



Diagnostics as essential tools for containing antibacterial resistance

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ABSTRACT

Antibacterial drugs are overused and often inappropriately selected. This exacerbates drug resistance and exacts a high burden from acute respiratory tract, bloodstream, sexually-transmitted, diarrheal and other infections. Appropriate use of existing diagnostic tests, and developing better ones, could avert these costs and would avoid selective pressure from unnecessary antibacterial use. Product profiles of resistance-averting tests would specify WHO 'ASSURED' (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Deliverable) criteria and request susceptibility as well as etiological information. Advances in genomics, nanoscience, microfluidics and bioengineering, as well as innovative funding paradigms can help to overcome research and development barriers for such diagnostics if they are deliberately and forcefully applied. Rapid uptake of new tests requires timely translation of research on cost-benefit analyses into policy, value-based subsidies and reimbursements, as well as behavioral change of health care providers and users.

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1. Diagnostics: the "Achilles Heel" of antimicrobial resistance containment

Antibacterials are among the 20th century's greatest innovations and are an invaluable resource for human and animal health today, but their non-indicated use provides needless selective pressure for resistance. Antibacterial stewardship to avert this adverse societal consequence has been described as "the use of the right antibiotic, at the right dose, route and duration, for the right bacterial infection at the right time" (Dryden et al., 2009b). Several inputs, including drug supply, pharmacokinetic and pharmacodynamic information discussed in the accompanying paper by Grundmann et al. (in press) are required for stewardship. An often overlooked but necessary input is objective diagnostic support. Berkelman et al. (2006) have referred to diagnostic oversight as "The 'Achilles Heel' of global efforts to combat [infectious diseases] and the antimicrobial resistance that accompanies them".

Recognizing diagnostics as an overlooked tool for containing resistance, the Uppsala conference on "The Global Need for Effective Antibiotics—moving toward concerted action" convened

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a workshop on “mobilizing the development of diagnostics”. This diagnostics development workshop was initiated from responses to a questionnaire administered to an expert working group. Expert replies were collated and discussed in two working-group meetings and a workshop including other participants with expertise, interests and stake-holding in the field. The meetings focused on the most important issues relating to diagnostics and drug resistance, identified knowledge-gaps and roadblocks to progress and proposed next steps for spurring the development and use of diagnostics to contain antibacterial resistance. A summary of conclusions was presented to 190 delegates from 45 countries and that included leading stakeholders from civil society, academia, industry, governments, authorities, supranational organizations – at The Global Need for Concerted Antibiotics meeting, inviting further comments. This paper comprises input from all these consultations.

Experts all agree that antibacterials are prescribed in a number of instances when a bacterial infection cannot be assured largely because clinicians cannot make a precise diagnosis soon enough. Overall, it is probable that 50% of human antibacterial use could be avoided without negative consequence (Dryden et al., 2009b). However, without suitable diagnostic support, clinicians will prescribe antibacterials just in case their patients might have a bacterial infection, to protect themselves from litigation or to satisfy patient demands. When patients do require an antibacterial, they may not receive the most cost-effective alternative (Sakoulas et al., 2009; Wise et al., 1998). The overall volume of antibacterial use is correlated with resistance and declines with diagnostic information (Goossens et al., 2005; van de Sande-Bruinsma et al., 2008). The precise contribution that diagnostics could make to resistance containment has not been sufficiently studied but available evidence suggests that diagnostics may be more effective than some other interventions in preventing over-prescribing of antibacterials (Cals et al., 2010), and as discussed later in the paper, better diagnostics will also boost antibacterial development.

1.1. Life-threatening pediatric infections

Over a third of child deaths occur in the first month of life and up to 70% of bacterial isolates from recently cultured neonatal infections in developing countries are non-susceptible to affordable first-line drugs recommended for serious pediatric systemic infections (Bell et al., 2009; Zaidi et al., 2005). Emergence and spread of extended spectrum β -lactamase-producing organisms is compromising more expensive second- and third-line drugs. Child survival depends on adequate laboratory support and on up-to-date surveillance data to inform initial empiric choices. Both are also necessary to prevent the unwarranted antibacterial use that drives resistance but are underused globally and typically absent in the most resource-limited settings (Ishengoma et al., 2009; Okeke, 2011; Opondo et al., 2009; Zaidi et al., 2005). Moreover, precise diagnoses are needed to pinpoint problem areas and roadblocks to reaching Millennium Development Goal #4, which aims to reduce the 1990 under-five mortality by two-thirds (Anonymous, 2007).

1.2. Respiratory tract infections (RTI)

Acute respiratory tract infections were recently identified as one area where diagnostics would have considerable impact for treatment and in preventing antimicrobial overuse (Lim et al., 2006). RTI are the leading reason for seeking medical care and are the most common reasons why antibacterials are prescribed in the community and hospitals in Europe (Amadeo et al., 2010; Ansari et al., 2009; Goossens et al., 2005). In Asia and South America, clinical diagnosis of RTIs by the Integrated Management of Childhood Illness (IMCI) protocols leads to substantial overuse of antibacterials. Diagnostics could reduce this overuse and would annually save

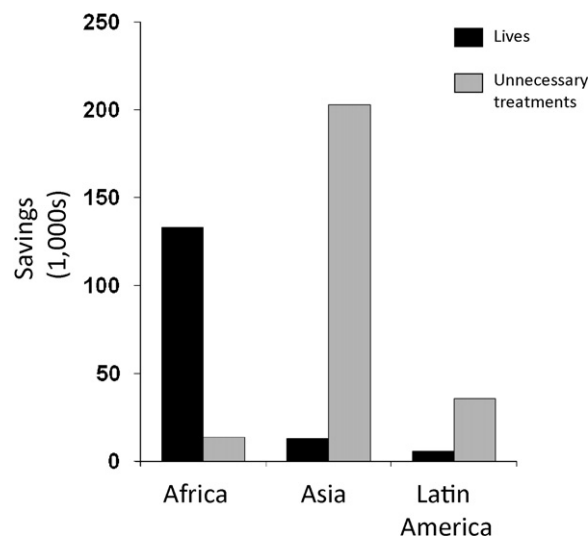


Fig. 1. Benefits of a new test for bacterial pneumonia in developing countries predicted by modeling the benefits of a new test for bacterial pneumonia for children under 5 (Giroi et al., 2006). The model assumes a population of 535 million children (based on 2004 estimates) and that each child has 5–6 acute respiratory tract infections a year. Black bars indicated lives saved from reducing disease burden and grey bars refer to unnecessary treatments saved.

almost 1,50,000 lives in Africa, where access to diagnostics and health professionals is poor (Fig. 1) (Burgess et al., 2007). There is insufficient knowledge on the etiology of RTI and almost no valid rapid diagnostic tests are available on the detection of bacterial infections. These uncertainties have resulted in prescriptive promiscuity, which largely explains the escalating antibiotic resistance of common bacterial respiratory pathogens.

