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Implementation and Evaluation of Antimicrobial Resistance Surveillance Systems in Sub-Saharan Africa

Dissertation

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" Antimicrobial Resistance is a global challenge that demands our collective understanding, vigilant surveillance, and unwavering commitment. In the face of this silent storm, let knowledge be our compass, surveillance our shield, and unity our greatest strength—forging a resilient defence against the erosion of our medical arsenal."

- Unknown

RESEARCH SIGNIFICANCE

In the face of the escalating global health threat posed by Antimicrobial Resistance (AMR), particularly in Low- and Middle-Income Countries (LMICs), gaining insights into the epidemiology and surveillance capacities of AMR in these regions is crucial for the effective implementation of intervention strategies.

The current thesis contributes to the AMR evidence base in Sub-Saharan Africa (SSA) through two comprehensive cross-sectional studies. The first, adopting a quantitative approach, analyzes clinical specimens from febrile patients hospitalized across Burkina Faso, Gabon, Ghana, and Tanzania, with emphasis on key AMR concerns highlighted by the World Health Organization, including carbapenem-resistant and extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, fluoroquinolone-resistant *Salmonella enterica*, and methicillin-resistant *Staphylococcus aureus* (*S. aureus*). This multi-center surveillance finds notable variations in susceptibility patterns across the regions studied with ESBL production and ciprofloxacin resistance notably prevalent in Burkina Faso, underscoring the need for region-specific AMR surveillance and improved reporting for tailored action.

The second study, utilizing a mixed methods approach, assesses the capacity for AMR surveillance in health laboratories across Kenya spanning public and private sectors, urban and rural settings, and various healthcare levels. The findings uncover gaps in laboratory information management technology, quality assurance, data management, and resources, particularly in rural areas. Notably, facilities performing bacterial cultures only and those conducting antimicrobial susceptibility testing (AST) show similar capacities, except in terms of equipment. These findings suggest that strategic investment in materials holds the potential to empower these laboratories to perform ASTs, offering a significant opportunity for improving AMR diagnostics and healthcare delivery within and beyond the country.

In essence, this thesis advances the knowledge of AMR in Sub-Saharan Africa, providing a roadmap for tailored policies, strengthened surveillance systems, and improved capacities in health laboratories, particularly in resource-limited settings. The findings can serve as a basis to gauge the potential impacts of future interventions, and the applied scoring tool can be utilized in similar contexts for comparative analysis. Moreover, the evaluation tool used in the study stands as a valuable resource for facilities to independently assess their capacities and practices, contributing to ongoing development efforts.

LIST OF SCIENTIFIC PAPERS

The thesis is based on the following manuscripts, each referenced within the text using Roman numerals.

III. **Moirongo RM**, Lorenz E, Ntinginya NE, Dekker D, Fernandes J, Held J, Lamshöft M, Schaumburg F, Mangu C, Sudi L, Sie A, Souares A, Heinrich N, Wieser A, Mordmüller B, Owusu-Dabo E, Adegnika AA, Coulibaly B, May J and Eibach D (2020) Regional Variation of Extended-Spectrum Beta-Lactamase (ESBL)-Producing Enterobacterales, Fluoroquinolone-Resistant *Salmonella enterica* and Methicillin-Resistant *Staphylococcus aureus* Among Febrile Patients in Sub-Saharan Africa. Front. Microbiol. 11:567235. doi: 10.3389/fmicb.2020.567235

IV. **Moirongo RM**, Aglanu LM, Lamshöft M, Adero BO, Yator S, Anyona S, May J, Lorenz E and Eibach D (2022) Laboratory-based surveillance of antimicrobial resistance in regions of Kenya: An assessment of capacities, practices, and barriers by means of multi-facility survey. *Front. Public Health* 10:1003178. doi: 10.3389/fpubh.2022.1003178

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ABSTRACT

Aim: To enhance the understanding of antimicrobial resistance patterns in Sub-Saharan Africa and evaluate existing laboratory capacity for AMR surveillance in the region.

Methods: The thesis comprises two cross-sectional studies: a quantitative approach in [I] and a mixed methods approach in [II]. In **Paper I**, clinical specimens were collected from febrile patients aged ≥30 days and ≤15 years in Burkina Faso, Gabon, Ghana, and Tanzania. Antimicrobial susceptibility testing was performed on Enterobacterales and *Staphylococcus aureus* using the disk diffusion method. ESBL production was confirmed via a double-disk diffusion test and gene detection. Multilocus sequence typing was conducted on ESBL-producing *Escherichia coli, Klebsiella pneumoniae*, ciprofloxacin-resistant *Salmonella enterica*, and *Staphylococcus aureus*. Ciprofloxacin-resistant *Salmonella enterica* isolates were screened for plasmid-mediated resistance genes and mutations. *S. aureus* isolates were tested for *mecA* and Panton-Valentine Leukocidin (*PVL*) presence and spa-typed. In **Paper II**, health facilities in Kenya were conveniently sampled. Online surveys were conducted with laboratory managers to assess quality assurance, data management, resources, staffing, competency, biosafety, and certification. Facility capacities were evaluated using a 0-1 scoring scheme and compared across facility types, settings, and governance levels.

Results: The study analyzed 4,052 specimens from 3,012 patients, finding 219 positive cultures. Prevalence of ESBL-producing Enterobacterales varied: highest in Burkina Faso (45.2%), followed by Gabon (25.8%) and Ghana (15.1%), while Tanzania had none. ESBL-positive *Salmonella* was found in Burkina Faso and methicillin-resistant *S. aureus* in Ghana. ST131 dominated ESBL *E. coli*, and ESBL *K. pneumoniae* had diverse sequence types. Ciprofloxacin-resistant *Salmonella* were common in Burkina Faso (50%), carrying qnrB genes. *PVL* was found in 81.3% of *S. aureus* **(I)**. Among 219 participating facilities, most did not offer bacterial culture testing (n= 135, 61.6%), while 47 (21.5%) provided culture services only, and 37 (16.9%) conducted antimicrobial susceptibility testing (AST). Major gaps in AST facilities were poor access to laboratory information management technology (LIMT) (score: 45.9%) and low participation in external quality assessment (EQA) programs (score of 67.7%). Urban facilities had more than two-fold higher access to laboratory technology compared to rural facilities (58.6% vs. 25.0%). Laboratories lacking culture services showed significant infrastructural gaps (average score 59.4%), whereas facilities performing cultures only and AST had notably high and similar scores (Average scores: 83.6%. & 82.9%). Lack of equipment was the main challenge for susceptibility testing in 46.8% of laboratories **(II).**

Conclusions: The findings highlight distinct susceptibility patterns in the study regions and underscore the need for local AMR surveillance and reporting. The study also identified gaps in laboratory information management technology, external quality assurance, and equipment in the surveyed health facilities in Kenya. The findings suggest that by investing in equipment, facilities performing cultures can be successfully upgraded to provide additional antimicrobial susceptibility testing, presenting a chance for a leap toward improved AMR diagnostics and surveillance in the country.

Contents

1.0 SYNOPSIS

1.1 Statement of the Problem

Antimicrobial resistance (AMR) poses a significant global health threat, jeopardizing the efficacy of treatment and prevention of infectious diseases across the world. Africa, especially Sub-Saharan Africa (SSA), emerges as a region of particular concern, with projections estimating 4.2 million annual deaths attributable to AMR by 2050 if no interventions are implemented [1]. Multiple factors contribute to the emergence and spread of AMR in SSA. The region bears a high burden of infectious diseases like diarrhoeal- and lower respiratory infections [2], which often necessitate extensive use of antibiotics [3–7] as common recourse. Limited access to professional healthcare, particularly in remote and underserved areas, encourages self-medication and reliance on drugstore owners, some of whom lack formal medical training. This unchecked consumption of antibiotics promotes the selection and spread of drug-resistant pathogens within communities and healthcare settings.

Robust surveillance systems are crucial for detecting AMR patterns and informing appropriate treatment regimens. However, SSA often lacks access to quality laboratory services, leading to empirical prescriptions, often involving broad-spectrum antibiotics [8–15]. The absence of reliable bacterial identification and susceptibility testing further hinders targeted therapy. Additionally, inefficient communication and data sharing due to inadequate infrastructure delay treatment initiation and potentially compromise patient outcomes. These challenges are exacerbated by poverty and limited governmental resources in SSA, creating persistent gaps in healthcare infrastructure.

Despite these obstacles, several global initiatives aim to combat AMR in the region. The World Health Organization's Global Antimicrobial Resistance and Use Surveillance System (GLASS) [16], the Africa CDC's Antimicrobial Resistance Surveillance Network (AMRSNET) [17], and the Fleming Fund [18], through partnerships like the African Society for Laboratory Medicine (ASLM) [19] work towards improved surveillance, laboratory capacity, and antibiotic stewardship. Regionally, the East African Community (EAC) launched the East African Integrated Disease

Surveillance Network (EAIDSNet) [20], to monitor infectious diseases, including those linked to AMR, across member states such as Kenya, Tanzania, and Uganda.

While these initiatives are promising, they remain in their early stages, and significant gaps in knowledge, data, and coverage, particularly outside urban centers, persist. This thesis aims to present actionable data on AMR patterns and resistance determinants of key bacterial pathogens in SSA, as identified by the World Health Organization [21]. Additionally, it will describe the capacities and limitations of laboratory infrastructure and AMR surveillance practices in healthcare facilities, aiming to contribute to sustainable AMR surveillance strategies in low- and middle-income countries.

1.1.1 Aims and Objectives

The overall aim was to contribute to the broader goal of enhancing the understanding of AMR and strengthening laboratory-based surveillance capacities in the Sub-Saharan African region.

1.1.2 The specific objectives of this research were twofold:

i. To investigate the antimicrobial susceptibility and resistance determinants of carbapenem-resistant and Extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, fluoroquinolone-resistant *Salmonella enterica*, and methicillin-resistant *Staphylococcus aureus* at hospitals in Burkina Faso, Gabon, Ghana, and Tanzania, which have been designated by the World Health Organization (WHO) as priority pathogens contributing to antimicrobial resistance [**Paper I**],

ii. To assess the capacities, practices, and barriers of laboratory-based surveillance of antimicrobial resistance in Kenya, while exploring potential opportunities for improvement [**Paper II**].

1.1.3 Research Questions:

i. What are the antimicrobial susceptibility and resistance determinants of carbapenem-resistant and Extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, fluoroquinolone-resistant *Salmonella enterica*, and methicillin-resistant *Staphylococcus aureus* at hospitals in Burkina Faso, Gabon, Ghana, and Tanzania?

ii. What are the current capacities, practices, barriers, and potential opportunities for improvement in laboratory-based surveillance of antimicrobial resistance in Kenya?

1.2 Methods

1.2.1 The Golden Thread of the Thesis

The methodological approaches employed to fulfil the specific objectives of the current thesis are outlined in Table 1.2.1. Detailed descriptions of the methods can be found in chapters 4 and 5. The thesis focuses on addressing antimicrobial resistance in Sub-Saharan Africa (Figure 1.2.1). Objective I investigated the antimicrobial susceptibility and resistance determinants of WHO-designated priority pathogens in selected hospitals (Figure 1.2.1), aiming to reveal the prevalence and mechanisms of resistance. On the other hand, objective II assessed capacities, practices, and barriers of laboratory-based AMR surveillance in multiple facilities within the region. These approaches sought to understand the state of AMR in Sub-Saharan Africa and identify potential strategies for promoting sustainable AMR surveillance in resource-limited settings.

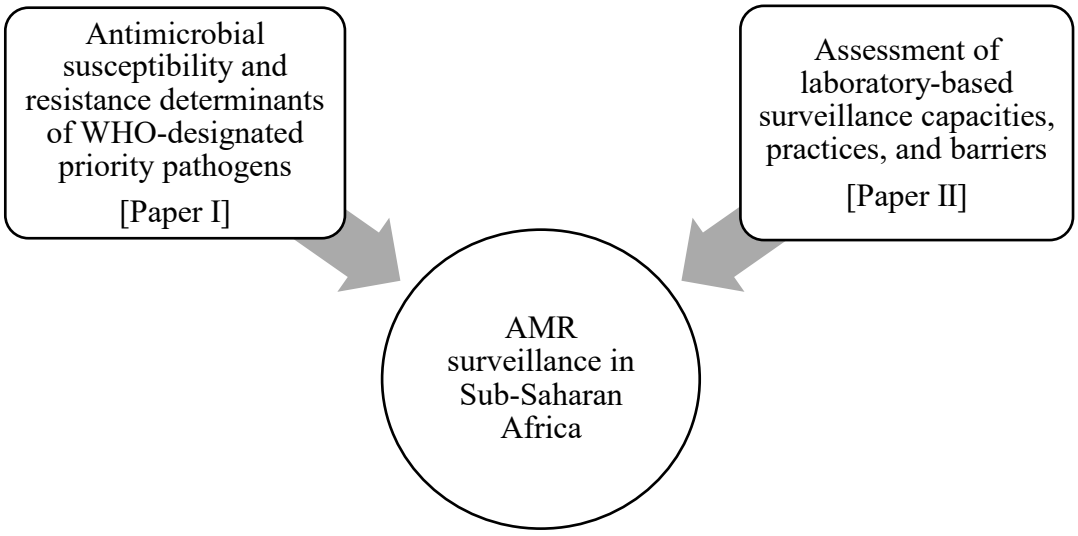

Figure 1.2.1: Study framework

Paper I- a quantitative multi-centric cross-sectional study investigating the antimicrobial susceptibility and resistance determinants of pathogenic bacteria among febrile patients hospitalized in Burkina Faso, Gabon, Ghana, and Tanzania.

