies rely on the use of standardized regimens. Although this approach is less logistically challenging and costly than individualized treatment, the need to ensure low levels of resistance to the drugs included in standardized regimens is essential. Additionally, surveillance for drug resistance can alert public health authorities to changing epidemic patterns and can trigger responses to eliminate the causes or mitigate the effects of rising levels of drug resistance. For example, increasing acquired drug resistance (i.e., resistance emerging under treatment pressure) requires actions that improve drug quality, delivery strategies, and mechanisms to support patient adherence to drug treatment. However, increasing transmitted resistance (i.e., primary infection by a resistant strain) should trigger different interventions, such as more timely diagnosis and better treatment of individuals with drug-resistant disease and improved infection control.

Coordinated work by international agencies, led by the World Health Organization (WHO), has resulted in guidelines for population-level surveillance of drug-resistant TB, HIV, and malaria.[6–11] These protocols provide detailed methodological guidance on the design of studies to measure drug resistance and have proven to be especially valuable for countries with limited technical capacities. Furthermore, the standardization of methods ensures comparability across countries and over time. As much as possible, these guidelines adopt simple study designs and integrate surveillance activities into the standard flow and patterns of health facilities to minimize the burden on already strained providers and clinics.

Here, we summarize six key activities currently used to monitor resistance to drugs used in standardized treatment regimens for TB, HIV, and malaria (Table 1). Although there are other surveillance strategies that support these health programs (for example, Malaria Indicator Surveys and Demographic Health Surveys), the activities highlighted here were selected because of their direct link to drug resistance detection and public health response. In the discussion, we compare the strengths of the different strategies and propose modifications that might improve their capacity to more efficiently identify and deter the emergence and spread of drug resistance.

DRUG RESISTANCE SURVEILLANCE STRATEGIES

TB. Although specific TB treatment strategies differ between national programs and may be modified for certain subgroups (for example, patients undergoing retreatment upon disease recurrence), all recommended regimens in international treatment guidelines use combinations of antibiotics administered for at least 6 months.[12] For newly diagnosed patients, this includes a 2-month intensive phase during which four drugs are administered and a 4- to 6-month continuation phase when two drugs are given. The guidelines emphasize direct observation of treatment to ensure adherence during these long courses of treatment.[13] The drug-resistant TB (DRTB) surveillance strategy includes two key activities: estimating DR prevalence in new cases (no previous treatment or previous TB treatment of less than 1 month) and DRTB prevalence in retreatment cases (previous TB treatment of at least 1 month).[11]

DRTB prevalence surveys in new cases. The DRTB prevalence survey aims to measure the proportion (with corresponding confidence intervals) of new sputum smear positive TB cases with evidence of drug resistance. All new cases at all public TB diagnostic facilities are eligible for inclusion. If inclusion of all facilities is not possible, a subset may be randomly selected, with the probability of each facility being included in the sample proportionate to the number of incident cases diagnosed at that facility. Surveys can be implemented in smaller regions or districts if a national activity is not feasible.

*Address correspondence to Bethany L. Hedt, Harvard School of Public Health, 677 Huntington Ave., Boston, MA 02115. E-mail: bethhedt@gmail.com

TABLE 1
Summary of drug resistance surveillance strategies for TB, HIV, and malaria

