**Clinical bacteriology in low resource settings – not tomorrow’s but today’s solutions**

Sien Ombelet 1 \* [sombelet@itg.be](mailto:sombelet@itg.be)

Jean-Baptiste Ronat 2 \* [Jean-Baptiste.RONAT@paris.msf.org](mailto:Jean-Baptiste.RONAT@paris.msf.org)

Janneke Cox 1 [jcox@itg.be](mailto:jcox@itg.be)

Cedric P. Yansouni 3 [cedric.yansouni@mcgill.ca](mailto:cedric.yansouni@mcgill.ca)

Timothy Walsh 4 [WalshTR@cardiff.ac.uk](mailto:WalshTR@cardiff.ac.uk)

Octavie Lunguya 5,6 [octmetila@yahoo.fr](mailto:octmetila@yahoo.fr)

Marie-France Phoba6 [mfphoba@yahoo.fr](mailto:mfphoba@yahoo.fr)

Lompo Palpouguini 7 [palponet@yahoo.fr](mailto:palponet@yahoo.fr)

Erika Vlieghe 1, 8 [evlieghe@itg.be](mailto:evlieghe@itg.be)

Delphine Martiny 9 [delphine.martiny@lhub-ulb.be](mailto:delphine.martiny@lhub-ulb.be)

Thong Phe 10 [thongphe@sihosp.org](mailto:thongphe@sihosp.org)

Samuel Kariuki 11 [samkariuki2@gmail.com](mailto:samkariuki2@gmail.com)

Paul N. Newton 12, 13 [paul@tropmedres.ac](mailto:paul@tropmedres.ac)

David A.B. Dance 12,13,38 [david.d@tropmedres.ac](mailto:david.d@tropmedres.ac)

Claude Muvunyi 14 [clmuvunyi@gmail.com](mailto:clmuvunyi@gmail.com)

Sayda El Safi 15 [shelsafi@yahoo.com](mailto:shelsafi@yahoo.com)

Barbara Barbé 1 [bbarbe@itg.be](mailto:bbarbe@itg.be)

Dadi Falay 16 [falaydadi@gmail.com](mailto:falaydadi@gmail.com)

Dissou Affolabi 17 [affolabi\_dissou@yahoo.fr](mailto:affolabi_dissou@yahoo.fr)

Maurice Page 18 [maurice-page@orange.fr](mailto:maurice-page@orange.fr)

Céline Langendorf 19 [Celine.LANGENDORF@epicentre.msf.org](mailto:Celine.LANGENDORF@epicentre.msf.org)

Yves Gille 20 [yv.gille@laposte.net](mailto:yv.gille@laposte.net)

Tjalling Leenstra 21 [tjalling.leenstra@rivm.nl](mailto:tjalling.leenstra@rivm.nl)

John Stelling 22 [jstelling@whonet.org](mailto:jstelling@whonet.org)

Thierry Naas 23 [thierry.naas@aphp.fr](mailto:thierry.naas@aphp.fr)

Thomas Kesteman 24 [thomas.kesteman@fondation-merieux.org](mailto:thomas.kesteman@fondation-merieux.org)

Yuan Qiong HU 25 [Yuanqiong.HU@geneva.msf.org](mailto:Yuanqiong.HU@geneva.msf.org)

Elsa Tran 26 [Elsa.tran@brussels.msf.org](mailto:Elsa.tran@brussels.msf.org)

Daniel Seifu27 [abbysinia2002@gmail.com](mailto:abbysinia2002@gmail.com)

Makeda Semret28 [makeda.semret@mcgill.ca](mailto:makeda.semret@mcgill.ca)

Elisabeth Delarocque-Astagneau 29 [elisabeth.delarocque-astagne@pasteur.fr](mailto:elisabeth.delarocque-astagne@pasteur.fr)

Constance Schultsz 30 [schultsz@gmail.com](mailto:schultsz@gmail.com)

Heidi Schütt-Gerowitt 31 [heidi.schuett-gerowitt@uk-koeln.de](mailto:heidi.schuett-gerowitt@uk-koeln.de)

Joanne Letchford32 [joanne.letchford@dmdp.org](mailto:joanne.letchford@dmdp.org)

Heiman Wertheim 33 [Heiman.wertheim@gmail.com](mailto:Heiman.wertheim@gmail.com)

Gunnar Kahlmeter34 [gunnar.kahlmeter@kronoberg.se](mailto:gunnar.kahlmeter@kronoberg.se)

Awa Aidara Kane 35 [aidarakanea@who.int](mailto:aidarakanea@who.int)

Olivier Vandenberg 8, 36 [olivier.vandenberg@ulb.ac.be](mailto:olivier.vandenberg@ulb.ac.be)

Jan Jacobs 1, 37 [jjacobs@itg.be](mailto:jjacobs@itg.be)

\* Equal contributors

1 Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

2 Médecins Sans Frontières, Operational Center Paris, France

3 J.D. MacLean Centre for Tropical Diseases, McGill University Health Centre, Montreal, Canada

4 Cardiff University, Cardiff, United Kingdom

5 Department of Clinical Microbiology, National Institute for Biomedical Research, Democratic Republic of the Congo

6 Department of Microbiology, University Hospital of Kinshasa, Democratic Republic of the Congo

7 Institut de Recherche en Science de la santé/Clinical Research Unit of Nanoro (IRSS-CRUN), Nanoro, Burkina Faso

8 Department of Microbiology, Antwerp University Hospital, Edegem, Belgium

9 Department of Microbiology, LHUB-ULB, Pôle Hospitalier Universitaire de Bruxelles, Brussels, Belgium

10 Infectious Diseases Department, Sihanouk Hospital Center of HOPE, Phnom Penh, Cambodia

11 Centre for Microbiology Research, Kenya Medical Research Institute (KEMRI)

12 Lao-Oxford-Mahosot Hospital, Wellcome Trust Research Unit, Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao People’s Democratic Republic