1.3. Hospital-acquired infections

Dissemination of multiply-resistant clones within and among hospitals is a principal reason why resistant nosocomial infections have attained the prominence and accrued the costs they have today (Eber et al., 2010; Enright et al., 2002; Klugman, 2003). New molecular typing methods allow tracking of resistant clones but are limited to hospitals that have access to molecular testing. Rapid screening methods for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) are now also available. Inexpensive tests that can identify and track the etiologic agents of hospital outbreaks due to other bacteria transmitted in hospitals are desirable components of clinical toolkits for containing the most deadly forms of resistant infection and should be achievable with recent genomic advances (Cooke and Holmes, 2007).

1.4. Community-acquired bloodstream infections in malaria endemic areas

Rapid diagnostic tests for malaria that perform well at the point-of-care in resource-limited situations are now being introduced into African health systems and will have application throughout the malaria-endemic world (WHO, 2009). In contrast to the long-standing protocol of treating all fevers as malaria, it is now possible to make precise diagnoses for this disease, illustrating that point-of-care testing for high-burden disease is feasible in the most remote and resource-limited situations, and that it saves on antimalarial costs (Hamer et al., 2007; Shillcutt et al., 2008; Uzochukwu et al., 2009). Studies performed soon after the introduction of malaria rapid diagnostic tests revealed that some community health workers continued to administer antimalarials to patients who tested negative (Bell and Perkins, 2008; Lubell

et al., 2007). More recently, interventions to curb this behavior are producing a decline in antimalarial prescription but patients who test negative for malaria now almost invariably receive one or more prescriptions for antibiotics, shifting the overuse problem from antimalarials to antibacterials (Reyburn et al., 2007). Some of these antibacterial prescriptions will be justified, since bacterial bloodstream infections are an important and overlooked cause of systemic illness in malaria-endemic areas (Berkley et al., 2005; Blomberg et al., 2007; Evans et al., 2004; Kayange et al., 2010; Nadjm et al., 2010; Reddy et al., 2010; Reyburn et al., 2004). Unfortunately, however, in the absence of informative diagnostics, this antibiotic use is poorly targeted, increasing the overall selective pressure toward antibacterial resistance.

1.5. Sexually-transmitted infections

Bacterial sexually transmitted infections are easy to treat in early stages, but have harmful long-term sequelae and are socially stigmatizing, leading the infected to evade care. Stillbirths or debilitation from congenital syphilis, or blindness from gonorrhea or Chlamydia, can arise when children are born to infected mothers. Such problems can be easily avoided by treating infected women before their babies are born. These factors have prompted clinical algorithms but these algorithms have poor specificity, resulting in the overtreatment. As sexual partners also need to be treated, over-diagnosis has important social consequences and amplifies the impact of selective pressure. In resource-limited areas, those who receive a clinical misdiagnosis will commonly be women who are less likely to present with symptoms than men, for whom laboratory detection cannot be achieved through microscopy, and who, as caregivers, are most likely to pass on drug-resistant commensals with the genes they harbor to other individuals (Aledort et al., 2006; Hawkes et al., 1999; Mukenge-Tshibaka et al., 2002; Peeling et al., 2007; Watson-Jones et al., 2005; Zaidi et al., 2003). Time-consuming culture and susceptibility testing is possible in at least some laboratories but because patients with sexually transmitted diseases are commonly lost to follow-up, diagnosis, prescription and dispensing must ideally take place within a single health-center visit. Currently, the repertoire of point-of-care diagnostics for sexually-transmitted diseases is limited and under-utilized. Although it is universally acknowledged that resistance is increasing among *Neisseria gonorrhoeae* (Tapsall, 2005), there are currently no means for determining drug susceptibility at the point-of-care and most developing countries have little no surveillance data to inform empiric prescribing. Cheaper and more accessible tests could help to curb antibacterial consumption as well as prevent the dissemination of resistant organisms by improperly treated patients.

1.6. Antibacterial development

(So et al., in press) spotlight the slow and slim pipeline for antibacterials and the need for new innovations. Historically, clinically available narrow-spectrum agents have been underutilized and drug development programs have de-prioritized or ignored narrow spectrum hits even though their impact on resistance is lower (Dryden et al., 2009a; Payne et al., 2007). In addition to narrow-spectrum bacteriostatic and bactericidal agents, small molecules targeting bacterial adherence, virulence or signaling (Cegelski et al., 2008; Dryden et al., 2009b; Rasko and Sperandio, 2010) may have chemotherapeutic potential if paired with appropriate and rapid diagnostics. The contributions that diagnostics could make to drug development go beyond enhancing the potential of narrow-spectrum agents. The most pressing need is for antibacterials that show good efficacy against organisms that are resistant to current therapies. Patients pre-selected with appropri-

ate diagnostics can be appropriately targeted to clinical trials of new antibacterials. This will make it possible to enroll fewer patients in clinical trials and to detect improved outcomes more robustly. The reduced clinical trial denominator will make trials cheaper, easier to evaluate and quicker to complete. Such trials will generate antibacterial medicines that require affordable diagnostics for appropriate use. Thus diagnostics have the capability to advance antibacterial development just as they promote evidence-based appropriate use of existing antibacterial drugs.

The examples above illustrate that resistance-promoting drug use, adverse outcomes for patients with resistant and susceptible infections as well as roadblocks to antibacterial development are all exacerbated by inadequate availability and use of appropriate diagnostics. Among equally compelling scenarios in which diagnostic insufficiency is compromising patient care and promoting antibacterial resistance are invasive bacterial diarrheas and preventive therapy for Group B *Streptococcus* in pregnant women, both of which currently foster antibacterial overuse. Expectedly, diagnostics will not address all interventions that can contain resistance. However, while there are no direct effects of diagnostics on non-prescription use of antibacterials and the dissemination of poor quality antibacterials, to give two pertinent examples, by ensuring that the first prescription is the appropriate one, diagnostics could help to reduce both practices by respectively engendering confidence in sanctioned health providers and detecting drug counterfeits.

Using appropriate diagnostics increases the likelihood that treatment prescribed will cure the patient. Thus diagnostics are a necessary part of quality health care delivery. To optimize the management of bacterial infections and minimize resistance, it would be ideal to have five pieces of diagnostic information relayed promptly, and preferably electronically, to each prescriber at consultation. The information would provide precise answers to the following questions:

1. Does the patient have a bacterial infection, and if not, what is the cause of his/her ill health?
2. In the case of a bacterial infection, what is the causative organism?
3. What is the susceptibility pattern of the organism (or which resistance genes does it carry)?
4. Does the organism have any uncommon or novel mechanism(s) of resistance?
5. If the organism is resistant to one or more 'last resort' agents, what is the minimum inhibitory concentration?