	Tuberculosis		HIV			Malaria
	DRTB prevalence surveys in new cases	DRTB prevalence surveys in retreatment cases	HIVDR threshold survey	HIVDR monitoring survey	HIVDR EWI report	Malaria drug efficacy study
Study population	All new TB cases	All retreatment TB cases	Individuals newly infected with HIV or a surrogate population	All patients initiating treatment	All patients at a site in the specified cohort for the measure	All eligible malaria-positive cases
Design						
	All TB diagnostic centers if possible or a subset sampled with probability proportionate to size	All TB diagnostic centers if possible or a subset sampled with probability proportionate to size	Unspecified number of sentinel sites where treatment has been available to 20% of eligible patients for at least 3 years	9–30 sentinel sites	All sites offering treatment or a representative sample	4–8 sentinel sites
	Cross-sectional sample of patients	Cross-sectional sample of patients	Cross-sectional sample of patients	Cohort of patients identified and prospectively followed for 1 year	Census of patient records from a retrospectively identified cohort	Cross-sectional sample of patients identified and followed a minimum of 28 days to determine efficacy of treatment
Frequency	Every 3–5 years	Every 3–5 years	Every 1–2 years, depending on previous levels of infection	Every 3 years	Every 1 year	Minimum every 24 months
Sample size						
	Based on expected level of resistance, restrict 95% CIs to the level of precision specified by the national TB program plus 10% for lost/ untestable samples	Based on expected level of resistance, restrict 95% CIs to the level of precision specified by the national TB program plus 10% for lost/untestable samples (note that the target sample size is rarely met for retreatment cases)	Based on a truncated sequential sampling technique, sites need a minimum of 14 samples and a maximum of 47 samples with potential need to adjust for compromised samples	Restrict the 95% CI to ± 10%, regardless of the expected level of resistance, inflated for expected losses; typical sample sizes will range from 99 to 129 patients	Census (all patients at a site should be included)	Based on expected level of failure, restrict the 95% CI to ± 5% plus 20% for loss to follow-up and those excluded from analysis
	Sequential sampling until target sample size is reached	Sequential sampling until target sample size is reached	Sequential samples up to the maximum sample size	Sequential sampling until target sample size is reached		Sequential sampling until target sample size is reached.
Results	Report point estimates for resistance to each drug (and MDR) with CIs	Report point estimates for resistance to each drug (and MDR) with CIs	Classification into one of three categories of resistance: low (< 5%), moderate (5–15%), and high (> 15%)	Point estimate for possible and definite HIVDR plus CIs	Report percentage for each of the indicators for each site	Report point estimate for failures (early treatment failure, late clinical failure, and late parasitological failure) with 95% CIs
Recommendations	None	None	Classification-based: low = continue program; moderate = increase frequency of surveillance and consider alternative therapies; high = consider switch of first-line therapies	Use results and patient/ site characteristics to identify factors that increase risk for emergence of drug resistance	If a site fails to meet the target for a particular indicator, the site should be targeted for special intervention; if a percentage of sites fails to meet the target, then national interventions should be considered	Change first-line therapy if 10% or more of patients fail

CI = confidence interval; MDR = multi-drug resistant.

Survey sample sizes are calculated with an overall aim of restricting the width of the 95% confidence interval for the national estimate of resistance. To determine sample sizes, countries must specify both the expected prevalence of resistance and the desired precision of their estimates. Target sample sizes are increased by 10% to compensate for samples that may be either lost or untestable. At each participating site, eligible patients are consecutively enrolled until the target sample size is reached. Sputum is collected at the time of enrollment and sent to a reference laboratory for testing.

The guidelines recommend conducting DRTB prevalence surveys of new cases every 3–5 years. A high prevalence of DR in new cases indicates high levels of transmission of DRTB or possibly misclassification of previous drug exposure. Additional individual-level variables—such as age, sex, HIV status, and alcohol and tobacco use—as well as site-level program factors can be recorded if programs seek to understand how these variables may be associated with drug resistance. Notably, the guidelines do not offer guidance about how programs should be modified or what interventions should be introduced based on the results of this drug resistance survey.

DRTB prevalence surveys in retreatment cases. Individuals requiring a second course of TB treatment have a higher risk for drug resistance. Ideally, all retreatment cases are tested for resistance, but most countries heavily affected by TB currently have insufficient laboratory capacity to support this. Until countries develop the capacity for routine resistance testing, guidelines recommend estimating the proportion of retreatment cases with DRTB using methods identical to the prevalence surveys of new cases, as described above. In practice, many sites do not have enough retreatment patients initiating treatment during the sampling window to achieve the target sample sizes. As a result, estimates of resistance among retreatment cases often lack desired precision.