13 Centre for Tropical Medicine & Global Health, Nuffield Department of Medicine, Churchill Hospital, University of Oxford, Oxford, United Kingdom

14 Department of Clinical Biology, College of Medicine and Health Sciences, University of Rwanda, Kigali, Rwanda

15 Faculty of Medicine , University of Khartoum, Khartoum, Sudan

16 Clinical Department of Pediatrics, University Hospital of Kisangani (CUKIS), Democratic Republic of the Congo

17 Centre National Hospitalier Universitaire Hubert Koutoucou Maga, Benin

18 Independent consultant on Biomedical Engineering

19 Médecins Sans Frontières, Epicentre, Paris, France

20 Biologie Sans Frontières, Lyon, France

21 World Health Organization Collaborating Center for Antimicrobial Resistance Epidemiology and Surveillance, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherland

22 World Health Organization Collaborating Center for Surveillance of Antimicrobial Resistance, Division of Infectious Diseases, Department of Medicine Brigham & Women's Hospital, Boston, USA

23 Service de Bactériologie-Hygiène, Hôpital de Bicêtre, Paris, France

24 Fondation Mérieux, Lyon, France

25 Médecins Sans Frontières, Access Campaign, Geneva, Switzerland

26 Médecins Sans Frontières, International Office, Brussels, Belgium

27 College of Health Sciences, Addis Ababa University, Ethiopia

28 St Mary’s Hospital Centre and McGill University Health Centre, Montreal, Canada

29 Institut Pasteur, Paris, France

30 Department of Global Health and Department of Medical Microbiology, Academic Medical Center of the University of Amsterdam, Amsterdam, the Netherlands

31 Institute of Medical Microbiology, University of Cologne, Germany

32 Diagnostic Microbiology Development Program, Phnom Penh, Cambodia

33 Oxford University Clinical Research Unit, National Hospital for Tropical Diseases, Hanoi, Vietnam

34 Department of Clinical Microbiology, County Kronoberg, Växjö/Karlskrona, Sweden.

35 WHO-AGISAR, Department of Food Safety and Zoonoses, World Health Organization, Switzerland

36 Center for Environmental Health and Occupational Health, Public Health School, Université Libre de Bruxelles, Brussels, Belgium.

37 Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium

38 Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK

## Abstract

Low resource settings (LRS) are disproportionately burdened by infectious diseases and antimicrobial resistance (AMR). Good quality bacteriology is a prerequisite for effective AMR control, but LRS face infrastructural, technical and behavioural challenges when implementing clinical bacteriology. Typically, general referral hospitals in low- or middle-income countries have basic laboratory services operated by non-microbiology professionals. This article explores the needs for successful implementation of clinical bacteriology in LRS. The majority of currently available microbiological techniques and equipment have not been developed with the specific needs of LRS in mind. Pending the arrival of new generation LRS-friendly diagnostics, we have focused on improving, adapting and implementing conventional, culture-based techniques. LRS priorities include: (1) harmonized, validated, quality-assured and “tropicalized” equipment, consumables and techniques (2) rationalized bacterial identification and AMR testing (grouping of species into clinically relevant groups, contextually appropriate panels of antibiotics, validation of single-substrate “spot-tests” for bacterial identification and detection of resistance markers), (3) integration of diagnostics into clinical care, (4) appropriate selection and prioritization of clinically relevant specimens, (5), developing and making accessible cost-free training materials and information management tools dedicated to end-users and (6) on-site validation and field-adoption of diagnostics in LRS, with considerable shortening of the translational pipeline. Herein we argue that clinical bacteriology in LRS (1) improves individual patient management, (2) provides adequate surveillance for local antibiotic treatment guidelines and national policies and (3) contributes to outbreak management, AMR containment and hospital infection control.

**Introduction**

Bacterial sepsis is a leading cause of mortality and critical illness worldwide, according to even conservative estimates (1-3). Antimicrobial resistance (AMR) is considered a major public health threat worldwide, and low- and middle-income countries (4) (LMICs) are disproportionally burdened (5, 6). Factors contributing to this excessive burden include patients’ vulnerability to acquire invasive bacterial illness, widely uncontrolled use of antibiotics, and lack of diagnostic support for clinical decision-making resulting in overuse of antibiotics. Further, practices aimed at prevention of healthcare-acquired infections are generally weak to non-existent in low resource settings (LRS) (6).

Clinical bacteriology contributes to three out of five strategic objectives identified by the World Health Organization for the containment of AMR, *i.e.* surveillance, appropriate use of antibiotics (antibiotic stewardship), and healthcare infection control (7). These objectives were recently reaffirmed at the UN General Assembly High-level Meeting on Antimicrobial Resistance (8). Clinical bacteriology contributes largely to the patient’s diagnosis and guides antibiotic treatment. For severe sepsis, inappropriate antimicrobial therapy is a major contributor to preventable deaths, and sepsis guidelines emphasize the importance of culture-guided therapy (9). For tropical LRS, this is particularly problematic as life-threatening bacterial infections such as non-Typhoidal *Salmonella* bloodstream infections are clinically indistinguishable from severe *Plasmodium falciparum* malaria (10-13) and are often misdiagnosed (14, 15). If promptly reported, identification and antimicrobial susceptibility testing (AST) results allow optimisation, de-escalation or discontinuation of antibiotic treatment, resulting in improved patient outcomes, reduced costs and reduced selection of AMR (16). Culture data from routine patient care can be aggregated to identify common pathogens and define their susceptibility patterns, which may differ by region. Such surveillance data support validation of locally-adapted empiric antibiotic treatment guidelines (17). In addition, daily reviews of clinical bacteriology data are vital for detection and management of hospital- and/or community-associated outbreaks and to monitor emergence of resistance (18, 19). Moreover, identification and serotyping of pathogens can be useful for vaccine development and surveillance (20, 21).