Paper II- a cross-sectional, mixed methods study using online surveys of laboratory managers to evaluate the capacities, practices, and barriers pertaining to the detection and monitoring of AMR across health facilities in Kenya.

Table 1.2.1: Overview of topics and methods

Paper	Topic	Study design	Sampling and data collection	Data analysis
I	Antimicrobial resistance patterns and resistance determinants of Enterobacterales and Methicillin-Resistant *Staphylococcus aureus*	– Quantitative 4-year hospital-based cross-sectional surveillance	– Multiple specimen collection for culture from febrile in- and ambulatory patients – Bacterial Identification by standard biochemical methods using API – Disk diffusion and E-test for antibiotic susceptibility testing – Double-disk diffusion for confirmation of Extended-Spectrum Beta-Lactamase (ESBL) production – Polymerase Chain Reaction for detection of antibiotic resistant genes – Multi-locus Sequence Typing (MLST) and *Spa*-Typing for isolate strain characterization	– Descriptive statistics – Prevalence of resistances to studied antibiotics – Multilocus sequence types for ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates by country – Distribution of mutations and genes conferring ciprofloxacin resistance among *Salmonella enterica.* – Sequence types and *spa* types of *Staphylococcus aureus* isolates by country – Exact Clopper-Pearson method
II	AMR laboratory capacities, practices and barriers	– Cross-sectional online survey; using a 2-dimensional structure with multiple sub-dimensions and indicators – Mixed method	– Convenience and Snowball sampling methods to identify public and non-public laboratories with human health services – Questionnaires among laboratory managers responsible for AMR surveillance and Microbiology – Data collection and Management using REDCap electronic data capture tools	– Infrastructure and Resource capacity score – AMR surveillance Practice score – Indicator Weighting – Stratified analysis

Abbreviations: MLST, Multi-locus Sequence Typing; ESBL, Extended-spectrum beta-lactamase

1.2.2 Study Settings and Population

The research was conducted in hospitals within pre-existing German Centre for Infection Research (DZIF) collaborative networks[3] in countries facing high incidences of infectious diseases: Agogo Presbyterian Hospital in Ghana (catchment area: 149,491 inhabitants); Albert Schweitzer Hospital in Gabon (annual service to 50,000 people); Nouna District Hospital in Burkina Faso (population: 374,239); Matema Lutheran Hospital in rural Kyela district, Tanzania; and Kiwanja Mpaka clinic in urban Mbeya, Tanzania (catchment population: 500,000). The geographic locations of the participating hospitals are indicated in Figure 1.2.2, along with their respective bed capacities at the time of the study. Patients admitted to these hospitals with an axillary, rectal, or tympanic temperature of ≥38°C were included in the study from November 2013 to March 2017. Eligible patients were ≥30 days to ≤15 years of age, except in Tanzania where individuals above 15 years were also included [Paper I].

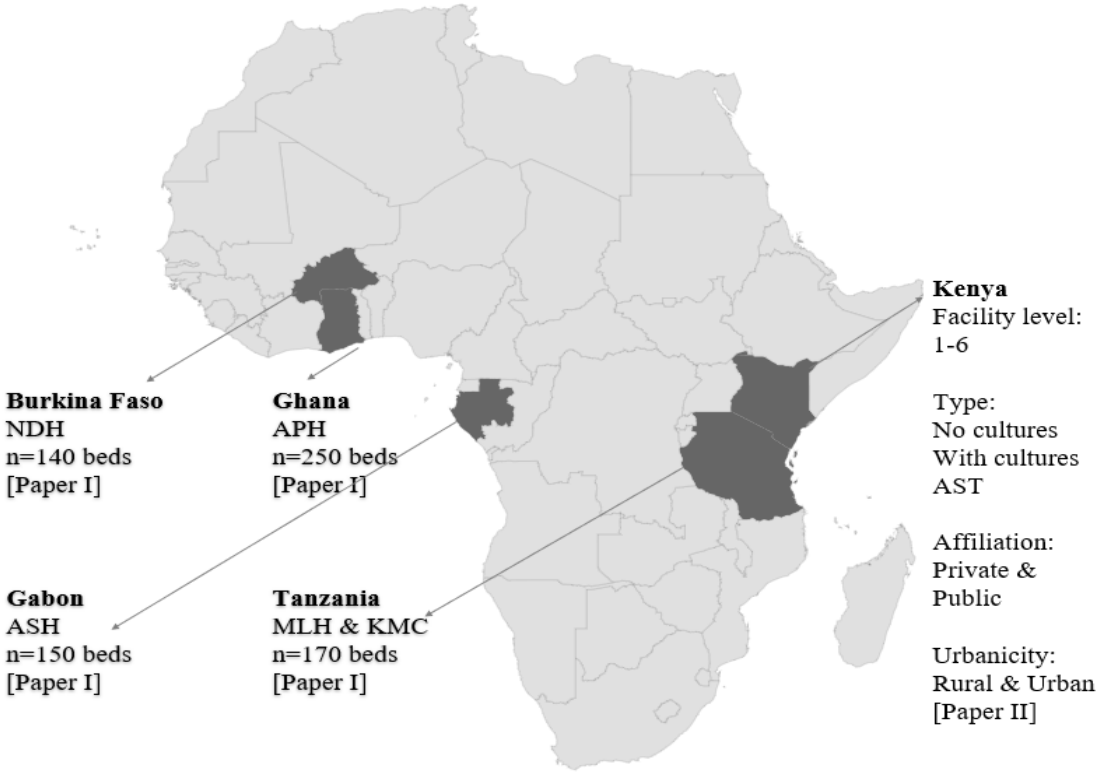

Figure 1.2.2: The map shows participating regions of Africa, with all countries represented in a uniform shade. NDH, Nouna District Hospital; APH, Agogo Presbyterian Hospital; ASH, Albert Schweitzer Hospital; MLH, Matema Lutheran Hospital; KMC, Kiwanja Mpaka Clinic; Facility type (No cultures; lacking culture testing,

[3] https://www.heidelberg-dzif.de/ttu-03-709

Cultures only; performing cultures but no antimicrobial susceptibility testing; AST, Antimicrobial susceptibility testing).

Paper II involved multiple laboratory facilities in Kenya. The Kenyan healthcare system is stratified into six hierarchical tiers. Lower-level facilities like community units (level 1) and health dispensaries (level 2) handle minor ailments, while county (level 5) and national (level 6) referral hospitals manage severe cases. Facility affiliation distinguishes between public and private ownership, the latter including non-public entities supported by faith-based organizations, non-government organizations, or operated for profit. Facility type was defined based on bacteriology activity, specifically the availability of culture services and antimicrobial susceptibility testing. The study area was classified as urban or rural based on population density and road network quality.

1.2.3 Sampling and Data Collection Snapshot

In **Paper I**, blood, stool, and urine specimens were collected from all patients at admission and cultured. Antimicrobial susceptibility testing was performed on Enterobacterales and *Staphylococcus aureus* using the disk diffusion method. ESBL production was confirmed via a double-disk diffusion test and gene detection. Multilocus sequence typing was conducted on ESBL-producing *Escherichia coli, Klebsiella pneumoniae*, ciprofloxacin-resistant *Salmonella enterica*, and *Staphylococcus aureus*. Ciprofloxacin-resistant *Salmonella enterica* isolates were screened for plasmid-mediated resistance genes and mutations. *S. aureus* isolates were tested for *mecA* and Panton-Valentine Leukocidin (*PVL*) presence and spa-typed.

In **Paper II**, health facilities were conveniently sampled. Online surveys were conducted with laboratory managers between 5th October and 8th December 2020. The survey combined two dimensions: (i) AMR surveillance practices and (ii) Laboratory infrastructure and resource capacity. Dimension 1, AMR surveillance practices, were further grouped into two subdimensions (quality assurance and management and dissemination of AMR data) of six indicators each. Dimension 2 combined six subdimensions with a variable number of indicators. Facility capacities were evaluated using a 0-1 scoring scheme and compared across facility types, settings, and governance levels. The areas addressed by the survey are summarized

in Figure 1.2.3. The data were collected and managed using REDCap electronic data capture tools.

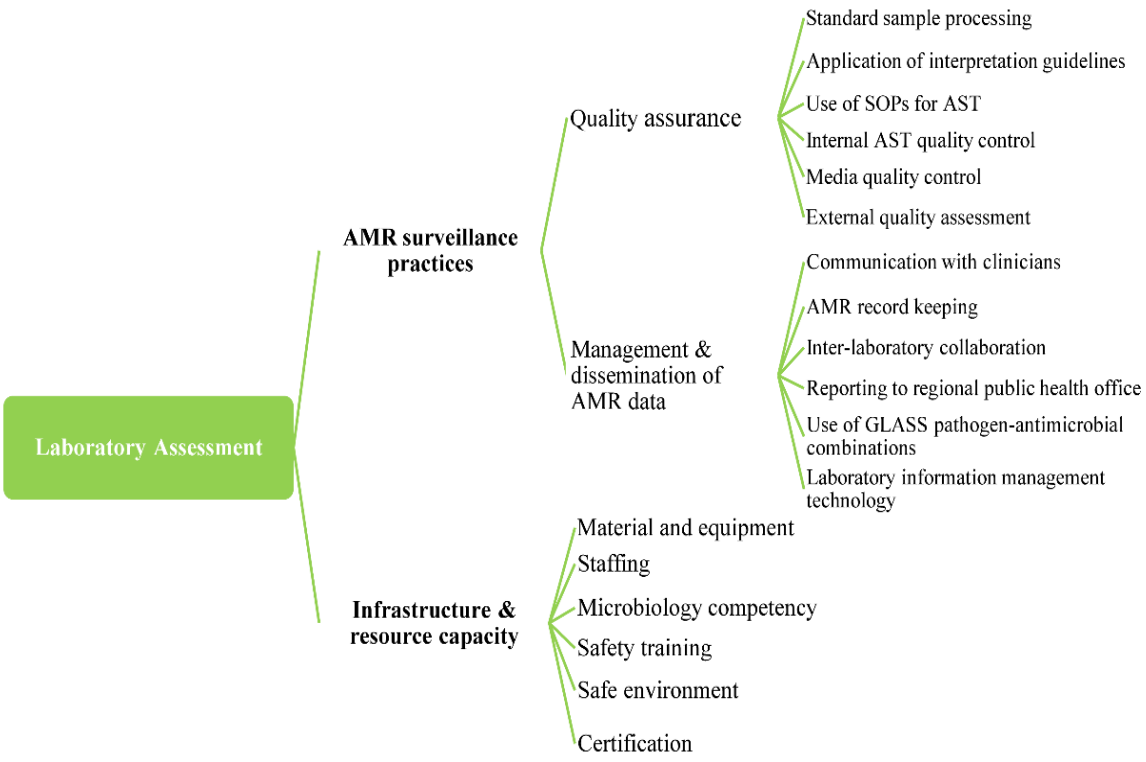

Figure 1.2.3: Dimensions, subdimensions, and indicators of assessment tool developed for health facilities in Kenya 2020. AMR, Antimicrobial Resistance; AST, Antimicrobial Susceptibility Testing; GLASS, Global Antimicrobial Resistance and Use Surveillance System; SOPs, Standard operating procedures.

1.2.4 Epidemiological Analyses

[Paper I] Descriptive statistics were used to present categorical variables as percentages and continuous variables as median and interquartile range (IQR). The statistical analysis was conducted using Stata version 14 software (StataCorp, College Station, Texas), and 95% confidence intervals were computed using the exact Clopper-Pearson method

[**Paper II**] A scoring system for the indicators was designed. Each indicator scored on a scale of 0-1, adapting established criteria. For the dimension “infrastructure and resource capacity,” indicators were reviewed and weighted based on their necessity for laboratory-based AMR surveillance. The weight values were assigned in indices and set from 0 to 1(**Appendix I**). All indicators of dimension “AMR surveillance practices” were weighted equally with value 1 as there are currently no standardized

guidelines pertinent to evaluating the indicators. The weighting criterion was defined by an expert team of the Department of Infectious Disease Epidemiology of the Bernhard Nocht Institute of Tropical Medicine and Kumasi Center for Collaborative Research. The questionnaire was piloted at a bacteriology laboratory in Germany before initiating the assessment. The total scores of all the indicators, subdimensions, and dimensions were converted into percentages. The total indicator scores were obtained as averages of all the participating facilities' indicator scores. For the dimension "AMR surveillance practices," overall scores per indicator were calculated as average indicator scores of facilities with susceptibility testing, whereas subdimension scores were obtained as average indicator scores. For dimension 2, laboratory infrastructure and resource capacity, we compared average subdimension scores for facilities without culture testing, those with cultures only, and those undertaking antimicrobial susceptibility testing, stratifying by affiliation, urbanicity, and level. Percentage values are interpreted as (80% and above) facility is adequate (60–79%) and requires some strengthening (<60%) needing significant strengthening, as similarly applied in other studies [22, 23].

1.2.5 Ethical Considerations

The study protocol was approved by the Institutional Review Board from each participating country. Informed consents were obtained from all participants.

1.3 RESULTS

This chapter presents the main findings derived from Papers I and II, focussing on two key areas: antibiotic susceptibility and resistance determinants across study countries [Paper I] and laboratory capacity, practices, and barriers to AMR surveillance [Paper II].