A high prevalence of resistance among retreatment cases indicates poor program implementation in the public sector, ineffectual treatment in the private sector with subsequent presentation in the public sector, or an initial infection with a resistant strain. The survey cannot distinguish between acquired and transmitted resistance because an individual could have either been infected with a resistant strain or developed resistance during the original course of treatment. The guidelines do not suggest appropriate program responses based on survey results.

HIV. Once eligible for antiretroviral therapy, an HIV-infected individual receives a lifetime course of treatment. National treatment programs generally offer one first-line option, with limited first-line alternatives for toxicities and second-line alternatives for treatment failure. The HIV drug resistance (HIVDR) surveillance strategy includes three activities: (1) a threshold survey to monitor the prevalence of HIVDR in newly infected individuals, (2) a monitoring survey to monitor the emergence of HIVDR under treatment pressure, and (3) an early warning indicator (EWI) report to monitor program factors linked to the emergence of HIVDR.[6–9] For the first two activities, specimens are tested for the presence of a pre-defined list of mutations that are validated markers of antiretroviral therapy (ART) resistance.[14,15]

HIVDR threshold survey. HIVDR threshold surveys result in the categorization of drug resistance among new HIV infections into three classes—low DR prevalence (< 5%), moderate DR prevalence (5–15%), or high DR prevalence (> 15%). A list of criteria has been developed to simplify the otherwise expensive and logistically challenging identification of individuals that are likely to be newly infected. These criteria include confirmed HIV infection, age less than 25 years (preferably less than 22 years), and if female, no previous pregnancy. When available, additional evidence, including recent high-risk behavior, early stage disease, or lack of exposure to ART, increases the confidence that a detected infection was acquired recently. The threshold survey uses sentinel sites to access these newly infected populations. Potential study sites are selected from health facilities that routinely test all patients for HIV (or offer and receive consent to test most patients) and that are attended by patients who adequately represent the entire population in the area. Selected sites should be busy enough to recruit at least 50 eligible patients over a short sampling period (< 6 months) and, as a means of providing a worst case scenario, be located in areas where ART has been accessible to at least 20% of eligible patients for at least 3 years.

The HIVDR threshold survey uses a truncated sequential sampling design that requires at most 47 completed tests to accurately classify an area into one of the three prevalence classes.[16] However, the target sample size should be increased to compensate for samples that are not viable for sequencing. Eligible patients are consecutively enrolled until the target sample size is reached. Samples are collected at the time of enrollment and sent to a reference laboratory for testing.

The threshold levels were established on the basis of historical observation, mathematical models, and expert opinion. Classification in each category links directly to a recommended programmatic response.[7] A low prevalence (< 5%) of resistance among new cases indicates that no changes to the standard drug regimen are needed, whereas a high prevalence (> 15%) suggests that modifications to the standard regimens are required. A moderate prevalence (5–15%) of resistance serves as a warning and should trigger consideration of viable alternative therapies. Additional information, such as availability and cost of alternative treatment regimens, will ultimately be factored into any policy decisions. The recommended frequency of surveillance also depends on the classification of the level of resistance—for example, biennial surveys are recommended for areas with low levels of drug resistance, and annual surveys are recommended in areas of moderate and high prevalence.