Answers to all the questions are not required for every patient but answers to any or some of the questions will reduce inappropriate antibacterial use. Importantly, information is most useful if it is available before the first prescription must be written.

2. Limitations of present-day diagnostics as relates to drug resistance

Most diagnosis and susceptibility testing for bacterial pathogens performed today depends on culture, biochemical species identification, and diffusion or dilution methods to determine susceptibility. These methods are based on principles that are over 75 years old. For rapidly growing bacteria, they work well, allow multiple pathogens to be identified in mixed infections, and allow for follow-on analyses to identify resistance genes and strain-interrelatedness, and require infrastructure and skill sets that are attainable by many laboratories. Unfortunately, because they require the bacterial growth, these methods are slow, typically returning a susceptibility profile in 48 h or longer. For slow-

Table 1
Settings in which diagnostic tests are used (adapted with permission from Girosi et al. (2006)).

Characteristics	No infrastructure	Minimal infrastructure	Moderate infrastructure	Advanced infrastructure	Research level infrastructure
Electricity	Not available	Not reliably available/accessible	Available	Available	Available
Clean Water	Not available	Not reliably available/accessible	Available	Available	Available
Physical Infrastructure	None	Physical space but no actual lab	Poorly or minimally equipped labs	Well equipped labs	State-of-the-art
Staff	No expertise	Minimal expertise available	Nurse, some physicians, poorly or minimally trained technicians	Nurse, physicians, well trained technicians	Clinical scientists, well trained technicians
Examples of actual locations	In the community or home	Health Clinics (Africa); Rural Health Clinics (Asia, Latin America); physician's office (Europe, North America)	Hospitals (Africa); Urban Health Clinics (Asia, Latin America), Primary care clinic (Europe, North America)	Hospitals (Latin America, Asia, Europe, North America)	Reference laboratories, Tertiary care hospitals

growing organisms such as *Mycobacterium tuberculosis*, this period stretches to weeks. Even with automated systems and technologies that can shorten this biological amplification time by up to 50%, culture-based testing does not provide susceptibility information in time to inform the first antibacterial prescription. Thus, although they are valuable accompaniments to clinical care (Cooke and Holmes, 2007), conventional tests cannot promote the most judicious antibacterial use. Outside hospitals and away from clinical microbiology laboratories, although the risk of mortality may be lower, culture and susceptibility testing is difficult to implement because patients would have to return for results and remain ill and infectious in the interim. More rapid diagnostics based on nucleic acid technologies such as PCR, microarrays and sequence based diagnostics, or on advances in protein science—for example MALDI-TOF now have clinical applications and are beginning to permeate clinical diagnostic laboratories in industrialized countries.

The settings in which diagnostic tests need to be used vary widely (Table 1). Many existing tests are currently most needed and least applied in settings with no or minimal infrastructure because they are too expensive and require sophisticated equipment and training. Routine culture and susceptibility testing can be provided in some resource-limited settings (Polage et al., 2006) but in many more, testing is not possible and the need for other technologies is even more pressing. A 2009 assessment of laboratories found that no laboratory in the Tanga region of Tanzania offered bacterial culture and susceptibility testing, even to support diagnosis of life-threatening infections like meningitis and bacteremia (Ishengoma et al., 2009). Similar assessments have come from other developing countries (Okeke, 2011; Tegbaru et al., 2004). In addition to the requirements for technical expertise, aseptic technique and infectious waste handling, many tests require elaborate sample preparation, which is not feasible at the point-of-care and difficult to implement without appropriate laboratory infrastructure.

Culture is a reference standard, but other, faster, options exist. These include immunoassays, nucleic acid detection by standard or real-time PCR, hybridization (including microarrays) as well as methods that identify 16S or other pathogen-specific methods directly in patient specimens, and tests for pathogen antigens or host biomarkers. First-generation immuno assays and nucleic acid tests may require sophisticated equipment and skilled expertise. Additional specialized expertise is needed to routinely use 16S rDNA sequencing, deep sequencing, MALDI-TOF, and other newer technologies for diagnostic purposes. Biomarker tests seek a host factor that is, for example, elevated when a bacterial infection is present, often using simple protocols and materials. Examples include C-reactive protein, a marker of systemic bacterial infection and leucocytes visible by methylene-blue staining or lactoferrin, which are inexpensively targeted markers of inflammatory diarrhea in stool. Biomarker tests have only recently become available

and although they show promise in some sub-populations, in others, significant cut offs are not yet known or have only been preliminarily investigated (Carrol et al., 2009; Opintan et al., 2010). Moreover, parasites as well as bacteria can elicit the inflammation on which such tests are based. These newer tests have reduced turn-around time and some have been shown to reduce antibacterial prescription (Cals et al., 2010; Lars et al., 2004). They however stop short of providing susceptibility information, which is needed to inform antibacterial selections when a bacterial infection is present. This deficit is partially ameliorated when these data are available from systematic epidemiological surveillance but rapid tests that could provide susceptibility information would be valuable.

3. Roadblocks associated with developing and using resistance-averting diagnostics

3.1. Roadblocks—research and development

3.1.1. Moving targets

Because microorganisms evolve rapidly, microbiological diagnostics need to evolve as well. This is particularly true for drug resistance, where new mechanisms are constantly emerging. In a hypothetical example for a β -lactamase diagnostic, which would have initially targeted TEM and SHV enzymes, it would have been necessary to adapt the test to detect OXA enzymes and then CTX-M and KPC extended-spectrum β -lactamases. One or more adaptations would be required to incorporate IMP, VIM and other metallo- β -lactamases and most recently, it might have been necessary to tinker with the test again to detect the NDM-1 β -lactamase. This ‘moving target’ is a disincentive for diagnostic test development, analogous to one of the many disincentives for developing antibacterial drugs, and makes it difficult to ensure that tests are also inexpensive and user-friendly. The moving target conundrum calls for hyperflexible platforms that can be adapted as and when new resistance genes or target microbes evolve. Robust but flexible platforms will also allow for tests to be adapted to the different disease ecologies that occur in different parts of the world.

3.1.2. Sample preparation

Sample preparation is one of the major roadblocks for developing sensitive tests because the microbial load in the sample can vary and in some cases is very low (Yager et al., 2008). Nucleic acid targets are among the easiest to identify and nucleic acid-based platforms are versatile. However these tests depend upon obtaining intact target nucleic acid from complex patient specimens, which inevitably contain nucleases. Similarly, many immunochromatographic tests require some level of antigen purification. Sample preparation is therefore a current bottleneck for converting many promising targets into the miniaturized diagnostics that hold the

greatest potential for use at the point-of-care (Dineva et al., 2007). Emerging technologies, such as sophisticated microfluidics offer some promise in this area, but still require considerable basic research to produce robust and versatile platforms that will perform in the most demanding settings (Yager et al., 2008). In order to overcome the sample preparation challenge, test developers must use specimens from real patients. These are often difficult to come by and could be accessed more easily through the development and use of specimen banks and, in the case of infectious diseases endemic to specific geographic localities, in-country research and appropriate research networks (Mabey et al., 2004; Okeke and Wain, 2008).