Diagnostic laboratories in LMICs face challenges of infrastructure, equipment, logistics, quality-assurance and human resources (22). During the past decade, considerable efforts have been made to strengthen laboratory systems in LRS (23-25) and successful dissemination of diagnostics has been achieved for tuberculosis, malaria, HIV and Ebola (6, 26, 27). In contrast, clinical bacteriology covers a wide spectrum of pathogens and cannot be captured by simple diagnostic and therapeutic algorithms. Moreover, it cannot be easily reduced to a few affordable rapid diagnostic tests or framed in powerful “vertical” control programs.

Design, proof-of-concept and even clinical validation of new diagnostics for clinical bacteriology can take between 2 – 10 years (28). For LRS, market penetration and dissemination pose additional problems including procurement, distribution and quality control, as has been illustrated for rapid diagnostic tests (29). In addition, manufacturers’ production capacity and compliance with ISO standards (ISO 13485) are frequently inadequate when addressing diagnostics marketed in LRS (30). Pending the implementation of new technologies, conventional culture-based techniques are the best option for implementing clinical bacteriology in LRS: they are well studied, robust, universally accepted, and most have regulatory certification. Moreover, culture-based techniques remain indispensable for AST, even in the presence of advanced molecular or biomarker tests.

We explore barriers and priorities influencing the implementation of clinical bacteriology in LRS. Our typical target is a general referral hospital in LMIC categorized as “moderate infrastructure” (31), *i.e.* including a basically equipped diagnostic laboratory operated by mostly non-microbiology-expert users. These target laboratories are at the core of any clinical bacteriology activity, including surveillance. We have focused on operational requirements for culture-based techniques and considered the interaction between clinicians and laboratory staff in LRS. General issues of laboratory services in LRS including supranational initiatives towards funding, accreditation, the landscape of diagnostic regulations and production are briefly discussed where relevant. References to more extensive sources are cited (22-24, 32-34).

**Implementation of clinical bacteriology in LRS: six building blocks**

With the proliferation of automated and mass spectrometry technologies for bacterial identification and AST in high resource settings, a widening gap between these systems and practices of clinical bacteriology in LRS is evident (Table 1). In order to bridge this gap, we suggest six essential “building blocks” needed for successful implementation of clinical bacteriology in LRS.

**1. Availability of “tropicalized” equipment and consumables**

The environmental conditions in LRS affect electronic equipment as well as basic consumables such as glassware and dehydrated culture media (35) (Table 1, Figure 1). Availability of quality-assured diagnostics is further compromised by a lack of on-site production and by an inadequate supply chain, incompatible with the shelf life and cold-storage requirements of many diagnostics (36). In addition, there is little commercial interest in developing diagnostics for the LRS market in view of low profit margins (6).

Borrowing a term from the car industry (37), equipment and consumables in LRS need to be “tropicalized”, which means resistant to harsh conditions of temperature, humidity, dust and sunlight as well as suitable for shipment and transport (*e.g.* compliant with international transport guidelines). In addition to being robust, safe and stable, equipment should require low energy consumption and maintenance. Consumables need to have a long shelf life and generate minimal waste. Where possible, internal quality controls should be embedded within reagents’ kits to ensure optimal product performance. Finally, quality assurance in manufacturing, market release, distribution, client support, post-marketing service and maintenance should be guaranteed.

A first step towards tropicalization of diagnostics is drafting clear product specifications (*i.e.* ‘target product profiles, TPP ‘). In view of their complexity, extensive expert and stakeholder consultations are needed, and they should also cover the pre- and post-analytical phases, such as thorough skin disinfection and efficient waste management (in the case of blood cultures). Manufacturers need to be engaged in discussions concerning TPPs from the start. Some established manufacturers have already launched research initiatives for ‘low-cost innovation’ targeting diagnostics in LRS (38); furthermore, manufacturers in growing economies (China, Viet-Nam, Thailand) are producing a wide range of diagnostics hitherto unknown outside their domestic markets (*e.g.* Nam Khoa Biotek blood culture bottles).

In order to adapt existing techniques, there are easy-wins. Some diagnostics used in clinical bacteriology have been long established but not extensively validated for stability and shelf-life, which may have been set arbitrarily at 25°C and 6 months. However, actual use (and some guidelines) suggests a reliable performance outside these specifications (39) and identical products from different brands show different storage requirements and shelf-life. Therefore, in line with current practice for pharmaceutical products (40), extended product stability testing (including thermal shock and shipment stability) should be performed to confirm the genuine limits of stability and shelf-life.

Other short-term goals are “tropicalized” packaging (*e.g.* vacuum-sealed package protecting against humidity), clear labelling and adaptation of instructions to the language and educational level of intended users (41, 42). Furthermore, production of quality-assured consumables (including culture media and stains) can be outsourced to a centralized facility as has been implemented in Cambodia (43).

Medium-term objectives include the development of ‘low-tech, low-cost, low-maintenance’ equipment such as electricity-free incubators (44), battery-operated centrifuges (45) and solar energy operated autoclaves (46-48). Alternatives for sheep blood, horse blood and rabbit plasma (which are historically embedded in the practice of clinical bacteriology) should be explored: for example, goat, pig and hair sheep (a breed of sheep adapted to tropical climates) blood are alternatives to sheep blood (49, 50) but lyophilized or synthetic media should also be considered. Likewise, agar (*i.e.* the solidifying substrate of culture media), is obtained from sea algae at a limited number of harvesting sites; alternatives such as cellulose (produced by engineered bacteria (51)) should be assessed for stability and commercial viability.