HIVDR monitoring survey. Successful deterrence of acquired resistance for an individual on treatment benefits not only the individual by maximizing the probability of sustained treatment response but also benefits the community by eliminating a potential source of transmitted HIVDR. The HIVDR monitoring survey aims to (1) estimate the proportion of patients initiating ART who have possible or definite HIVDR within the first year of treatment, (2) determine the specific drug resistance mutations that are common among individuals who do not maintain suppressed virus in the first 1 year of treatment, and (3) identify programmatic factors that are associated with the emergence of HIVDR. All treatment-naive patients as well as patients exposed to antiretroviral drugs through prevention of mother to child transmission programs, previous mono- or dual- therapy programs, or other informal mechanisms are eligible for inclusion at participating sentinel sites. Guidelines recommend a pilot phase at up to

four sentinel sites; after these sites are operational, the surveys should expand to include representative sentinel sites throughout the country. The goal is to have between 9 and 30 sentinel sites each implementing surveys on a 3-year cycle, such that one-third of the sites are conducting a survey in any given year. The sentinel sites are intended to be representative of the entire area under study; thus, the selection of sites to be included should be random and should not reflect the availability of resources for conducting studies within the sites.

The target sample size—96 patients—restricts the 95% confidence interval for the estimated proportion of patients with possible or definite resistance to less than ± 10%. Individuals who die or transfer out in the first year are unlikely to have resistance and therefore, are excluded from analysis. The sample size must be inflated to compensate for these expected losses (typical sample sizes range from 99 to 129 patients). Patients are consecutively enrolled and tested within 1 month of initiation to assess their baseline resistance profile. For subjects who remain on the first-line ART regimen, the endpoint is defined as 1 year after treatment initiation. Other analyzed endpoints include switching regimens, being lost to follow-up, and stopping treatment. Subjects that are still on first-line treatment at 12 months and who have detectable viral loads (defined as HIV RNA levels of at least 1,000 copies/mL) have their viral isolates retested for HIVDR mutations. Those with detectable HIVDR mutations are classified as definite HIVDR, whereas those with elevated viral loads but without detectable HIVDR mutations are classified as possible HIVDR.

Additional variables associated with HIVDR acquisition are collected, including individual patient factors (previous exposure to ART, baseline HIVDR mutations, ART regimens used, on-time clinic attendance, on-time drug pick-up, and ART adherence) and clinic factors (provider to patient ratios, presence of adherence support, factors related to ART access, drug supply reliability, prescribing practices, and drug quality). Analyses of the relationship of these factors to the development of HIVDR inform programs about possible target areas to reduce the risk of acquiring drug resistance. However, the guidelines do not recommend specific program changes for any observed relationships between individual/clinic factors and resistance or for any observed levels of resistance.

HIVDR EWI report. Many individual and site factors can increase the risk of developing HIVDR. The EWI report monitors these factors at HIV treatment sites using routinely collected patient or pharmacy data to identify sites vulnerable to the emergence of drug resistance. All ART patients are eligible to be included in this monitoring activity, and guidelines recommend that all (or a representative random sample of) sites be included.

The EWI reports should be compiled annually from routine program data. The following factors and corresponding targets are included in the EWI report: (1) the percentage of prescribed ART regimens that are consistent with existing guidelines (target = 100%), (2) the percentage of patients lost to follow-up within the first year of treatment (target < 20%), (3) the percentage of patients remaining on first-line treatment for at least 1 year (target > 70%), (4) the percentage of patients picking up their prescriptions on time (target > 90%), (5) the percentage of patients keeping appointments (target > 90%), (6) the percentage of patients for whom regimens were modified or interrupted because of problems with drug availability (target = 0%), (7) the percentage of patients who show > 90% adherence (target > 90%), and (8) the percentage of patients with undetectable viral load after 1 year of ART (target > 70%). The first six indicators are recommended, and the last two are optional for the EWI report. If specific sites fail to meet these targets, efforts to improve performance through education or provision of additional resources may be warranted. If many sites fail to meet these measures within a country, more comprehensive actions to improve delivery of care and thus delay the emergence of resistance are necessary.