1.3.1 Antimicrobial Susceptibility in WHO-Designated Priority Pathogens Across African Hospitals

Paper I describes resistance patterns of ESBL-producing Enterobacterales, Ciprofloxacin-resistant *Salmonella enterica,* and Methicillin-resistant *S. aureus*. Data for this study was collected from febrile patients, admitted to hospitals in Burkina Faso, Gabon, Ghana, and ambulatory patients in Tanzania between November 2013 and March 2017. The study included participants aged ≥30 days to ≤15 years, except in Tanzania where patients above 15 were also included. Blood, stool, urine, and wound specimens were collected from the febrile patients before drug administration and analyzed. A total of 3,012 fever cases were recorded, with patients' ages ranging from 30 days to 81 years old. The median patient age was 2.0 years in pediatric study sites, while in Tanzania it was 9.1 years. The sex ratio was balanced, and the median temperature was 38.9 °C. Table 1.3.1 provides demographic information on the individuals recruited.

Table 1.3.1 Participant Overview

Country	Burkina Faso	Gabon	Ghana	Tanzania	Total
Patients, N	478	600	1238	696	3012
Age (median; IQR)	1.0; 0.3-2.0	2.4; 1.0-5.7	2.0; 1.0-4.0	6.7; 3.4-17.6	2.4; 1.0-5.6
Female, N (%)	221 (46.2)	280 (46.7)	561 (45.3)	390 (56.0)	1452 (48.2)
Temperature (median; IQR)	38.8; 38.4-39.3	39.0; 38.4-39.7	39.0; 38.5-39.6	38.9; 38.4-39.4	38.9;38.4-39.5

Abbreviations: IQR, interquartile range; N, sample size

1.3.1.1 Positivity Rate

Among 3423 blood, 629 urine, and 412 stool cultures, a total of 219 tested positive. The highest culture positivity rate was observed in urine (15.4%), followed by blood (3.3%) and stool samples (2.2%). Positive urine cultures were most frequently detected in Ghana, followed by Burkina Faso and Gabon. Positive blood cultures were most in Burkina Faso, followed by Ghana and Gabon. Stool cultures for *Salmonella/ Shigella* infections were most common in Burkina Faso, followed by Gabon and Ghana (Appendix II).

1.3.1.2 ESBL in Enterobacterales Across Countries

The majority of positive cultures (88.1%) were attributed to Enterobacterales, with *S. enterica* (41.1%), *E. coli* (32.9%), and *K. pneumoniae* (10.0%) being the most prevalent species (Appendix III). Among Enterobacterales, 20.7% were ESBL-producing organisms, with the highest rates observed *in K. pneumoniae* (63.6%) and *E. coli* (31.9%), and the lowest in *S. enterica* (3.8%). The proportions of ESBL-producing organisms varied across countries, with Burkina Faso having the highest rate (45.2%), followed by Gabon (25.8%) and Ghana (15.1%), while none were found in Tanzania (Appendix III). All ESBL-producing Enterobacterales across countries harboured only *bla*CTX-M15 ESBL genes.

1.3.1.3 Resistance to Ciprofloxacin among Enterobacterales

The proportion of Enterobacterales with reduced susceptibility to ciprofloxacin was notably higher in Burkina Faso (51.6%) compared to Gabon (12.9%), Ghana (13.4%), and Tanzania (8.3%). Ciprofloxacin resistance among non-typhoidal *Salmonella* (NTS) was 50.0% in Burkina Faso, and 3.3% in Ghana (Appendix III). No ciprofloxacin-resistant NTS was found in Tanzania and Gabon. In Burkina Faso, all isolates were *Salmonella* Typhi (*S*.Typhi) with ST 313 and harboured *qnr*B genes. Ghana had two ciprofloxacin-resistant *S*.Typhi isolates with a single *gyr*A mutation. A ciprofloxacin-resistant *S*.Typhi with *gyr*A double mutations was found in Burkina Faso, with a second ciprofloxacin-resistant *S.* Typhi harbouring *gyr*A and *gyr*B mutations found in Tanzania. ESBL production and ciprofloxacin resistance concurrence were observed in Burkina Faso among NTS isolates. No mutations or resistance genes were found in *parC, parE*, *aac* (6') *Ib*(*-cr*), *qnrA, qnrC, qnrD*, and *qnrS* genes (Appendix V).

1.3.1.4 Methicillin-Resistant *S. aureus*

A total of 16 *S. aureus*, constituting 7.3% of all positive cultures, were isolated from thirteen blood and three urine cultures, primarily from Tanzania (*n* = 5) and Ghana (*n* = 5) (Appendix II). The isolates belonged to six *spa* types and five STs, with ST 152 being the most common clone, particularly prevalent in Tanzania, Burkina Faso, and Gabon. The most frequent *spa* types included *t*355, followed by *t*186 and *t*314. Panton-Valentine Leukocidin (*PVL*) was found in 81.3% of isolates, with 76.9% of these being ST152. All *S. aureus* isolates from Tanzania and Burkina Faso were *PVL*-positive. Two MRSA were found in Ghana, both belonging to ST 88, *spa*-type *t*186, *mecA* positive, and *PVL* negative (Appendix VI).

1.3.2 Capacities, Practices, and Barriers of Laboratory-based Surveillance of Antimicrobial Resistance Surveillance in Kenyan Laboratories

In paper II, a convenience sample of healthcare facilities in Kenya, encompassing public and private sectors, rural and urban settings, as well as national, county, and community units, were assessed using an online survey. A scoring system was applied to evaluate indicators of quality assurance, management and dissemination of AMR data, material and equipment, staffing, microbiology competency, biosafety, and certification. This section provides a summary of capacities and gaps in infrastructure and AMR surveillance practices across these diverse healthcare facilities.

1.3.2.1 Study Facilities

Between October 5th and December 8th, 2020, 466 REDCap survey links were distributed to health facilities nationwide. The data collection achieved a response rate of 73.2%, with 341 completed surveys. After reconciling duplicates and incomplete forms, 219 surveys (64.2%) of submitted forms were considered for analysis. Among these facilities, 61.6% did not offer culture testing, 21.5% conducted cultures only (without AST), and 16.9% performed AST. The majority of facilities were from the private sector (55.3%), and there was a balanced representation between urban (49.3%) and rural (50.7%) areas. Notably, a higher proportion of rural facilities (72.1%) lacked culture testing compared to urban facilities (50.9%). Susceptibility testing availability increased with the facility level, ranging from 0% in community health units and dispensaries to 100% in national referral (Appendix XI).

1.3.2.2 Strengths and Gaps in Quality Assurance and Management of Data among AST Facilities

The evaluation of antimicrobial susceptibility testing (AST) facilities involved two subdimensions: "quality assurance" and "management and dissemination of AMR data." AST facilities demonstrated overall high performance (average score: 86.5%) in "quality assurance," with scores above 80% (indicating adequacy) in four of six indicators Appendix X). However, a notable gap was identified in "external quality assessment" (score: 67.6%), where 32.4% of facilities reported non-participation in external quality assessment (EQA) programs for bacterial species isolation. The uptake of EQA programs was generally balanced across rural and urban settings and public and private sectors. In the subdimension "management and dissemination of AMR data" (average score: 73.9%), facilities excelled in "communication with clinicians" (score: 100%) and "AMR record-keeping" (score: 94.6%) but showed significant weakness in "laboratory information management technology" (LIMT) and the analysis and sharing of microbiology data (score: 45.9%). LIMT availability was particularly scarce in rural areas (25%) compared to urban areas (58.6%) but was similarly available in the public (35%) and private (47.1%) sectors. Regionally, LIMT was more available in facilities in Nairobi (92.9%) followed by the Central administrative region (50%) (Appendix VIII).

Regarding the Global Antimicrobial Resistance and Use Surveillance System (GLASS), specified pathogen-antimicrobial combinations were fully applied in about half of the facilities and partially applied in 10 (Appendix IX). In cases where GLASS guidelines were partially applied, modifications were made to the list of antimicrobial agents provided by the World Health Organization (WHO), and the priority pathogens for surveillance in Sub-Saharan Africa were altered.

1.3.2.3 Comparison of Infrastructural and Resource Capacities Across Study Facilities

The evaluation of health laboratories' infrastructure and resource capacities considered various aspects, including "material and equipment," "staffing," "microbiology competency," "biosafety training," "safe environment," and "certification," with multiple indicators (Appendix I). The capacities of laboratories exhibited variations across facility levels and types. Community units and dispensaries

showed a need for significant infrastructural strengthening (scores <60%), while county and national referral hospitals, as well as research centres, appeared to be performing well (scores >80%) (Appendix XI).

Among the investigated facility types, those lacking culture testing recorded the lowest average score (59.4%), requiring substantial improvement. Of all facilities, 53.4% were certified, and 13.7% were in the process of receiving certification. Interestingly, facilities offering only cultures (average score: 83.6%) or antimicrobial susceptibility testing (AST) (average score: 82.9%) demonstrated similar capacity scores across all categories, with "certification," "staffing," and "microbiology competency" ranking the highest.

Scores showed minimal variation between urban (73.3%) and rural (64.4%) facilities but were comparable between the public (68.9%) and private (68.7%) sectors. Facilities generally scored moderate to high in "safe environment" (73.6–87.7%) and "biosafety training" (65.0–80.1%). However, 11% reported never receiving biosafety training, while the majority (89%) received training at least once per year.

1.3.2.4 Obstacles to Antimicrobial Susceptibility Testing among Culture-Performing Facilities

Several reasons for the inability to perform AST among facilities with culture services were identified (Table 1.3.2). The leading challenge reported was the unavailability of equipment, with a higher prevalence in the public sector (62.5%). The most commonly lacking equipment included -70°C freezers, water distillation systems, blood culture machines, safety cabinet level 2, atmosphere generating systems, glass or disposable Petri dishes, warm air incubators, and manual pipettes. Lack of funds and challenges related to the acquisition and maintenance of supplies were cited more frequently in the public sector compared to the private sector. Inadequate competency among personnel was the least identified challenge reported.

Table 1.3.2 Barriers Hindering implementation of antimicrobial susceptibility testing among facilities with culture services in regions of Kenya

Barriers	All facilities (n=47)	Public (n=16)	Private (n=31)
Lack of equipment	22(46.8)	10(62.5)	12(38.7)
Samples processed at partner facility	22(46.8)	5(31.3)	17(54.8)
Lack of funds	14(29.8)	7(43.8)	7(22.6)
Challenges obtaining supplies of reagents and materials	10(21.3)	9(56.5)	1(3.2)
Lack of skilled personnel	2 (4.3)	0(0)	2(6.5)

Abbreviation: n, sample size

1.4 DISCUSSION

1.4.1 Antimicrobial Susceptibility in WHO-Designated Priority Pathogens Across African Hospitals

This research identifies Enterobacterales as a major cause of fever in rural and semi-urban Sub-Saharan Africa, with widespread and geographically variable resistance to commonly used antibiotics, including penicillins, cephalosporins, and fluoroquinolones. ESBL-producing Enterobacterales rates vary from 45% (Burkina Faso) to 26% (Gabon) to 15% (Ghana), aligning with similar studies in the respective countries [24–26]. Contrasting rates reported in large cities [27–29] suggest within-country AMR variations, potentially due to differing methodological approaches adopted in the studies. The subsequent evaluation study within this thesis [paper II] exemplifies this variability, revealing non-standardized AMR practices in the study country, particularly in sample processing, application of interpretation guidelines, protocol usage, and data management and dissemination. These findings further emphasize the necessity for standardization to enhance comparability and validity of AMR data.

As the predominant pathogen detected in blood cultures (70.8%; 69 non-typhoidal *Salmonella,* 11 *S.* Typhi) in the research, resistance rates of *Salmonella enterica* can potentially serve as a surrogate indicator for guiding empirical treatment for invasive bloodstream infections in the study regions. The detection of ESBL-producing non-typhoidal *Salmonella* in samples from Burkina Faso is consistent with previous reports [30]. Notably, no ESBL production was observed in *S.* Typhi, suggesting the continued susceptibility of these pathogens to recommended third-generation cephalosporins.

Remarkable regional variations in ciprofloxacin-resistant non-typhoidal *Salmonella* were identified, with Burkina Faso exhibiting over 10 times higher frequency than Ghana, consistent with previous findings [31]. While Kenya has reported high ciprofloxacin-resistant *S.* Typhi, none has been observed in Central or West Africa. Previous research suggests a potential spread of these resistances from the Indian subcontinent to East Africa [32]. In this study, ciprofloxacin-resistant *S.* Typhi was detected in Tanzania (1/10) and Burkina Faso (1/1), suggesting a possible dissemination to West Africa, warranting close monitoring.

High ESBL rates were found in *E. coli* and *K. pneumoniae*, raising concerns about transferrable antibiotic resistance and nosocomial outbreaks, particularly in regions with limited treatment options [18]. The study also reported methicillin resistance in *S. aureus* isolates from Ghana, with Burkina Faso exhibiting higher AMR levels than in Gabon and Ghana.

The geographical disparities in AMR levels may be associated with socioeconomic and health factors, as previously described [35]. Burkina Faso, ranking low in the Human Development Report, exhibits higher resistance levels than Gabon and Ghana [36]. The study acknowledges limitations, including variations in participants' demographics, and potential underrepresentation due to non-prescription antibiotic use, low blood sample positivity rates, and self-medication prior to hospitalization [37–39], emphasizing the need for cautious interpretation of the reported proportions. Moreover, it calls for expanded region-specific surveillance networks in Sub-Saharan Africa, a need resounded in the evaluation study [paper II], to promote representative AMR surveillance and improved clinical case management.