**2. Rationalized bacterial identification and antimicrobial susceptibility testing**

Due to recent technological advances in identification techniques and continuous refinements of AST guidelines, state-of-the-art identification and AST in LRS remains a major challenge (Table 1)(36). Importantly, the CLSI AST guideline has followed the example of the EUCAST guidelines and has become open-access (52, 53), but building local capacity to review and implement the annually revised standards can be challenging.

The relevance of identifying all bacteria to the species level is debatable (54) and grouping of genera and species according to their clinical relevance seems more pragmatic (55). Accordingly, a two-tier approach could be considered, consisting of a first-line identification to the ‘group’ level and preliminary AST, followed by an advanced identification and AST at the level of national reference laboratories when required. Pragmatic guidelines for grouping bacteria according to clinical significance, AMR profile, hospital epidemiology and public health importance should be undertaken. Table 2 gives an example of grouping for enteric bacteria.

Grouping of bacteria encourages technologies for abbreviated identification through “spot-tests”, which are single-substrate biochemical or enzymatic tests that can be read by colour, fluorescence or turbidity. Spot-tests are available for key-pathogens such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aerug*inosa; they are reliable and their rapidity can significantly contribute to clinical decision making (56-58). Valuable documents such as the CLSI guideline ‘Abbreviated Identification for Bacteria and Yeasts’ (57) should be updated, extended to ‘tropical’ bacteria (e.g. *Salmonella, Burkholderia pseudomallei*), preferably validated with geographically representative strains and made open-access.

AST panels should be adapted to requirements in LRS, *e.g.* harmonized with the country’s essential drug list. Disk diffusion testing has advantages of cost, flexibility, simplicity and reliability, but is demanding in terms of quality assurance. Lyophilized microbroth dilution methods, when appropriately protected from humidity (59), are less error-prone than disk diffusion and provide minimal inhibitory concentrations when clinically needed (*e.g.* fluoroquinolone susceptibility profiles for *Salmonella* Typhi (52, 60)). A simple reading apparatus would be helpful for accurate interpretations, particularly when coupled to an open-access “expert” system.

**3. Embedment into clinical care**

The interface between clinicians and the diagnostic laboratory has been seldom studied in LRS (61, 62). Relying more on clinical algorithms and syndromic approaches, clinicians are reluctant to request laboratory tests (Table 1), a tendency that is aggravated by their perception that tests are expensive, slow and often erroneous(63). In some sub-Saharan African regions, there is fear of blood sampling and invasive procedures among patients (63, 64)(Figure 2). Additionally, senior hospital management staff traditionally draws from the pool of academic clinicians, which in turn increases the communication gap between decision-makers, clinicians and laboratory staff. Laboratory staff frequently are left with poor job satisfaction and a sense of restricted professional development (61, 62).

In high resource settings, clinical bacteriology has become closely integrated with patient management and infection control (65, 66). This contrasts with LRS, where trained clinical microbiologists are rare and the main focus is on the analytical phase, with little professional collaboration and communication between laboratory and clinicians (22). Thus, even when good quality diagnostic laboratories in LRS are present, their impact is frequently compromised by under-utilisation, pre-analytic errors such as inadequate specimen collection or post-analytical issues such as ineffective reporting of results (6, 35, 61, 63). Person-to-person interactions between clinicians and laboratory staff are instrumental in improving communication and collaboration, and this collaboration should already feature in education and pre-service training (67, 68).

The “Strengthening Laboratory Management Toward Accreditation” (SLMTA) program of the World Health Organization’s Regional Office for Africa (WHO AFRO) provides useful key-messages on improving communication between clinicians and laboratory staff (69, 70). Likewise, the SLMTA toolkit modules for specimen collection and test result reporting can strengthen clinical bacteriology activities.

**4. Selection and prioritization of clinically relevant specimens**

Although key to performance (70), the minimal number of samples (“critical volume”) to be processed in order to acquire expertise in clinical bacteriology is not defined. Clinical bacteriology relies on the competence and experience of laboratory staff; for example, the Gram stain is notoriously error-prone in unexperienced hands (71). However, sample volumes in LRS tend to be low and laboratories often process inappropriately selected specimens (Table 1).

When resources are limited, prioritizing is critical (Table 3). Some clinical specimens, such as blood cultures, are more relevant than others for patient management and AMR surveillance (18). In contrast, culture of CSF, which has more stringent requirements, might offer less added clinical value since standard 3rd generation cephalosporin treatment in the post-neonatal period would cover most bacterial pathogens (once diagnosis of bacterial meningitis is made using cell count and Gram stain) (72-74). However, identification can be important in case of *Salmonella* meningitis, which requires extended treatment (75). In addition, culture results provide valuable data to support vaccine coverage (20, 21).

Processing of empyema, abscesses, bone fragments and surgical samples can guide appropriate clinical care but procedures for these specimens are considerably more complex (76). Of lowest priority for individual patient management are upper respiratory tract specimens, urine and stool, which, additionally, require considerable expertise. However, they may be useful for AMR surveillance and to identify patients in whom antibiotics are not indicated. Clear sampling procedures and sample acceptance criteria need to be formalized, communicated and implemented (68).

**5. Accessible and affordable training and reference materials**

Literature and training materials dedicated to clinical bacteriology in LRS are scarce and fragmented, seldom updated, and sometimes expensive. Moreover, culture type strains used as internal controls are expensive to procure and maintain (Table 1).

Training (both theoretical and practical) and reference materials should be dedicated to LRS, open-access and available in local languages. Attention must be paid to readability and comprehensibility for non-expert users of various cultural backgrounds. End-user feedback is essential to refine and improve teaching material and validating training materials should be compulsory (77).

Established guidance and toolkits should be linked to these reference materials. As stated above, the SLMTA toolkit’s modules as well as the SLIPTA (Stepwise Laboratory Improvement Towards Accreditation) checklist (78) can be complemented with real-life scenarios of clinical bacteriology. Leading clinical reference documents such as the WHO guidelines for hospital care of children and adults (72, 73) could include recommendations for clinical bacteriology (indications, sampling and transport) and antibiotic stewardship. New programs adapted to LRS, such as the Laboratory Assessment Tool (LAT) from WHO (79), can also offer guidance to evaluate laboratories and national laboratory systems.