Malaria. Unlike HIV and TB, which require lifelong or prolonged therapy, malaria is an acute infection with a 1- to 7-day treatment course. The specific therapy offered depends on the species of parasite that is found in the region or diagnosed by microscopy or rapid diagnostic test. For uncomplicated *Plasmodium falciparum* infection, the prevailing recommended therapy is artemisinin-based combination therapy (ACT). The recommended treatment of *P. vivax* infection is a blood schizonticide, such as chloroquine, followed by primaquine to eliminate hypnozoites (the parasites that are latent in the liver).[17] Malaria drug efficacy surveys measure the individual's response to therapy and do not assess *in vitro* drug susceptibility directly.[10]

Malaria drug efficacy study. Malaria drug efficacy studies quantify the proportion of patients receiving malaria treatment that experience treatment failure. These studies require that participants receive more intensive monitoring than is standard of care for patients with malaria. For individuals in the study, all drugs are administered under directly observed therapy, and active case detection persists beyond the end of drug treatment of both symptomatic and asymptomatic disease.

In areas of intense malaria transmission, children aged 6 months to 5 years with *P. falciparum* parasitemia of at least 2,000/mm^3 are eligible for inclusion in these studies. Adults are excluded because acquired immunity to malaria may contribute to successful treatment despite the presence of a drug-resistant infection.[18] In moderate- and low-transmission settings, individuals with lower levels of parasitemia and adults are eligible for enrollment because pre-existing immunity is less common. All sites providing malaria treatment are eligible for inclusion as long as they meet certain criteria. These include an adequate population density allowing enrollment of the desired sample size, infrastructure to support the required clinical and laboratory procedures, accessibility to allow for supervision, and an appropriate level of intensity of malaria transmission. The protocol suggests that sites represent all the epidemiological settings in the country; however, detailed transmission data are rarely available to inform such choices.

The target sample size restricts the 95% confidence interval for the estimated failure rates to less than ± 5% and requires a priori estimates of failure rates. The sample size should be increased by 20% to compensate for individuals that are lost to follow-up. Patients are consecutively enrolled on the day of clinical diagnosis (coinciding with treatment initiation) until the target sample size is achieved. Participants are monitored for 3 consecutive days after treatment and then one time per week for 4–6 weeks. Episodes of recurrent parasitemia require genotyping to distinguish new from recrudescent infections, and participants determined to have new infections are removed from the analysis at the time that recurrent parasitemia is detected. The guidelines describe special considerations for chloroquine efficacy studies with *P. vivax* infection, where episodes of recurrent parasitemia may be

caused by treatment failure with recrudescence of the original infection, new infection, or reactivation of hypnozoites (an occurrence that cannot be prevented without primaquine). At this time, the within-host parasite dynamics are poorly understood, and reliable genotyping techniques for *P. vivax* have only recently been reported.[19] As a result, there is currently no standardized method to distinguish between these three possibilities.

There are four possible classified outcomes in the malaria drug efficacy study: (1) early treatment failure (failed or slow clearing of parasites or the development of severe disease during the first 3 days of therapy), (2) late clinical failure (symptomatic infection occurring anytime between 4 days after treatment and the end of the study), (3) late parasitological failure (any parasitemia at least 1 week after the initial treatment), and (4) adequate clinical and parasitological response (absence of any of the failure criteria).[10] In a departure from previous protocols, the WHO now focuses on the recurrence of parasites in the blood as the key outcome of interest, even in the absence of symptoms.

In high-transmission settings, monitoring the efficacy of first- and second-line drugs is recommended every 2 years, and newly registered therapies may be evaluated in anticipation of changes to recommended drug regimens. In low-transmission settings, these surveillance activities may occur less frequently. Currently, the WHO recommends a change in national antimalarial treatment policy when combined failure rates are 10% or more. However, should ACTs reach this threshold, there are no readily available alternatives.[20]

DISCUSSION

Guidelines for surveillance of drug resistance in TB, HIV, and malaria outline methodological approaches for producing valid and useful assessments of resistance in areas that often have staggering logistical obstacles and resource constraints. There are many examples of successful implementation of TB and HIV surveillance programs (N. Wadonda-Kabondo, unpublished data and references 15 and 21–35 and references therein). Data from malaria drug efficacy studies are so abundant that there is a unified effort to both develop an organized database for assessing global antimalarial drug resistance and establish a Worldwide Antimalarial Resistance Network to coordinate efforts and collate results.[36] This paper reviews the methods for the DR surveillance guidelines rather than these observed results. The survey guidelines for each pathogen, as summarized in Table 1, differ substantially. We outline several important areas in which these studies differ and discuss how the comparison may generate alternatives for optimally balancing the validity and usefulness of the information obtained with the cost and feasibility of the study.