1.4.2 Capacities, Practices, and Barriers of Laboratory-based Surveillance of Antimicrobial Resistance Surveillance in Kenyan Laboratories

The evaluation found that health facilities in Kenya need improvement in key laboratory areas, particularly in quality assurance, information management, materials, and equipment. The lack of strong information management systems to support the surveillance of antimicrobial resistance (AMR) is widespread in Sub-Saharan Africa, limiting the ability to monitor, understand, and respond effectively to the growing threat of antimicrobial resistance. Only a few national AMR data systems exist, including the East Africa Public Health Laboratory Network (EAPHLN) sentinel site project [40] and the Mapping Antimicrobial Resistance and Antimicrobial Use Partnership (MAAP), which is operational in 14 countries across West, East, and Southern Africa [18].

Urban facilities exhibited more than two-fold higher access to laboratory technology than rural counterparts, reflecting the longstanding healthcare delivery imbalance common in low- and middle-income countries [41]. Since the disease burden entwined

with drug regulatory problems is prominent in remote and usually poor areas [42], mitigating this disparity is crucial for improving representative AMR surveillance.

The research identifies a gap in quality assurance, particularly the low uptake of external quality assessment (EQA) programs for bacterial species identification. Despite the WHO's regional EQA program's expansion, many peripheral laboratories lack EQA provision [43, 44]. Poor internal quality control mechanisms, including limited use of control strains and infrequent application of WHO-specified pathogen-antimicrobial combinations, were also revealed, raising concerns about result credibility.

Laboratories without culture testing, mainly serving Kenya's vast rural population, were identified as having the weakest infrastructure and resource capacity [45, 46]. Notably, facilities performing bacterial cultures only and those conducting antimicrobial susceptibility testing (AST) show comparable capacities, with the exception of equipment. These findings suggest that strategic investment in materials holds the potential to empower these laboratories to perform ASTs, offering a significant opportunity for improving AMR diagnostics and healthcare delivery within and beyond the country.

Limitations of the study include self-reported data and a lack of on-site visits due to COVID-19 restrictions, potentially affecting the accuracy of assessments. While not covering all geographical areas, it provides a diverse reflection of Kenya's laboratory capacity status.

1.5 CONCLUSION

The thesis highlights geographical variations in AMR levels across Sub-Saharan Africa, emphasizing the need for region-specific surveillance and improved reporting of AMR data for evidence-based local decision-making. The findings underscore the importance of national initiatives to strengthen antimicrobial surveillance systems in African countries to curb the spread of drug-resistant pathogens and preserve the effectiveness of available treatment. While the study found no resistance to carbapenems, indicating their potential as an alternative therapy for ESBL-producing bacteria despite their cost and limited availability, it is essential to remain vigilant in detecting emerging carbapenemase-producing pathogens promptly.

Furthermore, the research systematically assessed health laboratories across diverse regions in Kenya, identifying shortcomings in information management technology, external quality assurance, and the availability of materials and equipment. Notably, facilities performing bacterial cultures only and those conducting antimicrobial susceptibility testing (AST) were found to have similar capacities, except in terms of equipment. This implies that investing in equipment could enhance the capabilities of facilities conducting cultures, thereby facilitating expanded antimicrobial susceptibility testing, and improving AMR diagnostics and surveillance not only in Kenya but also across SSA countries.

Overall, this thesis represents a substantial advancement in understanding AMR in Sub-Saharan Africa, offering actionable insights that can guide policymakers, healthcare professionals, and organizations working towards effective AMR management in resource-limited settings

2.0 ABBREVIATIONS

AMR:	Antimicrobial Resistance
AMRSNET:	Africa CDC's Antimicrobial Resistance Surveillance Network
API:	Analytical Profile Index
APH:	Agogo Presbyterian Hospital
ASH:	Albert Schweitzer Hospital
ASLM:	African Society for Laboratory Medicine
AST:	Antimicrobial Susceptibility Testing
ATCC:	American Type Culture Collection
CDC:	Centers for Disease Control and Prevention
CDDEP:	Centre for Disease Dynamics, Economics & Policy
CLSI:	Clinical and Laboratory Standards Institute
CRE:	Carbapenem-Resistant Enterobacterales
COVID-19:	Coronavirus Disease 2019
DNA:	Deoxyribonucleic Acid
DZIF:	German Centre for Infection Research
EAC:	East African Community
EAIDSNet:	East African Integrated Disease Surveillance Network
EAPHLN:	East Africa Public Health Laboratory Network
EA-REQAS:	East African Regional External Quality Assessment Scheme
EQA:	External Quality Assessment
ESBL:	Extended-spectrum beta-lactamases

EUCAST:	European Committee on Antimicrobial Susceptibility Testing
GHPP:	Global Health Protection Program
GLASS:	Global Antimicrobial Resistance and Use Surveillance System
IQR:	Interquartile Range
KIPPRA:	Kenya Institute for Public Policy Research and Analysis
KMC:	Kiwanja Mpaka Clinic
LIMT:	Laboratory Information Management Technology
LMICs:	Low- and Middle-Income Countries
MAAP	Mapping Antimicrobial Resistance and Antimicrobial Use Partnership
MIC:	Minimum Inhibitory concentration
MLH:	Matema Lutheran Hospital
MLST:	Multilocus Sequence Typing
MOH:	Kenyan Ministry of Health
MRSA:	Methicillin-Resistant *Staphylococcus aureus*
N:	Sample Size
NACOSTI:	National Commission for Science, Technology, and Innovation
NAP:	National Action Plan
NCTC:	National Collection of Type Cultures
NDH:	Nouna District Hospital
NGO:	Non-Government Organizations
NTS:	Non-Typhoidal *Salmonella*
PCR:	Polymerase Chain Reaction

PVL:	Panton-Valentine Leukocidin
SLIPTA:	Improvement Process Towards Accreditation
SOPs:	Standard operating procedures
SSA:	Sub-Saharan Africa
ST:	Sequence Type
REDCap:	Research Electronic Data Capture
WHO:	World Health Organization

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4.0 PUBLICATION I: Regional variation of extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, fluoroquinolone-resistant *Salmonella enterica* and methicillin-resistant *Staphylococcus aureus* among febrile patients in sub-Saharan Africa

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4.1 Abstract

Background: Antimicrobial resistance (AMR) thwarts the curative power of drugs and is a present-time global problem. We present data on antimicrobial susceptibility and resistance determinants of bacteria the WHO has highlighted as being key antimicrobial resistance concerns in Africa, to strengthen knowledge of AMR patterns in the region.

Methods: Blood, stool, and urine specimens of febrile patients, aged between ≥ 30 days and ≤ 15 years and hospitalized in Burkina Faso, Gabon, Ghana, and Tanzania were cultured from November 2013 to March 2017 (Patients > 15 years were included in Tanzania). Antimicrobial susceptibility testing was performed for all Enterobacterales and Staphylococcus aureus isolates using disk diffusion method. Extended-spectrum beta-lactamase (ESBL) production was confirmed by double-disk diffusion test and the detection of *bla*CTX−M, *bla*TEM and *bla*SHV. Multilocus sequence typing was conducted for ESBL-producing Escherichia coli and Klebsiella pneumoniae, ciprofloxacin-resistant *Salmonella* enterica and S. aureus. Ciprofloxacin-resistant. *Salmonella* enterica were screened for plasmid-mediated resistance genes and mutations in gyrA, gyrB, parC, and parE. S. aureus isolates were tested for the presence of *mecA* and Panton-Valentine Leukocidin (*PVL*) and further genotyped by spa typing.

Results: Among 4,052 specimens from 3,012 patients, 219 cultures were positive of which 88.1% (n = 193) were Enterobacterales and 7.3% (n = 16) S. aureus. The prevalence of ESBL-producing Enterobacterales (all CTX-M15 genotype) was 45.2% (14/31; 95% CI: 27.3, 64.0) in Burkina Faso, 25.8% (8/31; 95% CI: 11.9, 44.6) in Gabon, 15.1% (18/119; 95% CI: 9.2, 22.8) in Ghana and 0.0% (0/12; 95% CI: 0.0,26.5) in Tanzania. ESBL positive non-typhoid *Salmonella* (n = 3) were detected in Burkina Faso only and methicillin-resistant S. aureus (n = 2) were detected in Ghana only. While sequence type (ST)131 predominated among ESBL E. coli (39.1%;9/23), STs among ESBL K. pneumoniae were highly heterogenous. Ciprofloxacin resistant nno-typhoidal *Salmonella* were commonest in Burkina Faso (50.0%; 6/12) and all harbored qnrB genes. *PVL* were found in 81.3% S. aureus.

Conclusion: Our findings reveal a distinct susceptibility pattern across the various study regions in Africa, with notably high rates of ESBL-producing Enterobacterales and ciprofloxacin-resistant non-typhoidal *Salmonella* in Burkina Faso. This highlights the need for local AMR surveillance and reporting of resistances to support appropriate action.

4.2 Introduction

Fever, caused by bacterial, viral, fungal and parasitic pathogens, is a leading complaint presented at healthcare centres in sub-Saharan Africa (sSA)[47, 48]. Ideally, effective individual treatment involves identifying the infectious agents through cultures, serological and molecular tests and subsequently targeting them with appropriate antimicrobials [49]. However, in several cases empirical treatment needs to be initiated before microbiological diagnostics to avert potential complications and to improve patient outcome [50].The inadequate diagnostic infrastructure in most African countries [51–53] further complicates targeted antimicrobial therapy resulting in heavy reliance on empiric judgement at hospitals, particularly those in remote areas.

As antimicrobial resistance (AMR) increases across the globe [54], adapting guidelines for empiric therapy to the changing drug susceptibility pattern is needed to allow better antimicrobial prescription decisions. At present, infections with AMR pathogens account for approximately 700,000 global deaths per year, and are estimated to cause up to 10 million annual deaths by 2050, with Africa expected to be one of the hardest hit continents (O'Neill, 2014). The mounting threat notwithstanding, only a few studies have so far addressed the growth of AMR in middle and low-income countries [55]. Not surprisingly, investigations have indicated that guidelines on empirical antibiotic therapy in these countries are seldom if ever, formulated in view of regional microbiology and drug susceptibility patterns. For example, Elias *et al* showed that only a third of recommendations on empirical antibiotic use for community-acquired pneumonia, urinary tract infections, acute otitis media, rhinosinusitis and pharyngitis considered the extent of local resistance, mostly in high-income settings [56]. Enhancing understanding of the actual epidemiology of the local resistance landscape, on a routine basis, is therefore critical for adequate treatment guidelines, antimicrobial control policies and appropriate action plans in the regions. Although effort has been made by the WHO to foster national AMR surveillance systems in low-and middle-income countries by introducing GLASS (Global Antimicrobial Resistance Surveillance System) [21, 57] only a few countries in sSA have functional AMR surveillance systems and for those that do, data from outside the major cities are scarce [58].

This multicentre surveillance in rural and semi-urban Burkina Faso, Gabon, Ghana and Tanzania aimed to identify the causative pathogen for bloodstream-, urinary tract-, and gastrointestinal tract infections among hospitalized febrile patients with a focus on carbapenem-resistant and Extended-spectrum beta-lactamase (ESBL)-producing

Enterobacterales, fluoroquinolone-resistant *Salmonella enterica* and methicillin-resistant *Staphylococcus aureus*, declared as WHO priority AMR pathogens. Results will be used to guide physicians and public health experts on empiric antibiotic treatment decisions and hospital medication guidelines.

4.3 Materials and Methods

4.3.1 Study Population and Study Sites

The study was conducted among patients with an axillary or rectal or tympanic temperature of ≥38°C, admitted at hospitals in Burkina Faso, Gabon, Ghana and ambulatory patients in Tanzania from November 2013 to March 2017. Participants were between ≥30 days and ≤15 years of age from all sites, except for Tanzania where patients above 15 were also included (n=188). The largest among the hospitals (250 beds), is Agogo Presbyterian Hospital (APH) situated in the capital of Asante-Akim North District in Ghana. Its catchment area has an estimated 149,491 inhabitants[4]. Albert Schweitzer Hospital (ASH) is in Lambaréné, Gabon. As of 2017, the facility had 150 beds and served around 50,000 people a year [59]. Nouna District Hospital (NDH) is located in the Kossi province, North West of Burkina Faso, with a total population of 374,239 inhabitants and 50 primary health care facilities[5]. The NDH has a bed capacity of 140, of which 50 are in the pediatric unit. In the Mbeya region, two health institutions were used: the Matema Lutheran Hospital, which is situated in a rural area in the Kyela district at the shore of Lake Nyassa and the Kiwanja Mpaka clinic which is in urban Mbeya, a town of 500,000 inhabitants.

4.3.2 Detection and Identification of Pathogens

Specimens were collected from all patients at admission following physical examination by a medical practitioner and preceding drug administration. Three millilitres (ml) of blood were drawn from each child and 8-10 millilitres from adults. Each blood sample was inoculated into an aerobic paediatric/adult blood culture bottle (BACTEC (Peds) Plus Culture Vials, (Becton Dickinson, Germany) and processed using a BACTEC 9050 blood culture system (Becton Dickinson, Germany) according to the manufacturer's instructions. Positive blood cultures were sub-cultivated on Columbia blood agar, chocolate agar, and MacConkey agar (all Oxoid, Basingstoke, United Kingdom) for species identification and antimicrobial susceptibility testing. If

[4] https://www.statsghana.gov.gh/
[5] http://www.insd.bf/n/

urinary tract infection signs (painful urination, renal angle tenderness, elevated leucocytes or nitrite in urine dipstick) were manifest, two ml urine was obtained from patients and plated on Chromogenic UTI Medium (Oxoid, Basingstoke, United Kingdom). From cases presenting with diarrhoea, defined as three or more loose stools in 24h, a tea-spoon size of stool was screened for *S. enterica* and *Shigella* spp. Samples were enriched overnight on selenite F broth and then cultured on xylose lysine deoxycholate agar (Becton Dickinson, Germany) and MacConkey agar.