3.1.3. Inadequate focus on surveillance

Resistance surveillance is critical to understanding the status and trajectory of antibacterial resistance and containing the problem. There are a number of general and disease-specific surveillance networks but most have little or no coverage in many parts of the world (Grundmann et al., *in press*). Global surveillance is a “weakest link public good” (Barrett, 2006) and the current uneven landscape means that we have limited capability to detect resistance emergence ahead of dissemination. There is very little focus on developing diagnostics for surveillance, which, in addition to identifying the causative organism and its susceptibility pattern, would need to determine similarities among isolates and resistance genes. Surveillance is also important for determining which diagnostics will be needed as well as when and where. Therefore, just as diagnostics are needed to bolster surveillance, surveillance boosts diagnostics development and use.

3.1.4. Fragmented expertise and the need of increased R&D exchange

Developing diagnostics is often wrongly perceived to be an endeavor with low innovation potential (Pettersson et al., 1987). It will be essential to induce the best scientists to the interdisciplinary enterprise of diagnostics development. In order to develop sensitive, specific and useable point-of-care, we will need significant advances in pathogen and biomarker biology for target-finding, microfluidics for sample processing, target amplification, component design and assembly, detection technology, as well as data collection, handling and dissemination. Multiplex diagnostics capable of detecting the most common pathogens associated with syndromes that cannot be resolved clinically, particularly fever, acute respiratory tract infections and diarrhea, require expertise in parasitology, virology and bacteriology at the front end of the development process. Application of all knowledge bases and earlier discoveries, to diagnostics will also require sophisticated handling of intellectual property challenges associated with multiple innovations, particularly ones that involve biological targets and processes. The Global Strategy of WHO's recently convened Inter-governmental Working Group on Innovation, Intellectual Property and Public Health could provide a way forward in this regard. There is also a better need for policymakers to understand the diagnostic development process, to characterize and document the pipeline and to identify innovation system gaps as well as the points in the process at which candidate tests are most likely to fail. Very few groups involved in test development are addressing all facets of the diagnostic challenge and we need better communication among groups, for example to ensure that optimal detection platforms are paired with optimal sample processing. Currently, there is insufficient exchange between the public and the private sector, or among diagnostic and pharmaceutical industries. Recent public-private initiatives have resulted in product development partnerships, such as those described in Box 1, may address some of these issues (Hunter, 2008; Mboya-Okeyo et al., 2009). A recent call for proposals from the Bill & Melinda Gates Foundation and Grand

Challenges Canada, aims to overcome the problems associated with linking different innovations by allocating funds for component building in phase 1 and then funding a second phase to support integration of the “best-in-class” from each component (Box 1). Clinicians also need to be more tightly connected to the diagnostics development process, to ensure that the most useable tests emerge. As an example, recently introduced rapid molecular tests for sepsis diagnosis were not thoroughly assessed for their ‘added clinical value’ compared to conventional existing gold standard tests such as blood culture, or clinical diagnosis (Mancini et al., 2010). The developers of nucleic acid tests for sepsis did not incorporate clinician decision-making nor did they estimate the potential impact of different test strategies on appropriate targeting and adequacy of antibacterial therapy for sepsis patients. Diagnostic test developers are also often unfamiliar with the nuances associated with test use in resource-limited settings.

3.1.5. Test evaluation and regulation

A 2004 report observed that 45 of 85 surveyed countries, virtually all of which regulated medicines and health professional practice, do not regulate diagnostics (Mabey et al., 2004). For those that do, there are no universal standards for test evaluation and most do not require clinical trials (Mabey et al., 2004; Peeling et al., 2006b). As such, diagnostic evaluations are often not predictive of in-use conditions. They may use disparate populations, impracticable facilities and small sample sizes (Bachmann et al., 2006; Peeling et al., 2006b; Smidt et al., 2006). The Standards for Reporting Diagnostics Accuracy adopted by about a dozen journals provides a checklist for evaluating diagnostic studies and aims to improve the quality of diagnostic evaluation overall (Bossuyt et al., 2003a,b,c). These Standards have led to a noticeable improvement in the quality of published diagnostic test evaluations but improvements are still needed (Smidt et al., 2006). When evaluations are properly performed, it is often unclear how products should be regulated and registered.

3.1.6. Funding

Funding and perceived return on investment is a primary roadblock to the development of diagnostics, particularly those that would have the most benefit in resource-limited settings. As the same disincentives for developing drugs for poor patients apply to diagnostics, and so will their solutions (So et al., *in press*; Usdin et al., 2006). Progress made in recent years has allowed some of the best advances for human diagnostics to develop innovative tests for malaria, tuberculosis and HIV in spite of market disincentives (Boehme et al., 2010; Larsen, 2008; Usdin et al., 2006). A pre-market commitment is presently lacking for many other diagnostics, particularly those that could assist in containing bacterial resistance. If the constraints associated with testing in resource limited systems are taken into account during development, a single assay platform should be able to serve both developed and developing country communities. Mechanisms are needed to encourage researchers to produce globally applicable tests where possible. There has been a recent increase in available funding for diagnostic development (Box 1), in part spurred by the rising costs of antimicrobial chemotherapy due to resistance. The parallel publication “explosion” (Yager et al., 2008) demonstrates that increased funding can promote research on diagnostics. However, levels of funding and resources for diagnostics research and development are still far below what is available for drugs and vaccines. Many recent calls that focus on diagnostics have been for short-term projects and do not acknowledge the long-term investment that may be needed to overcome the formidable technical challenges that must be overcome to make point-of-care diagnostics. Funding is also needed for basic microbiology, chemistry and nanoscience research, which could overcome technical roadblocks to diagnos-