Clinical, *in vitro*, and molecular monitoring. Choosing to monitor drug resistance by clinical outcomes, *in vitro* response to drugs, or testing for validated molecular markers not only affects the cost and need for laboratory technical capacity but also impacts the interpretation of survey results. Clinical outcome assessment of drug efficacy offers direct information about the ability of a drug to cure the disease (or to significantly improve survival in the case of HIV). For malaria, in which clinical efficacy rather than drug resistance is the measured outcome, drug resistance is largely responsible for treatment failure, but other factors, including host immune response and pharmacokinetics, can also influence clinical outcome.[37] *In vitro* testing measures the ability of drugs to inhibit pathogen growth in a laboratory setting, but failure of cultured organisms to respond to drugs delivered *in vitro* does not necessarily lead to failure of drug therapy in a human host or vice versa. Finally, because only a subset of the genetic mechanisms responsible for drug resistance are known, existing molecular markers of resistance to individual drugs may not completely characterize drug resistance and may not be perfectly correlated to actual patient response. Selection for type of testing depends on technological constraints and national capacity to collect, store, and ship samples.

Frequency of monitoring. The recommended frequency of monitoring varies from annually to one time every 5 years and is largely motivated by resource capacity considerations. In contrast, the frequency of the HIVDR threshold surveys is explicitly linked to previously observed levels of resistance in the newly infected population. At low levels of HIVDR, the surveillance activity can be implemented biennially, because an area will unlikely move from low- to high-prevalence categories in a single year. Upcoming changes to the HIVDR threshold survey methodology will likely reduce the recommended frequency of implementation at low-prevalence levels to every 4 or 5 years. However, at moderate levels, the frequency of monitoring will increase to annually so that areas surpassing the 15% threshold can be identified quickly as a high-prevalence setting. Adapting the frequency of surveys to the current epidemiological setting should allow for timely programmatic modification while conserving resources when changes are not imminent.

Selection of sites. The HIVDR EWI report and DRTB prevalence surveys aim to include all eligible sites or, when that is not feasible, a random and therefore representative selection of sites. In reality, the selected sites may not have the infrastructure to participate, thus compromising the true randomness of the sample. The DRTB surveillance activities explicitly exclude private sector sites. Although private sector sites are not formally engaged in national treatment programs, exclusion of these sites may greatly bias results.[38]

Other survey activities make use of sentinel sites, although the principles by which sites are selected vary greatly. The HIVDR monitoring survey aims for national representation in the selection of sites. Although this is the ideal scenario, in actuality, the selected sites may be those that are better equipped and not necessarily representative. The HIVDR threshold survey targets areas with established treatment programs, because they are most likely to have circulating resistance and serve as worst case scenarios for the overall treatment programs. This allows programs to detect transmitted resistance early but may overclassify the national levels of resistance. Furthermore, these sites may only include subpopulations that are not representative of the general population, like antenatal care clinics, limiting the interpretability of the results. The malaria drug efficacy study includes the selection of sites based on epidemiological representation as well as accessibility and feasibility of implementing the activity. Often, sentinel sites engaged in this study are used for other surveillance activities, such as malaria incidence monitoring and implementation of control efforts. This continued use of particular sites improves infrastructure, which is likely to improve the quality of the malaria treatment program and thus compromises the ability to generalize the results to the national scale. Despite concerns that they are

not truly representative, sentinel sites are frequently used for surveillance in resource-poor countries, especially when high-quality sample and data collection at randomly selected sites is impossible because of limited clinical and laboratory expertise and infrastructure.