Incubations were done overnight at 35°C-37°C. All bacterial colonies were further identified by standard biochemical methods using the Analytical profile Index test (API 20E, bioMérieux, Marcy L'Etoile, France). Environmental bacteria and bacteria belonging to the skin flora (eg, coagulase-negative Staphylococci except *S. saprophyticus* in urine and *S. lugdunensis* in blood culture, *Corynebacterium* species, and *Bacillus* species) were considered as contaminants. All isolates were sent to Germany on dry ice for species confirmation by MALDIToF MS (Bruker Daltonics, Bremen, Germany) and subtyping analysis.

4.3.3 Antimicrobial Susceptibility Testing

For all Enterobacterales and *S. aureus*, antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to the 2019 European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [60]. Enterobacterales were tested against ampicillin, cefotaxime, ceftazidime, meropenem, ciprofloxacin, gentamicin, tigecycline and sulfamethoxazole/trimethoprim (all Oxoid, Basingstoke, United Kingdom). Ciprofloxacin resistance was defined as a MIC > 0.06 mg/L for *S. enterica*, confirmed by E-test (Oxoid, Basingstoke, United Kingdom) and a MIC > 1 mg/L for other Enterobacterales. A positive ESBL phenotype was confirmed by the double-disk diffusion test with cefotaxime and ceftazidime alone and in combination with clavulanic acid (Becton, Dickinson and Company, Sparks, MD, USA) as described before by the EUCAST [61]. *S. aureus* isolates were tested against penicillin, cefoxitin, clindamycin, erythromycin, ciprofloxacin, tetracycline, sulfamethoxazole/trimethoprim, tigecycline, gentamicin, rifampicin, linezolid, teicoplanin and vancomycin (all Oxoid, Basingstoke, United Kingdom).

4.3.4 ESBL Genotyping and Ciprofloxacin Resistance Mechanisms

All isolates with ESBL phenotypes were screened for the presence of *bla*$_{CTXM}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ genes by polymerase chain reaction (PCR) and sequenced as previously described. [62]. Group specific primers were used to distinguish *bla*$_{CTXM}$ positive isolates [62]. The resulting sequences were compared with known sequences using NCBI BLAST[6] and the Lahey Clinic Database[7]. Screening of ciprofloxacin non-susceptible *S. enterica* isolates for point mutations in DNA gyrase and/or DNA topoisomerase IV genes *gyrA*, *gyrB*, *parC*, *parE* and plasmid-mediated *aac(6')Ib(-cr)*, *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS* resistance genes was performed as previously described [63–65].

4.3.5 *Spa*-Typing and Multilocus Sequence Typing (MLST)

MLST was conducted for all *S. aureus*, ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* and ciprofloxacin-resistant *S. enterica* according to previously published loci protocols [66, 67]. *S. aureus* isolates were genotyped by *spa*-typing as described before [33] with *spa*-types being determined using the StaphType software and the Ridom SpaServer[8].

4.3.6 Epidemiological Analysis

Categorical variables were displayed with percentages, and continuous data with the median and interquartile range (IQR). Data analysis was performed with Stata version 14 software (StataCorp, College Station, Texas). 95% confidence intervals were calculated using the exact Clopper-Pearson method.

4.3.7 Ethical Considerations

The study protocol was approved by the Institutional Review board of Nouna Research Centre and the National Ethics Committee for Health Research in Burkina Faso, the Comité d'Ethique Institutionel in Lambaréné, Gabon, the Committee on Human Research, Publications and Ethics from the School of Medical Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, the Ethics committee of the medical faculty of the Ludwig Maximilians University in Munich, the Tanzanian National Research and Ethics committee, and the Mbeya Medical Research and

[6] https://blast.ncbi.nlm.nih.gov/Blast.cgi
[7] https://omictools.com/the-lahey-clinic-database-tool
[8] http://spaserver.ridom.de/

Ethics Committee for Tanzania and the Ethics Committee of the Ärztekammer Hamburg, Germany. All participants were informed about the study's purpose and procedures. Written informed consents were obtained from all participants above 18 years. In older children, written informed assent and consent was obtained from the patients and their parents, respectively. In case of infants, the parents or legal guardian provided written informed consent prior to enrolment.

4.4 Results

Altogether, the study recorded 3,012 fever cases aged between 30 days and 81 years from Burkina Faso, Gabon, Ghana and Tanzania. Patients above 15 years were only included in Tanzania (n=188). For all study sites, the median patient age was 2.4 years (IQR 1.0-5.6). The sex ratio was balanced (Female: 48.2%, n=1,452) and the median tympanic temperature IQR was 38.4-39.5°C. Possible bacterial causes of fever were analysed in 3009 blood, 629 urine and 412 stool samples obtained. Table 1.3.1 provides demographic information on the individuals recruited.

4.4.1 Bacterial Cultures

Overall, 219 cultures were positive. The highest culture positivity rate was found in urine (15.4%; 97/629) (95% CI: 12.7, 18.5), followed by blood (3.8%; 113/3009) (95% CI: 2.7, 4.0) and stool samples (2.2%; 9/412) (95% CI: 1.0, 4.1). For Tanzania, blood culture positivity was 3.7% (7/188) among participants above 15 years and 2.0%; (10/508) among children up to 15 years of age.

The positive urine cultures were most frequently detected in Ghana (26.5%; 63/238) (95% CI: 21.0, 32.6), then Burkina Faso (9.5%; 10/105) (95% CI: 4.7, 16.8) and Gabon (8.4%; 24/286) (95% CI: 5.5, 12.2). Positive blood cultures were most prevalent among samples from Burkina Faso (5.6%; 27/482) (95% CI: 3.7, 8.0), then Ghana (4.9%; 61/1238) (95% CI: 3.8, 6.3), Tanzania (2.4%; 17/696) (95% CI: 0.9, 2.4) and Gabon (1.4%; 8/593) (95% CI: 0.6, 2.6). Stool cultures for *Salmonella/Shigella* infections were commonest in Burkina Faso (5.6%; 1/18) (95% CI: 0.1, 27.3), followed by Gabon (3.3%; 3/91) (95% CI: 0.7, 9.3) and Ghana (2.2%; 5/232) (95% CI: 0.7, 5.0). No pathogenic bacterium was isolated in stool samples from Tanzania. Uropathogens detected were mainly *E. coli* (62.9%; n=61) and *K. pneumoniae* (19.6%; n=19). Bloodstream bacteria included *S. enterica* (70.8%; n=80), *E. coli* (9.7%; n=11), *S. aureus* (11.5%; n=13) and *K. pneumoniae* (2.7%; n=3). Gastrointestinal pathogens

were *S. enterica* (88.9%; n=8) and *Shigella sonnei* (11.1%; n=1). All cultures processed and country distribution of the species isolated are presented in **Appendix II**.

4.4.2 ESBL in Enterobacterales Across Countries

Enterobacterales accounted for 88.1% (193/219) of the positive cultures, with *S. enterica* (41.1%; n=90), *E. coli* (32.9%; n=72) and *K. pneumoniae* (10.0%; n=22) being the most prevalent. The most active antibiotics against these bacteria were meropenem (1.6%; n=3 intermediate resistance), tigecycline (7.8%; n=15 resistance) and ciprofloxacin (19.2%; n=37 resistance). Resistance was highest against ampicillin (67%; n=130), trimethoprim-sulphamethoxazole (62%; n=119) and gentamicin (50%; n=96). Appendix IV shows resistance patterns of all the Enterobacterales to the antibiotics tested. ESBL-producing organisms constituted 20.7% (n=40) of all Enterobacterales with the highest rate being among *K. pneumoniae* (63.6%; 14/22) and *E. coli* (31.9%; 23/72), and lowest among *S. enterica* (3.8%; 3/79). In decreasing order, their proportions were: 45.2% (14/31) (95% CI: 27.3, 64.0) in Burkina Faso, 25.8% (8/31) (95% CI: 11.9, 44.6) in Gabon, 15.1% (18/119), (95% CI: 9.2, 22.8) in Ghana and 0.0% (0/12) (95% CI: 0.0, 26.5) in Tanzania (Appendix III). The ESBL-producing Enterobacterales across all countries harboured only *bla*CTX-M15 ESBL genes. Thirteen MLST sequence types (ST) were identified among ESBL *E. coli* and 13 among ESBL *K. pneumoniae.* The set of ST found was unique for each country (Appendix V), except for ST 131, which was found among nine ESBL *E. coli*, mainly in Burkina Faso (n=5), Gabon (n=1) and Ghana (n=3).

4.4.3 Resistance to Ciprofloxacin among Enterobacterales

The proportion of Enterobacterales with reduced susceptibility to ciprofloxacin was notably higher in Burkina Faso (51.6%; 16/31) (95% CI: 33.1, 69.8), relative to 12.9% (4/31) (95% CI: 3.6, 29.8) in Gabon, 13.4% (16/119) (95% CI: 7.9, 20.9) in Ghana and 8.3% (1/12) (95% CI: 0.2, 38.5) in Tanzania (Appendix III). Among non-typhoidal *Salmonella* (NTS), ciprofloxacin resistance was 50.0% (6/12, (95% CI: 21.1, 78.9) in Burkina Faso, 3.3% (2/60) (95% CI: 0.4, 11.5) in Ghana and 0.0% (95% CI: 0.0, 41.0) in Gabon (0/7) and Tanzania (0/0). All isolates of Burkina Faso were ST 313 and harboured *qnrB* genes (5 blood, 1 stool) while both isolates (blood) from Ghana had a single *gyrA* mutation at position D87G. A ciprofloxacin-resistant *Salmonella* Typhi was isolated in a blood sample from Burkina Faso. The pathogen had a *gyrA* double mutations (D87G and E113G). A second bloodstream ciprofloxacin-resistant *S.* Typhi with a *gyrA* mutation at position E113G and a *gyrB* mutation S464Y was found in

Tanzania. Among NTS, ESBL production and ciprofloxacin resistance concurrence was observed in a single stool and two blood isolates from Burkina Faso only. No *parC*, *parE* mutations as well as *aac (6') Ib(-cr)*, *qnrA*, *qnrC*, *qnrD* and *qnrS* genes were found (Appendix VI).

4.4.4 Methicillin-Resistant *S. aureus*

A total of 16 *S. aureus,* constituting 7.3% (n=16) of all positive cultures, were isolated from thirteen blood and three urine cultures, mostly from Tanzania (n=5) and Ghana (n=5) (Appendix II). The isolates belonged to six *spa*-types and five ST. ST 152 was the most common clone, which accounted for 80.0% (4/5), 100% (3/3), 66.7% (2/3), and 20.0% (1/5) of *S. aureus* from Tanzania, Burkina Faso, Gabon and Ghana, respectively. The most frequent *spa*-types included t355 (50.0%; n=8), followed by t186 (12.5%; n=2) and t314 (12.5%; n=2). Panton-Valentine Leukocidin (*PVL*) were found in 13 (81.3%) isolates, of which 10 (76.9%) were ST152. All *S. aureus* isolates from Tanzania and Burkina Faso, were *PVL* positive. Two MRSA were found in Ghana, both ST 88, *spa*-type t186, *mecA* positive and *PVL* negative (Appendix VII).

4.5. Discussion

The current study identifies Enterobacterales as a major cause of fever in the hospitals in rural sub-Saharan Africa and reports that resistance to widely used antibiotics including penicillins, cephalosporins and fluoroquinolones is common and geographically variable. We found unequal rates of ESBL-producing Enterobacterales, ranging from 45% (Burkina Faso) to 26% (Gabon) to 15%(Ghana). These proportions are in line with reports from similar studies in the respective study countries [26, 68, 69]. However, differing ESBL rates have been reported from previous studies conducted in large cities in the countries, suggesting that AMR levels also vary within a country. The regional differences may be due to ununiform methodological approaches adopted in the studies, such as, varying inclusion criteria (age groups, type of hospital, in-patient or out-patient, symptoms etc.). To allow better comparability of results and improve data validity, we underscore the significance of standardized AMR methods and harmonized interpretation procedures using tools such as the GLASS guidelines to this end (WHO, 2015).

Being the most frequently detected pathogen from blood cultures in the present study study (70.8%; 69 NTS, 11 *S.* Typhi), resistance rates of *S. enterica* are key to determine the proper empirical treatment for invasive bloodstream infections in the

countries. Notably, ESBL NTS were only found in blood and stool samples from Burkina Faso, similar to what has been reported before [30]. No ESBL production was detected among *S.* Typhi. These low frequencies of ESBL-producing *S. enterica* indicate that the pathogens remain largely susceptible to the recommended third generation cephalosporins.

Remarkable regional differences were detected for ciprofloxacin-resistant NTS, with a frequency more than 10-folds higher observed in Burkina Faso compared to Ghana. A similar observation has previously been described [31]. High levels of ciprofloxacin-resistant *S.* Typhi in Kenya and none in Central or West Africa have been reported. The authors postulated a spread of these resistances from the Indian subcontinent to East Africa [32]. We found ciprofloxacin-resistant *S.* Typhi in Tanzania (1/10) and Burkina Faso (1/1), hinting at a possible dissemination to West Africa that needs to be monitored closely.