Box 1: Examples of diagnostic development initiatives Funding and Technology

- The European Union has recently funded a number of diagnostic development projects. These include InTopSens (“A highly integrated Optical Sensor for point-of-care label free identification of pathogenic bacteria and their antibiotic resistance”), which aims to develop a tool for detection of sepsis pathogens and relevant antibiotic resistances using label-free biosensors; TheraEDGE, which will develop a viable molecular diagnostic test for respiratory bacterial and viral pathogens and relevant antibiotic resistances using single-molecule detection techniques with a target turnaround time of under an hour and RAPP-ID (“Development of RApid Point-of-Care test Platforms for Infectious Diseases”) to develop point-of-care platforms for respiratory infections, sepsis and TB.
- Europe’s Innovative Medicines Initiative (IMI), a public private partnership that aims to support more rapid discovery and development of better medicines for patients, has extended its focus and include some diagnostic development.
- Grand Challenges Canada has called for proposals to develop diagnostic technologies and plans a second call to ensure that different innovations are linked to produce workable point-of-care diagnostics.
- The Bill and Melinda Gates Foundation-supported Foundation for (FIND) is using modern technologies to develop diagnostics for resource-limited laboratories. FIND was the key player in a public-private partnership that resulted in the development and field testing of Xpert MTB/FIF, an automated molecular platform for detecting TB infection and identifying rifampicin-resistant (which are often multidrug resistant) strains (Boehme et al., 2010). FIND is also supported by the US National Institutes of Health (NIH) and the UK Department for International Development (DFID), both of which are increasing support for diagnostics development.
- NIH has issued calls to develop point of care diagnostics, including those for nontraditional health care settings that would include resource-limited areas. It has also promoted public-private consortial arrangements and offers contract research services for specific development tasks in which test developers may lack infrastructure or expertise.
- The Program for Appropriate Technology in Health (PATH) and investigators at the University of Washington are developing multiplex diagnostics for diarrheal disease and acute fever. The aim is to produce a microfluidics card or “lab on a chip” that would be suitable for point-of-care use in developing countries http://www.path.org/projects/microfluidics_card.php. PATH’s center for point-of-care diagnostics also provides funding and support for field testing of diagnostic test candidates that have promise for resource-limited health care systems.

Networking and Implementation

- The WHO TDR program, the African Development Bank, the EU and other partners inaugurated the African Network for Drugs and Diagnostics Innovation (ANDI) to promote local research and development (Mboya-Okeyo et al., 2009). Asian and South American counterparts of ANDI have also been recently launched.
- To assist developing countries with the challenge of regulating diagnostic products and selecting high-quality products for the public sector, the WHO has established prequalification programs for some diagnostics (WHO, 2009; WHO/TDR, 2008). It is hoped that new diagnostics that are developed to support antimicrobial containment will receive this type of support.
- Recent initiatives by the Global Fund, the Clinical Laboratory Standards Institute, the American Society for Microbiology, the African Society for Laboratory Medicine and other professional organizations to build laboratory capacity in developing countries are welcome and timely and will assist in the effort to boost diagnostic capabilities worldwide.

tics. Currently, most diagnostic research is performed in academic institutions and within small and medium sized enterprises, which are highly grant-dependent. Researchers in these environments, being more distant from markets than are large companies, incur greater risk and have fewer resources for field evaluation. In addition, small and medium sized enterprises are very dependent on venture capital and therefore their capabilities are easily influenced by fluctuations in the financial market.

3.1.7. Time to development

Interest and appreciation in the value for diagnostics has increased exponentially in the last five years. However users will have to wait many more years for research and development challenges to be overcome, and for necessary tools to reach the market because, as shown in Fig. 2, it can take up to 10 years to develop a priority diagnostic. This long-term investment is a roadblock for test development. It is also a barrier to test use because the absence of a needed test entrenches substitute behaviors and practices, which may be difficult to change when a suitable test becomes available.

3.2. Roadblocks—the use of diagnostics

3.2.1. Test cost

Appropriate diagnostics for resistance control will necessitate increased volume and diversity of work for clinical laboratories. In resource-limited areas, this means that new laboratories will have

to be built, equipped and staffed. In higher-income countries that already have good laboratory networks, increased volume must be accompanied by increased automation because staffing is costly. Ideally, diagnostics for infectious diseases would be less expensive than antibacterial drugs. However, many recently developed rapid tests are expensive, perhaps rightly so, given their absolute cost and cost of development. Costs could fall with market penetration and increased use but presently, high prices impede introduction of new tests into resource-constrained and budget-conscious health systems. Paradoxically, low uptake in turn reduces the incentive to develop diagnostics and keeps the price of diagnosis high.

3.2.2. Test speed

For diagnostics to impact selective pressure from antibiotic use, speed is critical. Most current tests require culture of the organism as an essential first step. This amplification typically takes 18 h or longer, for fast-growing species even though it is presently feasible to modify current protocols to reduce incubation times without compromising sensitivity or specificity. Thus, there is a pressing need to improve detection speed for culture-based methods and to develop tests that return etiology and susceptibility results without requiring prior organism culture. Nucleic acid amplification tests are rapid but have not, as predicted, replaced culture-based detection as predicted two decades ago. Most nucleic acid amplification tests are complex to operate, and require cumbersome DNA extraction steps which limits their feasibility in standard microbiology laboratories.