Within a country, both the prevalence of drug resistance and the associated risk factors may vary regionally. Therefore, selected sites (whether random or sentinel) should be chosen such that regional results may be obtained. Because of logistical and budget constraints, multi-region resistance studies are unlikely to occur. The HIVDR monitoring survey recommends rotating sentinel sites every year over a 3-year period. At any given time, only between 3 and 10 sites are under active surveillance, but 9–30 total sites are engaged—enough to provide a clear picture of regional variation. Designs that rely on smaller sample sizes, such as the classification scheme used for the HIVDR threshold survey, may also allow for inclusion of more sites to increase regional representation.

Measured parameters. The DRTB surveys, the HIVDR threshold survey, and the malaria drug efficacy study obtain cross-sectional measures of resistance to help national programs understand how patients may respond to standard drug regimens and allow for adjustment of treatment options if resistance levels are rising. The special eligibility criteria that aim to identify newly infected populations in the HIVDR threshold survey should allow programs to estimate the levels of resistance in currently circulating viruses. From the malaria drug efficacy study, we can infer the levels of resistance in currently circulating parasites because of both the short life cycle of mosquitoes and the short latency period of *P. falciparum* malaria in humans (even *P. vivax* hypnozoites usually reactivate within weeks to months after the initial infection). However, because of the long and variable latency periods between infection with *Mycobacterium tuberculosis* and the development of active TB disease, the levels of resistance observed in the TBDR studies do not reliably represent the levels circulating in the general population, limiting the usefulness of the results.[39]

Unfortunately, many of these surveys do not identify the causal factors leading to the emergence of resistance in a community, which would facilitate the design of more responsive public health strategies. For example, the DRTB prevalence survey in retreatment cases cannot distinguish between transmitted and acquired resistance. In contrast, the HIVDR monitoring survey enrolls a cohort of patients, measures the presence of DR mutations at baseline, and then follows these patients to assess the development of drug resistance under treatment pressure. Although the prospective design is more complicated and expensive than either retrospective or cross-sectional studies, the unambiguous distinction between transmitted versus acquired resistance improves decision making about which interventions should be used to respond to a high or growing burden of resistance.

Precision of measurement. The HIVDR monitoring survey, HIVDR EWI report, DRTB prevalence surveys, and malaria drug efficacy study all produce point estimates for the prevalence of each measure reported with corresponding confidence intervals. This information is useful for estimating the exact number of patients needing alternative therapies and tracking changes over time. The results can also be used to trigger public health action; in the example of the malaria drug efficacy study, 10% or more failures suggests a need to change the treatment regimen, although the precision of these estimates must also be considered.

Alternatively, the HIVDR threshold survey classifies an area into ranges of drug resistance prevalence. This type of outcome—classification instead of point estimation—generally requires a smaller sample size, and the HIVDR threshold design requires even fewer samples by employing a truncated sequential sampling design. Smaller surveys allow for shorter study windows and fewer tests, which may be important advantages in resource-constrained settings. Additionally, surveys that result in the categorization of areas may provide a more direct link to program response than surveys that produce point estimates of resistance. For the HIVDR threshold survey, classifying an area into the moderate-resistance category (5–15% DR prevalence) triggers two responses: an increased frequency in survey implementation and exploration of viable alternative treatment options. It seems feasible that malaria drug efficacy studies could use a similar classification scheme, with perhaps two categories ($< 10\%$ and $\geq 10\%$ resistance), as a means to save money while preserving programmatic recommendations similar to those that currently exist. For the DRTB prevalence surveys, developing a classification scheme and program guidance based on categories of drug resistance may help facilitate the translation of survey results into public health action.