We found high ESBL rates among *E. coli* and *K. pneumoniae.* These organisms being important sources of transferrable antibiotic resistance and nosocomial outbreaks, the observed rates raise concern in countries with limited antibiotic treatment options [69]. For *K. pneumoniae* a clear geographical segregation with no overlapping STs or any predominant *K. pneumoniae* strains across the participating countries was observed. We found ST 131 *E. coli* among blood samples in Burkina Faso and urine samples in Ghana, Burkina Faso and Gabon. ESBL ST 131 *E. coli* has been described as a major cause of extraintestinal infections worldwide (e.g. UTI, bloodstream infections, meningitis, soft tissue infections) [34].

This study also reports methicillin resistance in two *S. aureus* isolates from Ghana. Equally low numbers of *S. aureus* were found in Burkina Faso, Gabon and Tanzania although none was MRSA.

The geographical disparity of AMR levels observed in our study may be associated with socio-economic and health factors, as described in other studies. In one such study, a systematic segregation of high-income countries in Europe, North America, Oceania from low-income countries in Africa, Asia, South America according to AMR gene abundance was demonstrated [35]. The present study reveals a separation of countries within sSA with Burkina Faso, which ranks very low (rank 182) in the human development report showing higher resistance levels than Gabon and Ghana, ranking

at 115 and 142, respectively [70]. It is notable that these findings hardly provide a representative basis but expose the need for expanding region-specific surveillance networks for AMR in African countries. On this matter, a recent progress report shows that 11/31 countries in sSA, Ghana included, have a national action plan (NAP) approved by government, with an operational plan and monitoring arrangements. Tanzania is already in the implementation phase while NAPs in Burkina Faso and Gabon have just recently been developed [71].

Some limitations of this study need to be addressed. First, there was no age restriction for participants from Tanzania whereas the other study sites included only children, thus, comparability of results from Tanzania to those from other countries was undermined. In some instances, the culture positivity rate was low. Easy availability of non-prescription antibiotics [37] and self-medicating prior to hospitalization [38, 39] may have caused the bacterial cultures to remain negative. Also, drawing sufficient quantities of blood samples from children was difficult, hence the low positivity rates. In this light, the proportions reported here should be interpreted in view of the isolate numbers. Also important is that our study was performed in single hospitals and this does not necessarily reflect the antimicrobial resistance trends in other regions within the countries.

4.5 Conclusion

This study reveals high occurrence of ESBLs and ciprofloxacin-resistant Enterobacterales in clinical samples in sSA. Further effort to enhance reporting of regional epidemiological AMR data is needed for evidence-based local treatment guidelines. On a national level, initiatives to scale up antimicrobial surveillance systems ought to be supported in the African countries to drive interventions aimed at limiting the dissemination of drug resistant pathogens. On an encouraging note, the study found no resistance to carbapenems, meaning that these antibiotics, although costly and hardly available, hold potential as an alternative therapy for ESBL-producing bacteria. Nevertheless, vigilance to detect emerging carbapenemase-producing pathogens, in time, is required.

4.6 DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

4.7 ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review board of Nouna Research Centre and the National Ethics Committee for Health Research in Burkina Faso, the Comité d'Ethique Institutionel in Lambaréné, Gabon, the Committee on Human Research, Publications and Ethics from the School of Medical Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, the Ethics Committee of the medical faculty of the Ludwig Maximilians University in Munich, the Tanzanian National Research and Ethics committee, and the Mbeya Medical Research and Ethics Committee for Tanzania and the Ethics Committee of the Ärztekammer Hamburg, Germany. All participants were informed about the study's purpose and procedures. Written informed consents were obtained from all participants above 18 years. In older children, written informed assent and consent was obtained from the patients and their parents, respectively. In case of infants, the parents or legal guardian provided written informed consent prior to enrolment.

4.8 AUTHOR CONTRIBUTIONS

DE, JM, AA, BM, ASo, NH, and ML designed and coordinated the study. NN, JF, JH, CM, LS, BC, EO-D, and ASi conducted and supervised the fieldwork. FS, AW, DD, and DE supervised the laboratory work. EL and RM performed the epidemiological and statistical analysis. RM, EL, and DE wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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4.10 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5.0 PUBLICATION II: Laboratory-Based Surveillance of Antimicrobial Resistance in Regions of Kenya: An Assessment of Capacities, Practices, and Barriers by Means of Multi-Facility Survey

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5.1 Abstract

Background: Adequate laboratory capacity is critical in the implementation of coherent surveillance for antimicrobial resistance (AMR). We describe capacities and deficiencies in laboratory infrastructure and AMR surveillance practices among health facilities in Kenya to support progress towards broader sustainable laboratory-based AMR surveillance.

Methods: A convenience sample of health facilities from both public and private sectors across the country were selected. Information was obtained cross-sectionally between 5th October and 8th December 2020 through online surveys of laboratory managers. The assessment covered quality assurance, management and dissemination of AMR data, material and equipment, staffing, microbiology competency, biosafety and certification. A scoring scheme was developed for the evaluation and interpreted as (80% and above) facility is adequate, (60-79%) requires some strengthening and (<60%) needing significant strengthening. Average scores were compared across facilities in public and private sectors, rural and urban settings, as well as national, county and community levels.

Results: Among the participating facilities (n=219), the majority (n=135, 61.6%) did not offer bacterial culture testing, 47(21.5%) offered culture services only and 37(16.9%) performed antimicrobial susceptibility testing (AST). The major gaps identified among AST facilities were poor access to laboratory information management technology (LIMT) (score: 45.9%) and low uptake of external quality assessment (EQA) programs for cultures (score 67.7%). Access to laboratory technology was more than two-fold higher in facilities in urban (58.6%) relative to rural (25.0%) areas. Whilst laboratories that lacked culture services were found to have significant infrastructural gaps (average score 59.4%), facilities that performed cultures only (average score: 83.6%) and AST (average score: 82.9%) recorded significantly high scores that were very similar across areas assessed. Lack of equipment was identified as the leading challenge to the implementation of susceptibility testing among 46.8% of laboratories.

Conclusions: We identified key gaps in laboratory information management technology, external quality assurance and material and equipment among the surveyed health facilities in Kenya. Our findings suggest that by investing in equipment, facilities performing cultures can be successfully upgraded to provide additional

antimicrobial susceptibility testing, presenting a chance for a major leap towards improved AMR diagnostics and surveillance in the country.

5.2 Introduction

The growing public health threat of antimicrobial resistance (AMR) increasingly undermines our ability to treat and prevent infections caused by bacteria with existing antibiotic medication. AMR can be effectively minimized through coherent surveillance that facilitates continuous capture and onward sharing of reliable data for the development of targeted curtailing interventions on local, national, and global levels [1, 72, 73]. Primarily, laboratory testing is the foundation for detecting resistance [74] and providing essential information for clinicians to institute appropriate treatment regimens for patients, thereby limiting potential misuse of drugs. However, where quality laboratory services are not always available, treatment often involves untargeted empirical administration of antimicrobials, including broad-spectrum agents, accelerating the development and spread of drug resistant microorganisms.

In Kenya, AMR data are mainly generated by the Kenya Medical Research Institute [75], supplemented by central reference laboratories, large hospitals and sentinel sites set up to address specific pathogens of major public health concern. Surveillance for AMR extends to facilities run by individuals or corporations, and in some cases externally funded research units. The past decade has seen a significant increase in effort to describe and tackle the burden due to drug-resistant infections in the country [76], although overall nationwide surveillance is still at the early stages with AMR data generally remaining patchy [77]. Over the years, many studies have demonstrated variable resistance rates in microorganisms that are associated with unfavorable outcomes in hospital and community settings, such as those that cause among others; tuberculosis, meningitis, pneumonia, and gastrointestinal diseases [78–84]. Findings from these studies and other initiatives fighting AMR highlight the need for horizontal [51, 85–87] as well as vertical [88] strengthening of laboratory capacity to promote widespread detection of resistance and to create strong evidence for optimal AMR response.

Our study applies quantitative scores to assess health laboratories in Kenya to identify deficiencies in resources, infrastructural and operational capacities regarding dimensions of surveillance systems emphasized by the WHO strategy on the

containment of AMR [89]. Recognizing resource scarcity, this assessment could guide planning, prioritization and implementation of project activities to support progress towards broader sustainable laboratory-based AMR surveillance in low-income settings.

5.3 Methods

5.3.1 Survey Tool

We composed a detailed online survey based on the WHO Antimicrobial Resistance Surveillance Questionnaire for Assessment of National Networks [90] and the Stepwise Laboratory (Quality) Improvement Process Towards Accreditation (SLIPTA) checklist[9]. The survey combined two dimensions: (i) AMR surveillance practices and (ii) Laboratory infrastructure and resource capacity. Dimension 1, AMR surveillance practices, was further grouped into two subdimensions (quality assurance and management and dissemination of AMR data) of six indicators each. Dimension 2 combined six subdimensions with a variable number of indicators (Appendix I). The areas addressed by the survey are summarized on figure 1**.2.3.**

A scoring system for the indicators was designed, adapting previously established criteria [22, 23, 91]. Each indicator was scored on a scale of 0 -1 as follows: A 'yes' or 'present and functional' gave an index value of 1, 'partial' or 'other' or 'present and non-functional' 0.5 and a 'no 'or 'absent' 0. For the dimension 'infrastructure and resource capacity', indicators were reviewed and weighted based on their necessity for laboratory-based AMR surveillance. The weight values were assigned in indices and set from 0 to 1 as described in Appendix I. All indicators of dimension 'AMR surveillance practices' were weighted equally with value 1 as there are currently no standardized guidelines pertinent to evaluating the indicators. The weighting criterion was defined by an expert team of the department of Infectious Disease Epidemiology of the Bernhard Nocht Institute of Tropical Medicine and Kumasi Centre for Collaborative Research. We piloted the questionnaire at a bacteriology laboratory in Germany before initiating assessment**.**

[9] https://apps.who.int/iris/handle/10665/204423

5.3.2 Sampling and Data Collection

A combination of convenience and snowball sampling methods was used in the study, taking advantage of previously established in-country networks. Only laboratories with human health services were included in the assessment covering elements such as their level, affiliation, type and urbanicity. Facility level refers to the six hierarchical tiers of the Kenyan healthcare service delivery system [92]. In the tier structure, the lower-level facilities including community units (level 1) and health dispensaries (level 2) are typically the first points of care for the management of minor ailments like common cold, uncomplicated malaria and diarrhoea. On the other hand, county (level 5) and national (level 6) referral hospitals, handle more severe cases that require specialized care[10]. Facility affiliation relates to ownership i.e., public or private. In this study, non-public entities include those supported by faith-based and non-government organizations as well as those run for profit by private companies or individuals. We described facility type based on bacteriology activity, particularly the availability of culture services and antimicrobial susceptibility testing (AST). The study area was defined as either urban; densely populated regions with compact road networks, or rural; moderate to sparsely populated regions with poor road network. Information was obtained cross-sectionally between 5th October and 8th December 2020, through online surveys of laboratory managers responsible for AMR surveillance, microbiology, and laboratory systems.

5.3.3 Data Management and Analysis

The data were collected and managed using REDCap electronic data capture tools hosted at the Bernhard Nocht Institute for Tropical Medicine, Germany[11]. Reconciliation of inconsistencies and missing data was done before conducting statistical analyses. The total scores of all the indicators, subdimensions and dimensions were converted into percentages. The total indicator scores were obtained as averages of all the participating facilities indicators scores. For the dimension 'AMR surveillance practices', overall scores per indicator were calculated as average

[2] https://roggkenya.org/2019/07/22/kenyas-health-structure-and-the-six-levels-of-hospitals-an-overview/
[3] https://redcapinfo.ucdenver.edu/citing-redcap.html

indicator scores of facilities with susceptibility testing, whereas subdimension scores were obtained as average indicator scores. Performance strengths and proportions of facilities across the AMR surveillance areas are displayed on a stacked bar chart. For dimension 2, laboratory infrastructure and resource capacity, we compared average subdimension scores for facilities without culture testing, those with cultures only and those undertaking antimicrobial susceptibility testing, stratifying by affiliation, urbanicity and level. Percentage values are interpreted as (80% and above) facility is adequate, (60-79%) requires some strengthening, (<60%) needing significant strengthening, as similarly applied in other studies [22, 23].

5.3.4 Ethical Considerations

The study was reviewed and approved by the National Commission for Science, Technology, and Innovation (NACOSTI) License No. NACOSTI/P/20/4083 and authorization to carry out the assessment granted by the Kenyan Ministry of Health (MoH). To ensure confidentiality, respondent identification information was only accessed by authorized people of the study.

5.4 Results

5.4.1 Study Facilities

Between 5th October and 8th December 2020, 466 REDCap survey links were sent to health facilities across the country. A response rate of 73.2% (n=341) was recorded at the end of the data collection period. Following cleaning and reconciliation of duplicates, incomplete and inconsistent forms, surveys from 219 (64.2%) of the submitted forms were considered for analysis. Most of the participating facilities are located in the country's densely populated areas, mainly the capital city Nairobi, the Lake Victoria, and the Coastal regions whilst the sparsely settled areas of north and eastern regions of the country are scarcely covered. Figure 2 shows the geographical locations of the health facilities that completed the survey. Of the total facilities (n=219), majority (61.6%; n=135) offered no culture testing, 21.5% (n=47) had cultures only i.e. no antimicrobial susceptibility testing and 16.9% (n=37) performed antimicrobial susceptibility testing (Appendix IX). There were slightly more facilities from the private (55.3%; n=121) relative to the public sector (44.8%; n=98), whereas the representation between urban (49.3%; n= 108) and rural (50.7%; n= 111) areas was balanced. A

notably higher proportion of facilities in rural areas (72.1%) lacked culture testing compared to those in urban areas (50.9%). Similarly, only 7.2% (n=8) of the participating facilities in rural areas performed susceptibility tests compared to 26.9% (n=29) of those in urban areas. Availability of susceptibility testing increased with advancing facility level from 0% in community health units and dispensaries to 100% in national referral hospitals. Further details on differences across laboratory affiliation, level, urbanicity and administrative region are represented on Appendix IX.