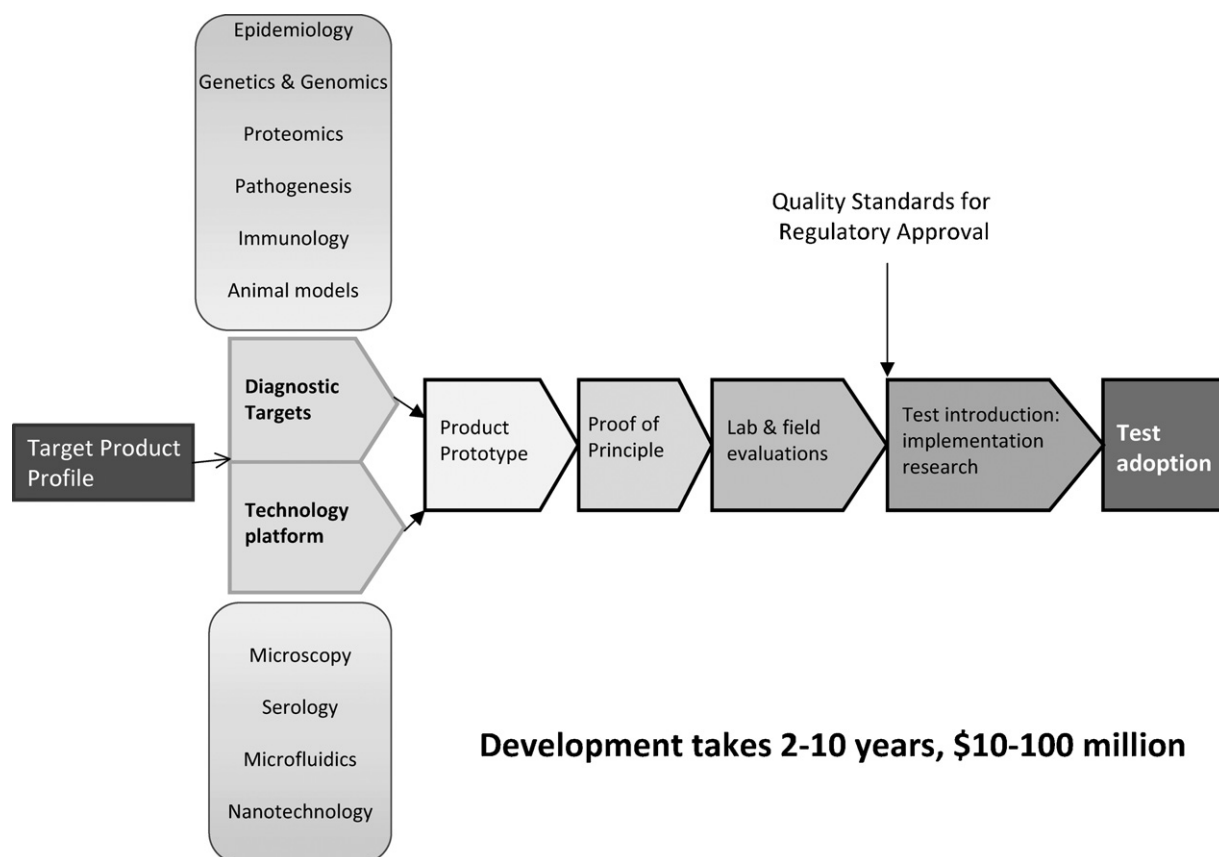


Fig. 2. Innovation pathway for appropriate diagnostics.

3.2.3. Test spectrum

Diagnostics are most useful clinically when they can inform patient care (Wootton, 2006). Many recently developed rapid diagnostics identify only a single pathogen (e.g. rapid malaria and MRSA tests). Single tests are an important first step, particularly for very common pathogens, but they limit the overall diagnostic value. Diagnostic flowcharts or multiplexes may increase the cost-effectiveness of testing and treatment since they offer more patients a precise diagnosis and they reduce the chance that an antibacterial will be prescribed when a single negative test is returned. Only when the indirect but heavy cost from drug resistance is considered will the true value of such tests be visible. In resource-limited settings, the absence of alternate treatments may also deter the use of diagnostics. Multiplex tests however, are even more challenging to develop than single pathogen tests and needed testing panels may vary geographically (Yager et al., 2008). While they may reduce the cost of diagnosis for uncommon infections, they increase the absolute cost for diagnosing more common ones. They may also be more difficult to set up and interpret.

3.2.4. Sample collection and test complexity

Many of the specimens needed for today's tests are difficult to access. In areas where trained physicians or nurses are not available, collecting spinal fluid, blood, vaginal swabs and other invasive or semi-invasive specimens may be impossible. Just as the availability of trained health professionals will dictate specimen accessibility, test accessibility is also determined by the level of training laboratory technicians have received. Tests yielding multiple or quantitative end-points (such as titers) may be particularly hard for semi-trained technicians to perform and clinicians to interpret. Even when optimally trained personnel attend a patient, the sample sent to the lab on occasion lacks diagnostic value because

extraneous contamination was not avoided, the sample container was inappropriate or the patient was too ill to provide sufficient sample.

3.2.5. Test limitations

Many rapid and molecular tests can only detect known mechanisms of resistance so that newly emerged mechanisms will be missed. Molecular methods may provide false positives since they detect unexpressed genes. Tests that do not require isolation and identification of a causative organism may reduce the chance that an unusual strain or specimen is sent to a reference laboratory for follow-up, unless specific protocols are put into place to ensure this. In many cases, the true limitations of tests are unknown. Studies evaluating diagnostic tests are often not rigorous enough and in some cases are not performed (Banoo et al., 2006; Peeling et al., 2006a,b). In some cases, evaluations are difficult to perform, particularly in those instances when reference standards are not sensitive or specific and protocols for evaluations do not exist. Tests may perform differently in different parts of the world due to variations in pathogen prevalence, disease severity or host genetics or immune status (Leeftang et al., 2009; Peeling et al., 2006b). Moreover, the nature of clinical expertise paired with a test may influence false-positive and false-negative rates. These factors make it challenging for health systems to select the tests that will optimize patient care and correctly inform prescribing.

3.2.6. Biosafety

Testing exposes individuals other than the patient and his or her caregiver to potentially pathogenic organisms, often in a biologically amplified form. Wherever testing is to be introduced, it is key to provide protection for health workers during sample collection and processing, and to assure safe disposal. These cannot

be guaranteed in resource-limited settings, where health workers have become infected as a result of testing (Mason, 2008; Yager et al., 2008). Thus, biosafe alternative methods, such as molecular testing following sample inactivation, or accessories to ensure safe test use, such as solar disinfection systems, need to be developed and used (Nathavitharana et al., 2007).

3.2.7. Access

Diagnostics are less accessible than medicines, even for disease conditions where they have been prioritized, such as HIV. Recent years have seen some strengthening of global laboratory infrastructure and the development of rapid tests for TB, HIV and malaria that are being put into use in resource-limited settings. Many large programs supporting drug access, particularly those that are donor-driven, tend to be vertical, whereas diagnostic development to contain resistance will have to be a horizontal process. To have optimal impact, tests must be performed and their results used. Worldwide, most outpatients can only afford to see a consulting physician, health-worker, or in some instances unlicensed practitioner, once. Thus, diagnostic information that is not available at the point of care may not influence drug choice or contain resistance.

3.2.8. Supply chain management, technology transfer and local production

Governments and health care aid programs for developing countries that distribute medicines require similar, integrated programs for diagnostics and accessories. Supply chain failures in any area negatively impact evidence-based health care delivery. For example, in a Uganda clinic, stock outs of gloves prevented malaria diagnostic testing when antimalarial drugs and rapid diagnostic tests were in stock (Kyabayinze et al., 2010). Although diagnostics may be introduced through donor-supported programs, their availability needs to be assured irrespective of donor commitment. For resource limited health systems, particularly in the case of tests for which the market elsewhere is small, these objectives may best be achieved by local manufacture. There are notable exceptions in emerging economies but in many of the least affluent countries, a bouquet of roadblocks – ranging from start-up and operating costs to shortage of biomedical and bioengineering expertise and regulatory bottlenecks – will be needed to make local production and distribution possible.

Table 3

Diagnostics as tools for limiting antibacterial resistance: next steps.

Need for action	Next steps
Strengthening the case for diagnostics	In depth situation analysis to better understand the challenges for diagnostic development and use State a compelling case for diagnostics in resistance containment in multiple venues
Product profile and development	Develop target product profiles that incorporate the meet the <u>A</u> ffordable, <u>S</u> ensitive, <u>S</u> pecific, <u>U</u> ser-friendly, <u>R</u> obust and <u>R</u> apid, <u>E</u> quipment-free and <u>D</u> eliverable to areas of need (“ASSURED”) criteria concept and will promote maximal antibiotic resistance containment Ensure susceptibility is included in diagnostic product profiles for bacterial infections Research suites of tests for given localities Develop local surveillance systems to ensure appropriate product development
Increased research and development, collaborations and information exchange	New funding programs that promote longer cycles and public-private initiatives Create appropriate networking platforms and resources Apply modern technologies and evaluating diagnostics Joint academia – health care – industry initiatives that include researchers from developing countries Closer collaboration between the pharmaceutical and diagnostics industries
Uptake by health systems	Advocating routine use of existing diagnostics Harmonized regulation for diagnostics between different countries Research to identify behavioral determinants influencing diagnostics use and interventions to promote effective integration of testing into health practices
Making the cost-effectiveness of bacterial diagnostics more visible	Cost-benefit analyses Offering equivalent or greater subsidies and reimbursements for diagnostics, as compared to medicines

Table 2

Modeling demonstrates that tests requiring less infrastructure produce large health benefits, even with less than perfect performance. Results assume access to testing.