Clinician and nursing staff on-site training is expected to have the highest efficiency and retention. Experiences from implementing clinical medicine in LRS highlight the value of on-site support through educational outreach and mentoring visits (80). Remote learning with video (*e.g.* the recently introduced instruction videos on disk diffusion by EUCAST (81)) is a valuable complement to bench-side exposure and coaching (82). Tele-microbiology (transfer of images of cultures and microscopy) allows for real-time analysis, expert consultation, training and education as well as quality assurance at an affordable cost (83, 84).

**6. Diagnostic research should address on-site validation and field-adoption in LRS**

As was emphasized in the 2016 O’Neill report on AMR, more effort should be put in the development of new diagnostics (85). Promising new technologies are making their way to clinical bacteriology (Table 1), but when applied to LRS, they are confronted with budgetary, technical and human constraints and rarely diffuse beyond reference laboratories (28). Furthermore, a major bottleneck for developing diagnostics for LRS occurs at the level of clinical performance studies (86).

Some manufacturers are investing research in so-called ‘low-cost innovation’ (38, 87) and some of those technologies under investigation may be turned into valuable diagnostics for LRS. Rather than awaiting accreditation in resource-rich settings, new diagnostics should be evaluated in field settings in LRS. It is to be expected that some of these innovations will diffuse to high resource settings, a process which is called “reverse innovation” (88, 89).

Well-functioning quality-assured diagnostic laboratories may constitute reliable study sites to carry out clinical diagnostic studies in the target population. Beyond strict diagnostic performance, such studies should address field adoption of new diagnostics, their integration into clinical care and cost-effectiveness analysis (90).

**Beyond the six building blocks**

Beyond the scope of this paper, there are other factors influencing the implementation of clinical bacteriology in LRS. Primarily, political commitment is essential to install and equip clinical laboratories at all levels of health care and to strengthen health systems. It is therefore worth noting the recent resolution of the UN General Assembly, wherein Heads of State have committed to a broad, coordinated approach to address the root causes of AMR (8). In addition, the professional, academic and regulatory environment should facilitate implementation of clinical bacteriology in the healthcare organization and biomedical curricula.

Further opportunities to boost implementation of clinical bacteriology diagnostics are linkage to the WHO Prequalification program of *in-vitro* diagnostics (WHO PQ Dx), which promotes good quality diagnostics in poorly regulated regions, as well as the “Expert Review Panel of Diagnostics (ERP-D)”, which allows time-limited access to diagnostics (91, 92). These tools may promote affordable and quality-assured bacteriology diagnostics in LRS, and contribute to their refinement and dissemination.

Furthermore, in response to the 2016 O’Neill’s report on AMR (85), wherein a Global Innovation Fund for non-commercial research is proposed, there has been advocacy for the foundation of a Global Antimicrobial Conservation Fund (93). We strongly endorse this initiative, and believe that in collaboration with the British Fleming Fund, this can further strengthen the support for basic bacteriology services in LRS.

**Conclusion**

Given the global attention for AMR and several recent “calls to action” (7, 94, 95), we believe it is timely to address clinical bacteriology in LRS. The benefits of clinical bacteriology are numerous, not only for individual patient care, but also for surveillance of outbreaks, emerging resistance, management of hospital infections and effective antimicrobial stewardship. We therefore advocate an extension of the WHO’s Maputo declaration on laboratory strengthening for HIV, TB and malaria diagnosis to include clinical bacteriology (25).

We have outlined some major challenges encountered when implementing clinical bacteriology in LRS, and we provide guidance as to how these difficulties could be overcome, but are aware of the fact that considerably more research, resources and advocacy are needed. The substantial progress in HIV, TB and malaria diagnosis and management has demonstrated that non-expert staff can effectively deliver services that were previously considered too complicated and demanding (96). We are confident that with similar concerted international efforts, the same accomplishment is in reach for clinical bacteriology.

Tables

Table 1: Clinical Bacteriology in low resource settings compared to high resource settings. Abbreviations: AMR: antimicrobial resistance, AFRO: Regional Office for Africa, AST: antimicrobial susceptibility testing, CLSI: Clinical and Laboratory Standards Institute , EUCAST: European Committee for Antimicrobial Susceptibility Testing, HIV: human immunodeficiency virus, ISO: International Organization for Standardization, JCI: Joint Commission International, LIMS: Laboratory Information Management Systems , LRS: low resource settings, MALDI-TOF: Matrix Assisted Laser Desorption Ionization – Time of Flight, MIC: minimum inhibitory concentration, SLIPTA: Stepwise Laboratory Improvement Process Towards Accreditation, SOP: Standard Operating Procedure, TAT: turn-around time, WHO: World Health Organization