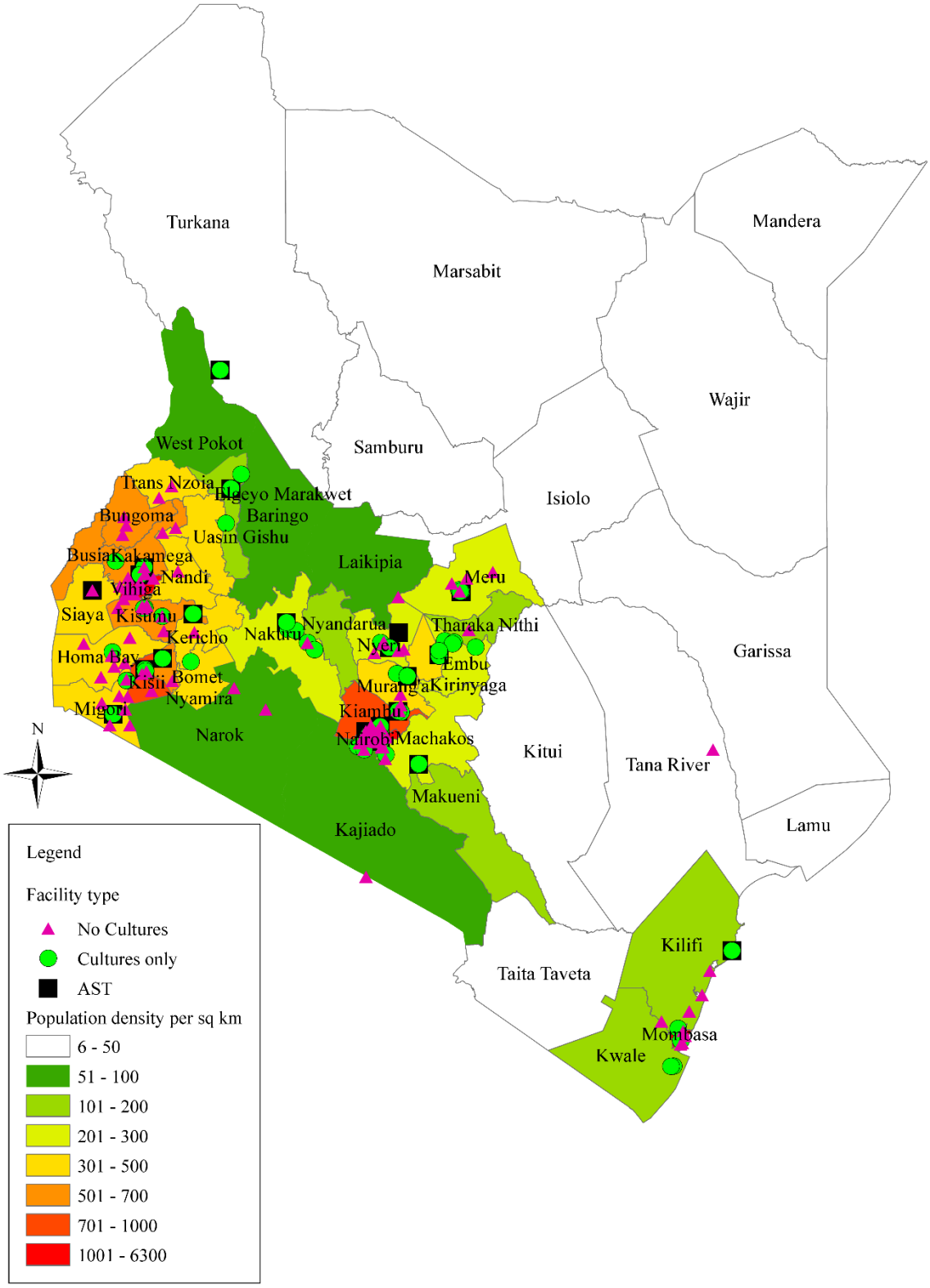

Figure 2: Map of Kenya representing the geographic locations of the health facilities completing the survey and the survey and the population density by location. Facility type (No cultures; lacking culture testing, Cultures only, performing cultures but no antimicrobial susceptibility testing; AST, Antimicrobial susceptibility testing).

5.4.2 Strengths and Gaps in Quality Assurance and Management of Data among AST Facilities

Indicators to evaluate antimicrobial susceptibility testing facilities were distributed across 2 subdimensions: ‘quality assurance’ and ‘management and dissemination of AMR data’.

The AST facilities recorded an overall high performance (average score: 86.5%) in ‘quality assurance’ with scores >80 % (facility is adequate) in four of six indicators (Appendix X). However, a substantial gap was identified in ‘external quality assessment’ (score 67.6%) as 12 (32.4%) facilities reported non-participation in external quality assessment (EQA) programs for bacterial species isolation. Uptake of the EQA programs was generally balanced in facilities in rural and urban settings and those in public and private sectors (Appendix VIII).

For the subdimension ‘management and dissemination of AMR data’ (average score: 73.9%), facilities were strong in ‘communication with clinicians’ (score: 100%) and ‘AMR record keeping’ (score: 94.6%) but significantly weak in ‘laboratory information management technology’ (LIMT) i.e., software to support systematic collation, analysis and sharing of microbiology data (score: 45.9%) (Appendix X). LIMT was particularly scarce in rural (25%) relative to urban (58.6%) areas but similarly available in the public- (35%) and private (47.1%) sectors (Appendix VIII). The availability of LIMT also varied regionally, being available in more facilities in Nairobi (92.9%) followed by the Central (50%) administrative regions (Appendix VIII). GLASS (Global Antimicrobial Resistance and Use Surveillance System) specified pathogen-antimicrobial combinations [93] were fully applied in about half of the facilities (score: 51.4%; n=19) and partially applied in 10 (score: 13.5%) (Appendix X). Where GLASS guidelines were partially applied (n=10), the list of antimicrobial agents provided by WHO and the priority pathogens for surveillance in Sub-Saharan Africa were modified.

5.4.3 Comparison of Infrastructural and Resource Capacities Across Study Facilities

Health laboratories’ infrastructure and resource capacities were evaluated in terms of ‘material and equipment’, ‘staffing’, ‘microbiology competency’, ‘biosafety training’, ‘safe environment’ and ‘certification’ based on multiple indicators as detailed in Appendix I. Generally, the laboratories demonstrated varied capacities across facility

level and type (Appendix IX). Community units and dispensaries required the most significant infrastructural strengthening (scores <60%) whereas county and national referral hospitals as well as research centres seemed to be performing well (scores > 80%). Across the three facility types investigated, those that lacked culture testing recorded the lowest average score (59.4%), with the subdimensions 'material and equipment' (score 44.8%), and 'certification' (score 39.0%) requiring significant strengthening. A total of 117 (53.4%) of all facilities were certified whereas 30 (13.7%) were in the process of receiving certification. Interestingly, in facilities where cultures only (average score: 83.6%) or AST (average score: 82.9%) were available, capacity scores were quite similar in all categories, with 'certification', 'staffing' and 'microbiology competency' ranking the highest. Scores varied minimally across facilities in urban (73.3%) and rural (64.4%) areas, but were similar between the public (68.9%) and private (68.7%) sectors. Facilities had moderate to high scores in 'safe environment' (73.6% - 87.7%) and 'biosafety training' (65.0% - 80.1%) although 11% (n=24) reported to never receiving any training in biosafety. The other 89 % (n= 195) receives the training between once in two years (n=16) to twice a year (n=100).

5.4.4 Obstacles to Antimicrobial Susceptibility Testing among Culture-Performing Facilities

Several reasons for the inability to perform antimicrobial susceptibility testing among facilities with culture services were provided (Table 1.3.2). Unavailability of equipment was identified as the leading challenge to testing for resistance by 46.8% of the facilities, particularly those in the public sector (62.5%). Most of the laboratories (68%; n=32) lacked -70°C freezers, followed by water distillation systems (38.3%; n=18), blood culture machines (29.8%; n=14), safety cabinet level 2 (23.4%; n=11), atmosphere generating systems (23.4%; n=11), glass or disposable petri dishes (21.3%; n=10), warm air incubators (21.3%; n=10) and manual pipettes (12.8%; n=6). Besides, lack of funds (43.8%) and the acquisition and maintenance of supplies (56.5%) were cited as challenges for the public sector in comparison to the private sector. Inadequate competency among personnel was the least identified challenge across the facilities, at only 4.3%. Aside the outlined challenges, 46.8% of the facilities reported to refer samples to other facilities for susceptibility testing.

5.5 Discussion

According to the present assessment, health facilities in multiple regions of Kenya require strengthening in key laboratory areas including, but not limited to, laboratory information management technology, external quality assurance and material and equipment. In sub-Saharan Africa, robust information management structures to support AMR surveillance are limited. National AMR data systems are few and examples include the East Africa Public Health Laboratory Network (EAPHLN) sentinel site project [40] and Mapping Antimicrobial Resistance and Antimicrobial Use Partnership (MAAP), now covering 14 countries across West, East and Southern Africa [18]. In high-income settings where well-functioning AMR surveillance systems exist, technologies for data management are integrated into most health systems as is in several European countries [94, 95]. In such settings, inter-country benchmarking of AMR trends [95] is possible and reliable AMR information is available for action. Thus, bridging the technological gap in health facilities in Kenya could enhance effective analysis and output of credible results for clinical case management and policy use.

Access to laboratory technology was more than two-fold higher in facilities in urban relative to rural areas. This finding mirrors the longstanding maldistribution of health-care delivery common in low- and middle-income countries [41]. Since disease burden entwined with drug regulatory problems are prominent in remote and poor areas [42], mitigating the inequitable access to laboratory technology is essential for improved representative AMR surveillance.

The study also identified a key gap in quality assurance, particularly low uptake of external quality assessment (EQA) programmes for bacterial species identification. Within Africa, WHO launched a regional microbiology EQA programme in 2002 that initially supported 39 national public laboratories from 30 member states. As of 2009, participating laboratories had doubled and 18 more member states had enrolled [43]. Although this suggests that implementation of EQA programmes in Africa has improved over time, a vast majority of peripheral laboratories still lack EQA provision [44] . In Kenya for instance, the WHO program serves two national facilities [96], a pattern that is likely to be similar throughout sub-Saharan African countries. There is therefore a need for the establishment of effective EQA schemes for bacterial identification and antimicrobial susceptibility testing in developing countries in order to ensure accuracy of laboratory investigations.

Poor internal quality control mechanisms were found among the participating facilities. This was evident in the limited use of control strains for cultures in several facilities, posing a challenge over the credibility of results generated by the laboratories. Whereas the use of unified international guidelines (CLSI or EUCAST) for interpretation of susceptibility results was noted in almost all facilities, the application of WHO specified pathogen-antimicrobial combinations was infrequent or partial in some cases, which could undermine uniformity and comparability of AMR data on multiple levels.

Infrastructure and resource capacity was rather weak among laboratories that lacked culture testing, particularly health centres, dispensaries and community units. Addressing the inadequacies would be of great benefit to an estimated 36% of Kenya's population [45], comprising the vast rural population primarily served by these facilities [46]. Notably, laboratories with cultures only and those with AST showed similar strengths in capacities. These findings hint at a potential target opportunity of upgrading facilities that perform cultures to implement antimicrobial susceptibility testing, with minimum investment. Such investments through the national and county governments in collaboration with development partners would greatly improve healthcare provision as well as AMR surveillance. Obstacles to the implementation of AST were lack of equipment and funding, while trained personnel seemed to be available. With the existing infrastructure and trained workforce in place, we suggest that future healthcare projects prioritize investment and procurement of new low-maintenance and easy to repair equipment to help enhance overall laboratory capacity. Moreover, upgrading facilities could help circumvent transport costs and reduce turnaround time for facilities that send out samples to external laboratories for testing.

Our findings highlight that facilities in the private sector did not face significant challenges in obtaining and maintaining supply of reagents and materials, yet more than half of those in the public sector cited this problem. This finding suggests that supplies can be obtained in Kenya, although it also exposes potential procurement obstacles in the public sector. Therefore, revisiting laboratories in this sector to identify supply constraints and institute corrective measures is recommended.

The study has some limitations beginning with that it was not designed to investigate the capacity of laboratories to confirm and interpret unexpected phenotypes. Secondly, data were self-reported, a limitation brought about by strict COVID-19 restrictions that prevented on-site visits and minimized independent survey verification.

Also, binary responses may lead to overly optimistic assessments with regards to true capacity and true performance. Finally, the generalizability of the current findings is limited as some geographic regions are barely represented among the facilities that participated in the study. Since the data was collected via a web-based program, limited internet access, unreliability of email addresses and lack of electronic appliances may have contributed to the disproportionate representation.

Although not all geographical areas are covered, the survey includes health facilities in very diverse settings of Kenya; from rural to urban sites, from Lake Victoria to the Indian Ocean, providing a good reflection of the country's laboratory capacity status. In resource limited settings, strengthening of health facilities require effective planning towards achieving universal coverage. It is therefore important to note that all clinical laboratories offering some microbiology services, especially microscopy, need not be able to provide culture and susceptibility testing capabilities. Ideally all geographic regions and patients should have access to culture and susceptibility tests, but not necessarily within each laboratory facility.