	Lives saved by new test for bacterial pneumonia	
	Good performance	Perfect performance
Minimal infrastructure	405,000	596,000
Advanced infrastructure	142,000	261,000

3.2.9. Testing environment and culture

Hospital laboratories are being downsized and medical and allied health education programs are changing, de-emphasizing microbiology and thereby compromising testing and depreciating the importance of test results in clinical diagnosis. In the US, medical students no longer have to take a practical (wet) microbiology laboratory and in many developing countries, such laboratories have been cut or discontinued due to funding constraints. In high-income countries, diagnostic facilities are increasingly being centralized. This has the advantages of reducing costs and increasing the scope of testing available, particularly for rarely performed tests. It also offers ‘out of hours’ testing to patients at institutions that cannot offer such a service. Centralization however adds transportation time to the time-to-diagnosis and hampers communication between laboratory personnel and physicians (Raoult et al., 2004). Bacteriology laboratories are uncommon in some countries and new HIV and/or TB laboratory programs often do not improve capacity in basic bacteriology even though such methods are inexpensive and easier to set up. Many developing countries have no accredited laboratories or routes to accreditation (Olmsted et al., 2010). Reassuringly, an African Society for Laboratory Medicine will be launched in March 2011 with the aim of promoting quality of laboratories in Africa. The Society will carry out accreditation for laboratories at different levels of the health care system.

4. Diagnostics as tools for limiting antibacterial resistance

We have identified a number of steps that should be taken to move toward better use of diagnostic technologies in limiting antibacterial resistance. These steps, summarized in Table 3 and described in more detail below, cover a breadth of areas, from pol-

icy and economics, to technical and behavioral. Concerted efforts by multiple stakeholders – governments, health care providers, funding agencies, private industries, regulatory authorities, academic researchers, as well as patients and the general public – will be required to complete these actions.

4.1. Strengthening the case for diagnostic development

Diagnostics point to a cure but do not produce one, leading many clinicians, patients and policymakers to undervalue them. There are several, potentially high-impact interventions that could contain resistance by effecting disease and infection control. Diagnostics are often rightly ranked below these strategies in terms of prioritization. However diagnostics are not merely preventive interventions, they are essential components of curative ones and their use should therefore be considered in the context of drug use, as well as for prevention. In the absence of a concerted interest in containing resistance, diagnostics may be perceived as cost-ineffective. Thus diagnostics need to be ‘marketed’ as part of the effort to conserve medicines because their benefits often accrue to health systems and regions, and not just to individual patients, particularly where they address antibacterial resistance. In addition to making the case for diagnostics, it is necessary to identify the areas that will produce the most gain. This can be done through the convening of experts and through modeling approaches, such as those recently performed by the RAND Corporation, in combination with a regular market analysis for bacterial infections diagnostics (Giroi et al., 2006; Urdea et al., 2006).

4.2. Product profile and development

Developing product profiles (functional requirement specifications) to meet clearly defined needs, including agreed roadmaps for point-of-care test development is a priority for advancing resistance-averting diagnostics. This should be done with a broad range of expertise and include stakeholders from academia, health care, industry and regulatory authorities. At the very least, point-of-care diagnostics, particularly those that will be used in resource limited settings, must be Affordable, Sensitive, Specific, User-friendly (requiring minimal training), Rapid and Robust, (possible to transport, store and use at high ambient temperature and humidity), Equipment-free and Deliverable to areas of need (“ASSURED”) (Mabey et al., 2004; Peeling et al., 2006a,b). To impact resistance, they must rapidly – within 30 min – delineate bacterial infections from those that are viral, parasitic, fungal or non-infectious, with high specificity and sensitivity, and at a price that is cheaper than the most commonly used antibacterial treatments. For community-acquired infections in resource limited areas, there is a pressing need for rapid diagnostics that can be used with limited amount of training and ideally no requirement for equipment, electricity, extraneous reagents (including water) and employing patient specimens that can be collected non-invasively. They should have some form of internal quality assurance, and results should be available in less than an hour. In principle, many diagnostics that are used in primary care settings elsewhere could also be of use in resource-poor settings. However, it must also be possible to transport, store and use tests at high ambient temperature and humidity levels. Finally, any target product profile for a diagnostic to be used in resource-limited settings, must contain input from practitioners working at such locations.

Many existing point-of-care tests identify or point to an etiologic agent but do not return a susceptibility test result. Profiles for products that provide susceptibility information, even if they overlook etiology will be important for containing resistance. Point-of-care tests along this line are now foreseeable: it should be possible to develop a point-of-care test that detects extended spectrum β -

lactamases in urine or sputum, for example. Affordability is vital in all settings. If laboratories in high-income countries are to increase the volume of specimens handled substantially, automated testing platforms may be necessary. Overall, the profile of an ideal product will be difficult to meet and therefore insisting on all criteria in a single product could stifle development. It is therefore important for experts to weigh criteria and to be willing to compromise on non-essential features when profiles are developed. The FDA Clinical Laboratory Improvement Amendments (1988) propose that lower performance levels may be acceptable for “simple tests”, such as automated instrumentation or point-of-care diagnostics. The idea is that a sensitivity as low as 70% may be accepted for tests used in physicians’ offices in high-income countries, as long as they minimize the chance of human error (FDA, 2008). In resource-limited countries where point-of-care tests are likely to be performed by partially trained personnel, there is every chance that tests will perform below their stated accuracy. However, even though such tests will be applied to life-threatening infections, modeling has shown that in areas where access to care is limited and laboratory facilities are minimal or non-existent, lower test performance may be tolerable (Table 2) (Burgess et al., 2007).

Suites of essential diagnostic tests, that is, region-specific ‘essential tests lists’, must be locally tailored to ensure that common endemic diseases are covered. This in turn requires surveillance at levels that currently do not occur in many low-income countries and a requirement for reference laboratories with superior diagnostic facilities. Current and future evidence-based medical practice depends on the quantity and quality of available surveillance data. Diagnostics can improve both. The rapid development and clinical introduction of HIV laboratory diagnostics and point-of-care malaria diagnostics in many parts of Africa demonstrates that both laboratory-based and point-of-care diagnostic tests can be used in resource-limited health systems and that they do improve the quality of care and precision of antimicrobial chemotherapy (Hopkins et al., 2009; Kyabayinze et al., 2010; Larsen, 2008). Tests that can identify patients with bacterial infections would have a similar potential.