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| **High resource settings** |  | **Low resource settings** |
| Infrastructure and equipment | | |
| Covered by ISO15189, JCI, CLSI QMS01-A4, CLIA or equivalent Quality Management System (97-100) |  | Basic requirements of infrastructure frequently not met (6, 33): power, climate control and ventilation (humidity and high temperatures affect electronic equipment), dust (sludge filters of biological safety cabinets, contamination of culture media), water quality, light sources and internet connectivity |
| Biosafety needs and occupational health protection are met |  | (Bio)safety requirements frequently not met (101) |
| Security needs (electrical equipment, fire escape, …) are met |  | Security needs frequently not met |
| Equipment is available as required |  | Equipment not adapted to environmental and technical constraints of LRS  Issues with procurement, training, validation, certification, calibration, maintenance (6, 33) |
| Consumables and diagnostics in general | | |
| Diagnostics identified as an essential tool in the containment of antibiotic resistance (32) Increasing development and market expansion of diagnostics (102, 103) |  | Sales in LRS are not lucrative enough for adequate return on investment (6) , therefore little commercial incentive to create rapid diagnostic solutions for LRS |
| Quality-assured production and supply by ISO 13485 certified manufacturers (104) |  | Limited quality assurance of process and products (36) Diagnostics manufacturers not always ISO13485 certified and client-centred (105) |
| Procurement and supply chain of diagnostics short and reliable |  | Lack of on-site production of diagnostics (106) Long supply chain with short shelf-life  High stress conditions (heat, humidity), limited environmental stability of critical diagnostics (33) |
| Storage, stock management and distribution systems in place |  | Paucity of in-country distributors  Need for cold chain (2 - 8°C or even -20°C)  Short shelf-life of critical products (*e.g.* blood cultures)  Stock management and inventory systems not in place or inappropriate (33) |
| Standard Operating Procedures, Documents, Training and Reference Materials | | |
| Standards for Standard Operating Procedures (SOPs) covered by ISO15189 or equivalent Quality Management System (97)  Clinical Bacteriology requires numerous SOPs (*e.g.* identification & Antimicrobial Susceptibility Testing (AST)) |  | Creating and updating SOPs and other documents are a significant obstacle (33) SOP templates and formats are designed for resource-rich settings (*e.g.* long and demanding (105) Multiple language and cultural barriers to understanding of SOPs (105) |
| Training materials are available and easily accessible |  | Training and resource materials are scarce and fragmented, *e.g.* limited to pathogens relevant for public health(107), focused on the analytical phase and targeted to public health laboratories |
| Reference materials are easily accessible |  | Reference materials such as culture type strains are expensive, subject to strict shipment requirements (104) and difficult to maintain; they are scarcely available even in reference laboratories (36) |
| Guideline documents, standards and norms are affordable |  | ISO documents (108) and CLSI guidelines are for sale but expensive. Of note, CLSI has put their key-document M100:S26 freely available online (53).  Failure to comply with CLSI guidelines (36) |
| Culture-based growth and identification of bacteria | | |
| Automated blood culture systems with continuous monitoring of growth and automated detection (109) |  | Manual blood culture systems requiring visual inspection which is only acquired through intensive training and experience  When available, the use of automated blood culture systems is limited by cost and availability of bottles |
| Culture media: highly harmonized through technical guidelines |  | On-site home-made preparation of culture media  Difficulties to procure products or to assure quality (*e.g.* distilled water, sheep blood (49)) |
| Staining reagents of high quality, supply chain is short and reliable |  | Locally produced stains: poor quality assurance, irregular availability, substandards (110) Some reagents are subject to "Dangerous Goods Regulations" when shipped by air (111) |
| Matrix Assisted Laser Desorption Ionisation – Time of Flight (MALDI-TOF) identification: simple and quick |  | Automated equipment and MALDI-TOF poorly penetrated in LRS: high cost, stringent requirements for infrastructure (6, 112) |
| Identification systems have been designed and validated with bacterial collections obtained in high resource-rich settings |  | Identification methods not yet validated with bacterial collections from LRS (112, 113)  Some "tropical" bacteria cannot be reliably identified with commercial identification methods (114, 115) |
|  |
| Interest in dichotomous identification and non-automated bacteriology has waned |  | Many so-called "spot-tests" (single substrate identification tests with a short incubation time) and manual diagnostic products are no longer commercialised. |
| Antibiotic susceptibility testing (AST) | | |
| Automated methods for incubation, reading and reporting have substantially decreased turnaround time (TAT) (55, 116) |  | Automated equipment poorly penetrated in LRS: high cost, stringent requirements for infrastructure (6, 112) Service contracts unavailable or unaffordable in many LRS settings (6) |
| Harmonized criteria by international guidelines (CLSI, EUCAST) integrated in automated AST methods assuring correct and updated use of breakpoints and expert rules (117, 118) |  | Guidelines are short-lived, not well disseminated, only available in English and poorly followed (36)  Expert rules too complicated to be mastered by non-expert microbiology staff Complex interpretation for some important pathogen/antibiotic combinations (*e.g.* *Salmonella* Typhi and fluoroquinolone susceptibility) Antibiotic panels used are not appropriate and without internal quality control (36) |
| When appropriate, minimal inhibitory concentration (MIC) value assessment is available |  | MIC determination out of range of laboratories in LRS (complexity, cost) Requirement for MIC-methods for some pathogen/drug combinations (*e.g.* for penicillin with *S. pneumoniae* isolates from blood or CSF) complicates the implementation of the simplest disk-diffusion methods |
| Laboratory processes and sample/results flow | | |
| Streamlined and automated trajectory with shortening of pre-analytical phase and post-analytical phase (119, 120)  Procedures designed to improve accuracy and speed (121) |  | Piecemeal sampling, manual work-up and administration  Accuracy of results is seen as important more than speed and short turnaround time (TAT), no communication of preliminary results (*e.g.* Gram stain of grown blood culture) |