Use of routine data. Many of the activities use routinely collected patient data for additional analyses but primarily rely on laboratory testing or observed patient responses to estimate levels of drug resistance or drug efficacy. These latter elements are not part of the standard of care and therefore increase the logistical complexity and costs of these surveys. As an alternative, the HIVDR EWI report relies exclusively on routinely collected program data, thus minimizing costs and allowing data to be monitored more frequently. Although these routine data (especially in areas without viral load testing) cannot definitively identify areas with drug resistance problems, the EWI report highlights problematic areas that are likely to develop resistance. This information can be used to strategically target sites for increased monitoring or interventions to reduce the risk of creating drug resistance.

Routinely collected data cannot completely replace more intensive activities, but maximizing the use of existing data may complement current drug resistance surveillance activities for malaria and TB. The use of routine program data can be limited by the quality and comprehensiveness of data collected by TB, HIV, and malaria programs. Surveillance teams must work directly with national programs to ensure the collection of relevant data elements in a high-quality and timely fashion.

CONCLUSION

Guidelines for monitoring and deterring drug resistance are critical to the success of national TB, HIV, and malaria treatment programs in resource-constrained environments. These guidelines ensure that results are comparable, meet needs for international monitoring of resistance, and minimize in-country technical support required for implementation. Additionally, the guidelines have been developed by panels of content experts and help countries interpret the clinical impact of the results, a common challenge in surveillance activities. This review provides a summary of the six most prominent

activities for drug resistance surveillance currently supported by the WHO. Each method balances the need for technical rigor with resource and capacity constraints.

Several key differences between the surveillance strategies are evident, including the types of assays used to define drug resistance, frequency of monitoring, selection of sites, measured parameters, measurement precision, and use of routine data. Future refinements of these protocols should reevaluate these choices to ensure that the results provide the necessary information for guidance of national policy. Complementary surveillance strategies that monitor patient populations excluded from surveys (e.g., patients in private or remote sites) and distinguish origin of resistance (i.e., acquired and transmitted resistance) may contribute actionable information. Furthermore, using existing program data to support surveillance strategies may provide an inexpensive means to identify sites at high risk for drug resistance.

We cannot discuss methodological considerations for drug resistance surveillance without addressing concerns about quality of implementation. Integrating surveillance into routine clinical activities often creates obstacles to high-quality implementation and interpretation. Eligible patients may be missed, samples may be compromised by poor storage and shipping, poor laboratory infrastructure can lead to inadequate testing sensitivity and specificity, and results may not be passed to policy makers for consideration. Well-developed protocols and procedures are essential for supporting drug resistance surveillance in resource-constrained countries, but protocols alone are meaningless without investing resources to ensure high-quality implementation and continuous quality management.

This paper discusses the methodological differences among existing drug resistance surveillance strategies. Specific examples of implementation of these strategies are outside the scope of this paper. Although these strategies are available and appropriate for countries needing to use population-level surveillance for drug resistance monitoring, the implementation of many of these activities is concentrated in sub-Saharan Africa. The disease epidemics and risk factors for emergence of resistance vary by region as do the obstacles for high-quality data collection and analysis. As such, one single protocol for each resistance activity may not be suitable for every context. Multiple protocols, covering a wide variety of epidemics and logistical constraints, may be necessary, provided that the interpretation and comparability of results are preserved across countries and time. Most importantly, protocols should provide clear guidance to countries on how to interpret and act on the results of these surveys.

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Authors' addresses: Bethany L. Hedt, Department of Biostatistics, Harvard School of Public Health, Boston, MA, and Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA, E-mail: bhedt@hsph.harvard.edu. Miriam K. Laufer, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, E-mail: mlaufer@medicine.umaryland.edu. Ted Cohen, Division of Global Health Equity, Brigham and Women's Hospital, Boston, MA and Department of Epidemiology, Harvard School of Public Health, Boston, MA, E-mail: tcohen@hsph.harvard.edu.

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