4.3. Research funding

One of the most obvious needs is a further augmentation of existing funding initiatives (Box 1), and in particular, initiating long-term support programs that will allow a concerted battle against impeding roadblocks. Many of the market-based mechanisms for research and development highlighted in the accompanying paper by (So et al., in press) could, and therefore should, be applied to diagnostics. Incentives and granting programs that encourage the development of flexible diagnostic platforms, integration of multiple targets per syndrome into a single test, as well as developing diagnostics that provide information on antibacterial susceptibility are especially needed. Existing programs that support drug development should be recast as supporting health innovations that include diagnostics. This will encourage investigators working at the cutting edge to be attracted to diagnostic innovation. We also need new business models to make diagnostic development more attractive to industry. Models that have been successful in promoting anti-infective drug discovery would mostly apply but there is also need for further incentive building, with the ultimate goal of developing diagnostics that are cheaper than medicines.

In addition to supporting applied research directly focused on diagnostics, there is need to invest in basic science projects that will fill knowledge gaps. Examples include microbiology research on the nature and density of pathogen material in infected specimens, microfluidic strategies for processing specimens and amplifying targets at point-of-care, nanoscience and bioengineer-

ing innovations that could make it possible to miniaturize tests and biophysical detection systems that obliterate the need for sophisticated equipment. Finally, very little is known about health-seeking and health practice behaviors that promote or retard the introduction of diagnostic tools into different types of health systems. Programs are needed to support social and behavioral research as well as modeling studies that assess diagnostic needs and cost-effectiveness.

4.4. Applying modern technologies and evaluating diagnostics

A wide range of new technologies are applicable to diagnostics research (Fig. 2). Genomic and proteomic methods increase the efficacy of finding diagnostic targets and other technologies will result in faster, cheaper and more reliable tests. For example, nanotechnology and microfluidics may make it possible to develop molecular tests on small, disposable and cheap platforms that can be used at the point-of-care. Other technologies often perceived as high cost, such as surface plasmon resonance, MALDI-TOF, automated molecular tests, microarray-based methods, become more cost effective if used routinely and intensively. These and other technologies have the potential to decrease the time required for detection of diagnostic targets, such as pathogen-derived proteins and DNA, from hours to minutes and will revolutionize the development of diagnostics in the next few years. A number of in-progress diagnostic initiatives using these technologies are currently in progress (Box 1). The resulting new diagnostics must be rigorously evaluated according to appropriate standards (Banoo et al., 2006; Bossuyt et al., 2003a,b,c; Peeling et al., 2006b).

4.5. Collaboration and information exchange

There is a need for closer collaboration between the pharmaceutical and diagnostics industries and better interactions among all stakeholders. We envision joint academia-industry initiatives recruiting broad diagnostic expertise to develop, evaluate, validate and implement new resistance-averting diagnostics. Therefore, it is essential to create appropriate networking and information-exchange platforms and resources. Special attempts must be made to include developing-country researchers, who work in areas with the greatest burden of disease (Okeke, 2011; Okeke and Wain, 2008; Peeling and Mabey, 2010). A global diagnostics database that includes information on potential and tried targets and technologies, which also offers networking opportunities for investigators, may be the option.

4.6. Uptake by health systems

Where possible, it would be advantageous to develop some tests that apply to different health systems irrespective of resource and location. This will make it possible to introduce differential pricing schemes that could make such tests globally accessible. Regulatory pathways for diagnostics need to become faster, more uniform, more transparent and easier to navigate. Global or regional harmonization of regulatory requirements will make it unnecessary for companies to conduct a clinical trial in every country to obtain approval and the WHO's bulk procurement scheme, which lists tests with acceptable performance, can offer tests to Ministries of Health in developing countries at negotiated pricing.

The potential benefit of optimized diagnostic procedures in current clinical practice should be modeled. Social, ethical, environmental, economical, and political factors, that influence the adoption of new diagnostic technologies and delivery into health systems, should be identified. When available, diagnostic tests and services are typically underutilized (Polage et al., 2006), pointing to a need for input from behavioral scientists and social marketing

experts to identify and address barriers for acceptance diagnostics, particularly at the point-of-care, as well as to understand motivational factors which may help overcoming hurdles to effectively use appropriate diagnostics in patient management. These findings must be used to develop and implement better education of policy makers, prescribers and patients. This can be done as part of antibacterial resistance containment initiatives as well as by bolstering existing resources on diagnostics.

More immediately, existing diagnostics have an important but underexploited role in containing antibacterial resistance today. Although bacterial culture followed by diffusion or dilution testing is typically too slow to inform the first empiric prescription, in the current era of multiple resistance, pre-emptive culture of initial specimens can inform a second prescription in the event that one is necessary (Sundqvist and Kahlmeter, 2009). At the point of care, a C-Reactive Protein (CRP) test has been shown to be effective in reducing antibiotic prescribing for acute respiratory tract infections (Andre et al., 2005; Cals et al., 2010; Jakobsen et al., 2010; Takemura et al., 2005), as have streptococcal antigen tests in the US and France. Other existing biomarker, microscopy and pathogen antigen tests can produce rapid results to inform the first prescription and all illustrate that it will be worthwhile to develop tests that return even more information (Charles and Grayson, 2007).

4.7. Costs and cost-effectiveness

Antibacterial drugs are currently often underpriced, in that their sticker price does not include the cost of resistance. Nonetheless, many patients that need these life-saving therapies cannot afford them and they are therefore often further subsidized. Treatment, reimbursement and subsidy costing need to be revised so that diagnostics are cheaper than drugs. This can be done by offering equivalent or greater subsidies and reimbursements for diagnostics, as compared to medicines. Also, the costs and benefits should be studied by performing cost-effectiveness analysis of new diagnostics compared with standard approaches for diagnosis of infectious disease.

5. Conclusion

Antibacterial resistance can only be contained by an integrated approach that includes all stakeholders. Diagnostics are an under-recognized and underexploited tool for resistance containment. In industrialized countries, they represent only 2% health expenses of but influence 60–70% of health decisions and in developing countries, spending on diagnostics ranges from negligible to 6% (Lewin, 2005; Peeling and Mabey, 2010). As antibacterial resistance containment receives the attention it deserves, the message to clinicians, scientists and patients alike needs to shift from recommending “prudent use” of antibacterials to enabling development and appropriate use of antibacterials through diagnostics. Maintaining antibacterial efficacy should be presented as a patient safety concern and diagnostics are an important part of this paradigm.

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