| Clinically relevant criteria for sampling (56) Quality indicators for sampling processing and reporting embedded in Quality Management systems |  | No laboratory guidance for selection, sampling and transport of specimens Sampling of severely ill and after failing initial treatment patients (biased to resistance) or patients who can afford testing No rejection criteria: all specimens are accepted and worked-up  Quality indicators not systematically monitored  Inadequate sampling: secretions from fistula, fluid collected through drains, urine through indwelling catheter, long transport delays, insufficient sample volume, wound swabs |
|
| Consolidation and merging of clinical laboratories in centralized facilities (65, 122) |  | Clinical bacteriology services are expected to remain decentralized and in-hospital, benefiting from easy accessibility and personal interactions |
| Laboratory Information Management Systems (LIMS) connected to the hospital medical record systems contributes to short TAT surveillance and outbreak detection (119) |  | Paper-based systems, with hardcopy results collected too late or not at all (123) No automated alerts for surveillance and/or infection control |
| 24/7 IT support for LIMS, internet connectivity ubiquitous |  | Lack of IT support, internet connectivity frequently not present |
| Proactive communication and reporting by the clinical microbiologist (66) |  | Poor communication between laboratory staff and clinicians |
| 7/7 days and 24/24 hours specimen reception and processing, consultative role including out-of-hours call for the clinical microbiologist (124) |  | Laboratory activities mostly limited to office hours |
| Laboratory staff | | |
| Professional standards for clinical microbiologist and biomedical staff (125)  Professional societies at the international and national levels Accessible programs of Continuous Medical Education |  | No professional standards or profiles for clinical microbiologists; clinical bacteriology is mostly supervised by general laboratory managers  Few professional societies and post-graduate activities International societies mainly experienced in malaria, tuberculosis and HIV |
| Dedicated and well-trained biomedical staff experienced in clinical bacteriology  Clinical microbiology experts are involved in training of staff and management of lab  Expertise in “manual” (equipment-free) bacteriology fading away (122) |  | Obstacles in staff management in clinical bacteriology with frequent understaffing, difficulties with staff retention (6, 33)  Clinical microbiology experts non-existent or scarcely involved  Lack of pre-service training and education  Few available training/teaching sites for clinical bacteriology |
| Quality management systems (*e.g.* ISO norms) offer recommendations to optimize the relation between clinicians and laboratory departments (62) |  | WHO SLIPTA guideline mainly addresses the formal "Client Management and Customer Service" items but does not address *e.g.* communication of intermediate and alert laboratory results (78) |
| The patient, the nursing staff, the clinician | | |
| The patient is mostly well knowledgeable and has a health insurance |  | Out-of-pocket payment resulting in reluctance to sampling from the patient's and the clinician's side (63) |
| Clinical and nursing staff familiar with indications and samples and the patient is well informed about the interventions |  | Reluctance from medical staff or patients to sample (63, 64, 126) |
| Clinicians  - are familiar with indications and interpretation of laboratory results - have low threshold to contact the clinical microbiologist - trust and rely on the laboratory results as part of evidence-based clinical algorithms and care bundles |  | Clinicians have a negative perception of the laboratory:  TAT too long (14, 61, 127), poor accuracy of laboratory tests (14, 61, 127), inadequate laboratory capacity and unavailability of consumables (22, 126) |
|  | Clinicians have a high reliance on (own) clinical judgment and are reluctant to request laboratory tests and tend to deny laboratory results (123, 126, 128) |
| High awareness of antimicrobial resistance (AMR) and infection control among clinicians, nursing and hospital staff |  | AMR is considered mainly a worldwide or nationwide problem, but considered to be less of a problem of the own hospital (129, 130) |
| Patients educated towards appropriate and restrictive use of antibiotics |  | Patients' pressure to prescribe antibiotics (129, 130)  Auto-medication because of widespread availability of antibiotics |
| Support to clinical decision making, antibiotic stewardship, infection control and surveillance | | |
| Shortened TAT of identification & AST combined with proactive reporting has shown to improve time to optimal treatment, clinical outcome and cost-effectiveness (16, 131) |  | Often long TAT, poor communication between laboratory and clinician, no data about patient outcomes and cost-efficiency |
| Clinical microbiologist is active member of the antibiotic stewardship committee (132) |  | AB stewardship committees or activities largely absent; lack of studies assessing non-use of antibiotics or de-escalation using the support of clinical bacteriology in LRS (6) |
| AMR surveillance reports generated by the results of routinely submitted samples and aggregated in (supra-)national or regional networks |  | AMR surveillance data based on poor data quality and representativeness, especially in Africa and the western Pacific (6, 133, 134)  AMR surveillance in LRS has been focused on intensive care units and vulnerable populations such as children and HIV-patients (6) |
| Real-time alert function for infection control (community and hospital-based outbreaks) (135) |  | Laboratory data hardly exploited for community or hospital-based outbreak investigation and management (6, 133); infection control committees are rarely working with laboratory data |
| Surveillance role for public health issues such as (re)-emerging pathogens and vaccine-preventable diseases (136) |  | Public health surveillance is mostly confined to reference laboratories |
| Professional and Regulatory environment | | |
| Certification, accreditation and regulation of clinical laboratories |  | In 2010, 340 diagnostic laboratories in Africa were accredited ( 93% in South Africa) (70) WHO Regional Office for Africa (AFRO) provides an accreditation process as an interim pathway to realize international laboratory standards (70)  "Stepwise Laboratory Improvement Process Towards Accreditation" focuses on malaria, tuberculosis and HIV and has few applications to clinical bacteriology (78) |
| External Quality Control Programs available |  | Existing external quality are expensive and address mostly haematology, clinical chemistry and serology  Moderate to low scores for external quality assessment of microbiology in public health laboratories in the WHO African Region, 2002 - 2009 (36) |
| Functional reference laboratories |  | Few reference laboratories, mainly oriented to research and outbreak management  Referral of specimens for clinical bacteriology is more demanding (sampling, shipment) than specimens for tuberculosis and HIV testing |
| Consolidation of Clinical Bacteriology laboratories into large core laboratories (137) |  | Laboratories mostly confined to urban areas; if rural, occasionally poor accessibility and geographic coverage |

Table 2: Proposal of grouping of bacterial species according to clinical and infection control relevance (54, 55, 138, 139), example of the Gramnegative enteric bacteria. Detail and level of identification can depend on technical and economic feasibility of the identification system used. Focus is on isolates recovered from bloodstream pathogens.

|  |  |  |  |
| --- | --- | --- | --- |
| **Name for lumped group** | **Examples of species in lumped group** | **Clinical relevance** | **Infection control relevance/Notes** |
| *Enterobacter/Citrobacter/Serratia* | *Citrobacter (freundii, koseri, braakii…)* | Pneumonia  Post-operative site infections  Urinary tract infections  Wound colonization/infections | Mostly healthcare-associated  Multidrug resistant |
| *Cronobacter (sakazakii, malonaticus)* |
| *Enterobacter (cloacae, aerogenes…)* |
| *Hafnia alvei*  *Serratia (marcescens, liquefaciens)\** |
| *Klebsiella* | *Klebsiella (pneumoniae, oxytoca…)*  *Raoultella spp.* | Bloodstream infections  Pneumonia  Pyogenic infections (e.g. liver abscess)  Urinary tract infections  Wound colonization/infection | Often healthcare-associated  Multidrug resistant  Hospital outbreaks (neonatal care)  Hypervirulent community-associated strains (pyogenic infections) |
| *Escherichia coli* | *Escherichia coli* | Bloodstream infections  Enteritis  Urinary tract infections | Mostly community-associated |
|
| *Salmonella* Typhi  *Salmonella* Paratyphi A | *Salmonella* Typhi  *Salmonella* Paratyphi A\* | Enteric fever | Note: *Salmonella* Paratyphi A very rare in Africa |
| Non-Typhoidal *Salmonella spp.* | Non-Typhoidal *Salmonella* | Bloodstream infections  (< 5 years old – malaria and malnutrition; HIV-infection)  Enteritis | Community-associated  Hospital outbreaks |
| *Proteus/Providencia /Morganella* | *Morganella morganii*  *Proteus (mirabilis, vulgaris, …)*  *Providencia spp.* | Post-operative site infections  Urinary tract infections | Healthcare-associated |
| *Shigella spp.* | *Shigella (boydii, dysenteriae, flexneri…)* | Dysentery  Enteritis  Haemolytic-Uremic Syndrome (HUS)◊ | Community-associated  Note 1: rarely isolated from other specimens than stool  Note 2: *S. dysenteriae* serotype 1 has public health relevance (epidemics) |

\* “nice to have” : if technically feasible and affordable, separate identification of this species would be desirable for clinical as well as epidemiological purposes

Table 3: Prioritization of specimens submitted as part of routine patient care in low resource settings: overview of the practical feasibility and relevance both for the individual patient management and surveillance purposes (see introduction) of different clinical specimens: grading is done by “+” (+++ = high, ++ = moderate, + = low, ± = very low). Pre-analytical feasibility refers to indications, sampling and transport; analytical refers to technical feasibility (*e.g.* selective culture media, incubation conditions) and human (training, expertise) requirements. Abbreviation: AMR = antimicrobial resistance, TAT = turnaround time.

|  |  |  |  |
| --- | --- | --- | --- |
| **Specimen** | **Pre-analytical and analytical feasibility** | **Relevance for individual patient management** | **Relevance for surveillance** |
| Blood | **+++**  Transport and technical requirements feasible  Mostly one pathogen, top-5 pathogens comprise majority of isolates (140-143) | **+++**  Unequivocal interpretation (except contaminants)  High impact on patient outcome (9)  Amenable to antibiotic stewardship (9) | **+++**  Indications for sampling standardized Quality indicators allow for inter-laboratory harmonization and over-time surveillance (144) |
| Cerebro-spinal fluid | **++**  Sampling requires training, skills and expertise  Cold-vulnerable pathogens Transport (delay, light, room temperature) | **++** Standard empirical treatment: value of culture over white blood cell count and Gram stain is limited (74) | **++**  Epidemics (*Neisseria meningitidis*) or serotype distribution (*Streptococcus pneumoniae)* (20, 21) |
| Empyema, closed abscess, joint fluid | **+++**  Mixed, fastidious and anaerobic flora possible  Transport demanding  Selective media and considerable expertise required | **++**  Can guide diagnosis and treatment, particular in case of severe infections and when complete drainage is not possible | **++**  Variability in patients' selection and previous interventions |
| Bone tissue | **+**  Surgically obtained samples  Need for grinding of specimens (76)  Mostly one pathogen (except trauma, prosthesis and chronic infections..) (145) | **+**  Guidance for treatment  Some flora difficult to interpret (e.g. coagulase-negative staphylococci) | **+**  Variability in patients' selection (e.g. trauma, prosthesis material) |
| Respiratory tract (non-tuberculosis) | **±**  Mixed flora, cold-vulnerable pathogens  Contaminating oral flora  Need for selective media and expertise  Low reproducibility of semi-quantitative cultures (146) | **±**  Difference between colonization and infection difficult to make (intensive care) | **±**  Variability in patients' selection and contaminating/ colonizing flora |
| Urine | **±**  Long delay of transport, requiring cold chain | **±**  Contaminating flora | **+**  Variability in patients' selection (more complicated cases and bias to AMR) |
| Stool | **±**  Transport requirements (delay, temperature)  Selective culture media, microaerophilic incubation conditions (*Campylobacter* spp)  Considerable expertise required, long TAT | **±**  Diarrhoea frequently of non-bacterial origin (147)  Long turnaround time  Relatively low sensitivity for bacterial pathogens of greatest interest (147) | **+**  Outbreaks suspected of dysentery or cholera (confirmation of epidemic and antibiotic resistance patterns) (148, 149) |

Figures



Figure 1: Need for "tropicalized" consumables (DR Congo). At high relative humidity, glass slides for microscopy become opaque by the development of “haze” or “fog” within the glass. A “tropicalized” vacuum-sealed package is needed to prevent this deterioration.



Figure 2: Lumbar puncture, DR Congo. Need for sterile gloves, dedicated needle, training and assisting nursing team. It is not surprising that in a busy clinical setting, clinical staff tends to be reluctant to sampling

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