5.6 Conclusion

We effectively applied a quantitative evaluation among health laboratories in multiple regions of Kenya and found gaps in information management technology, external quality assurance and material and equipment. Our findings suggest that by investing in equipment, facilities performing cultures can be successfully upgraded to provide additional antimicrobial susceptibility testing, presenting a chance for a major leap forward towards improved AMR diagnostics and surveillance in the country. Based on the gaps identified, we recommend increased access to laboratory information management technology for enhanced AMR data management and communication. As a national commitment, targeted quality assurance mechanisms for microbiology facilities are likely to greatly improve overall healthcare delivery. Also, long-term financing mechanisms are needed to improve testing capacity particularly at health center, dispensary and community facility levels where infrastructural deficiencies were most notable. In essence, our findings can serve as a basis to gauge the impact of these interventions and the scoring tool developed for the study could be applied in comparable gap contexts. Moreover, the evaluation tool applied in this study can be used by facilities to independently assess their infrastructure and resource capacities and evaluate their practices.

5.7 DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

5.8 AUTHOR CONTRIBUTIONS

RM, DE, and EL contributed toward conceptualization and study design. RM, BA, and SA conducted the survey. RM processed, analyzed, and interpreted data and wrote the first draft of the paper. DE and EL supervised study and contributed toward data interpretation. DE, LA, and EL supported the writing. SY took part in editing. JM and ML contributed toward funding acquisition. All authors read and approved the final manuscript.

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5.11 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.12 Publisher's Note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

5.13 Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1003178/full#supplementary-material

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6.0 EXECUTIVE SUMMARY

The thesis aims to enhance the understanding of antimicrobial resistance (AMR) patterns in Sub-Saharan Africa and evaluate existing laboratory capacities for AMR surveillance in the region. It comprises two comprehensive cross-sectional studies. The first, conducted between November 2013 and March 2017, analyses clinical specimens from febrile patients hospitalized in Burkina Faso, Gabon, Ghana, and Tanzania, with focus on key AMR concerns highlighted by the World Health Organization, including carbapenem-resistant and extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, fluoroquinolone-resistant *Salmonella enterica*, and methicillin-resistant *Staphylococcus aureus*. Notable variations in susceptibility patterns were found, with Burkina Faso exhibiting high prevalence in ESBL production and ciprofloxacin resistance, emphasizing the importance of region-specific AMR surveillance and improved reporting for targeted interventions. The study found no resistance to carbapenems, indicating their potential as an effective option against infections caused by ESBL-producing bacteria, despite their cost and limited availability. However, the thesis advocates for sustained vigilance in detecting emerging carbapenemase-producing pathogens to ensure timely response and management.

Paper two assessed a convenience sample of healthcare facilities in Kenya, encompassing public and private sectors, rural and urban settings, as well as national, county and community units. The assessment was conducted through online surveys of laboratory managers between October 5th and 8th, 2020, with the aim of evaluating laboratory capacity for AMR surveillance and identifying areas for improvement. A scoring scheme was applied to evaluate indicators of quality assurance, management and dissemination of AMR data, material and equipment, staffing, microbiology competency, biosafety and certification. Gaps in laboratory information management technology, quality assurance, data management, and resources were identified, especially in rural areas. Interestingly, facilities performing bacterial cultures only and those conducting antimicrobial susceptibility testing (AST) were found to have similar capacities, except in terms of equipment. This implies that investing in equipment could enhance the capabilities of facilities conducting cultures only to also perform ASTs, presenting a noteworthy opportunity for expanding AMR diagnostics and improving healthcare delivery within Kenya and potentially beyond its borders.

The thesis advances AMR knowledge in Sub-Saharan Africa, providing a roadmap for tailored policies, strengthened surveillance systems, and improved capacities in health laboratories. The findings can serve as a basis to gauge potential impacts of future interventions and the applied scoring tool can be utilized in similar contexts. Furthermore, the evaluation tool in the current research stands as a valuable resource for facilities to independently assess their capacities and practices, contributing to ongoing development efforts in combating AMR.

7.0 ZUSAMMENFASSUNG

Die vorliegende Arbeit hat zum Ziel, Antibiotikaresistenzmuster in Subsahara-Afrika (SSA) zu beschreiben und bestehende Laborkapazitäten für die AMR-Überwachung in der Region zu bewerten. Sie umfasst zwei umfassende Querschnittsstudien. Die erste, durchgeführt zwischen November 2013 und März 2017, analysiert klinische Proben von Patienten mit Fieber, die in Burkina Faso, Gabun, Ghana und Tansania hospitalisiert waren. Der Fokus liegt dabei auf bedeutenden Resistenzmechanismen, die von der Weltgesundheitsorganisation als kritisch eingestuft wurden. Dazu zählen carbapenemresistente und erweiterte Spektrum-Betalaktamase (ESBL)-produzierende Enterobacterales, fluorchinolonresistente *Salmonella enterica* und methicillinresistente *Staphylococcus aureus*. Bemerkenswerte Unterschiede in den Resistenzraten wurden festgestellt, wobei Burkina Faso eine hohe Prävalenz von ESBL-Produktion und Ciprofloxacin-Resistenz aufwies, was die Bedeutung der regionalen AMR-Surveillance unterstreicht. Die Studie zeigte keine Resistenz gegen Carbapeneme, was auf deren Potenzial als alternative Therapie für ESBL-produzierende Bakterien trotz ihrer Kosten und begrenzten Verfügbarkeit hinweist. Dennoch plädiert die Arbeit für eine anhaltende Wachsamkeit bei der Erkennung aufkommender Carbapenemase-produzierender Erreger, um eine rechtzeitige Reaktion sicherzustellen.

Die zweite Arbeit analysierte eine Stichprobe von Gesundheitseinrichtungen in Kenia, die öffentliche und private sowie ländliche und städtische, nationale, Bezirks- und Gemeindeeinrichtungen umfasst. Die Bewertung wurde durch Online-Umfragen bei Laborleitern zwischen dem 5. und 8. Oktober 2020 durchgeführt, mit dem Ziel, die Laborkapazitäten für die AMR-Surveillance zu bewerten und Verbesserungsmöglichkeiten zu identifizieren. Ein Bewertungsschema wurde entwickelt, um Indikatoren für Qualitätskontrolle, Management und Verbreitung von AMR-Daten, Material und Ausrüstung, Personal, mikrobiologische Kompetenz, Biosicherheit und Zertifizierung zu bewerten. Lücken in der Informationstechnologie der Labore, der Qualitätssicherung, der Datenverwaltung und den Ressourcen wurden identifiziert, insbesondere in ländlichen Gebieten. Interessanterweise wurde festgestellt, dass Einrichtungen, die ausschließlich bakterielle Kulturen durchführen, und solche, die zusätzlich Antimikrobielle Empfindlichkeitstests (AST) durchführen, ähnliche Kapazitäten haben, außer in Bezug auf die Ausrüstung. Dies legt nahe, dass eine Investition in Ausrüstung die Fähigkeiten von Einrichtungen, die nur Kulturen durchführen, verbessern könnte, auch ASTs durchzuführen. Dies bietet eine bemerkenswerte Gelegenheit zur Erweiterung der AMR-Diagnostik und zur Verbesserung der Gesundheitsversorgung in Kenia und möglicherweise darüber hinaus.

Die Arbeit trägt dazu bei, das Wissen über AMR in SSA voranzubringen, liefert einen Fahrplan für maßgeschneiderte Richtlinien, gestärkte Surveillancesysteme und verbesserte Kapazitäten in mikrobiologischen Laboren. Die Ergebnisse können als Grundlage dienen, um potenzielle Auswirkungen zukünftiger Interventionen abzuschätzen, und das angewandte Bewertungstool kann in ähnlichen Kontexten genutzt werden. Darüber hinaus stellt das Evaluierungstool in der vorliegenden Forschung eine wertvolle Ressource für Einrichtungen dar, um ihre Kapazitäten und Praktiken unabhängig zu bewerten und somit zum Kampf gegen AMR beizutragen.

8.0 CO-AUTHOR CONTRIBUTION TO THE PAPERS

The thesis incorporates previously published materials in manuscript format, cited as follows:

Paper I:

Moirongo RM, Lorenz E, Ntinginya NE, Dekker D, Fernandes J, Held J, Lamshöft M, Schaumburg F, Mangu C, Sudi L, Sie A, Souares A, Heinrich N, Wieser A, Mordmüller B, Owusu-Dabo E, Adegnika AA, Coulibaly B, May J and Eibach D (2020) Regional Variation of Extended-Spectrum Beta-Lactamase (ESBL)-Producing Enterobacterales, Fluoroquinolone-Resistant *Salmonella enterica* and Methicillin-Resistant *Staphylococcus aureus* Among Febrile Patients in Sub-Saharan Africa. Front. Microbiol. 11:567235. doi: 10.3389/fmicb.2020.567235

In relation to this paper, the co-author contributions were as follows:

- DE, JM, AA, BM, ASo, NH, and ML: Designed and coordinated the study
- NN, JF, JH, CM, LS, BC, EO-D, and ASi conducted and supervised fieldwork
- FS, AW, DD, and DE supervised laboratory work
- **RM** performed epidemiological and statistical analysis and wrote initial manuscript
- EL and DE reviewed manuscript and data analyses

Paper II:

Moirongo RM, Aglanu LM, Lamshöft M, Adero BO, Yator S, Anyona S, May J, Lorenz E and Eibach D (2022) Laboratory-based surveillance of antimicrobial resistance in regions of Kenya: An assessment of capacities, practices, and barriers by means of multi-facility survey. *Front. Public Health* 10:1003178. doi: 10.3389/fpubh.2022.1003178

Contributions for Paper II were as follows:

- **RM** conceptualized and designed the study, drafted the protocol, established survey tools, managed sampling, fieldwork, and data collection, processed, analysed, and interpreted data, wrote the initial manuscript, and serves as the corresponding author for this paper.
- BA and SA conducted fieldwork.
- DE reviewed the protocol, supervised the study, and reviewed the manuscript.
- LA and EL supported the writing process and data interpretation.
- SY participated in editing.
- JM and ML acquired funds**.**

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10.0 CURRICULUM VITAE

Lebenslauf entfällt aus datenschutzrechtlichen Gründen

11.0 EIDESSTATTLICHE VERSICHERUNG

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: RMM

Appendix I: Scoring Scheme Developed for Assessment of Health Facilities in Kenya, 2020

Dimension	Subdimension and Indicator	Question frame	Score	Weighting value
1. AMR surveillance practices	**1.1 Quality assurance**			
	1. Media quality control	-use of specific bacterial control strains for culture	No=0, yes=1	1
	2. Standard sample processing	-utilization of standard internal operating procedures for processing of samples for bacterial culture	No=0, yes=1	1
	3. External quality assurance	-participation in external quality assessment system for isolation of bacteria	No=0, yes=1	1
	4. Standardized antimicrobial susceptibility testing	-use of internal standard operating procedures for assuring the quality of ASTs	No=0, yes= 1	1
	5. Internal AST quality control	-Use of ATCC/NCTC or other reference strains	No=0, other= 0.5, yes = 1	1
	6. Application of international interpretation guidelines	-Use of CLSI/ EUCAST or other	No=0, other= 0.5, yes = 1	1
	1.2 Management and dissemination of AMR data			

	7. Communication with clinicians	- AST results reported to healthcare provider	No= 0, yes=1	1
	8. AMR record keeping	-AST records kept longterm	No=0, yes= 1	1
	9. Inter-laboratory collaboration	- Information exchange between laboratories	No=0, yes= 1	1
	10. Reporting to regional public health office	- Regular data submission to regional health office	No=0, yes= 1	1
	11. Application of GLASS guidelines	-AST performed according to GLASS pathogen-antimicrobial combination	No=0, partial=0.5, yes=1	1
	12. Laboratory information management technology	- Use of data management software such as WHONET. Other refers to local databases with no exchange of information	No=0, other =0.5, yes=1	1
2. Infrastructure & resource capacity	2. 1 Materials & Equipment			
	Category 1	-Availability and function	Present and functional=1, present and non-functional=0.5, absent =0	1
	1. Adequate glassware for media preparation (flasks, cylinders, etc) 2. Atmosphere generating systems or CO2 tanks and CO2 incubator or candle jars			

3. Autoclave (manual or electrically controlled)
4. Blood culture machine
5. Bunsen burner or heater or lamp to sterilize loops and needles
6. Disposable loop/needle handles or Loop/needle handles or 0.01 and 0.001ml calibrated loops
7. -70°C Freezer
8. Manual pipettes (e.g Eppendorf)
9. Microscope with oil-immersion objective
10. Petri dishes (glass or disposable)
11. Refrigerator
12. Safety cabinet- level 2 (protects operator and material from contamination)
13. Scale or balance
14. Slides
15. Staining facilities- sink and slide rack
16. Warm air incubator
17. Water distillation system
18. Other anaerobe jar

The question frame, score and weighting value in category 1 applies to indicators 1-18. The response type ‘non-functional’ is not applicable for indicator 1, 6, 10, 14, 18.

Category 2	-Availability and function	Present and functional=1, present and non-functional=0.5, absent =0	0.8

1.Test tube racks
2.Vortex Mixer
3.Magnifying lens
4.Safety cabinet-level 3 (protects operator, material, and environment)

The question frame, score and weighting value in category 2 applies to indicators 1-4. The response type ‘non-functional’ is not applicable for indicator 1, 3.

Category 3	-Availability and function	Present and functional=1, present and non-functional=0.5, absent =0	0.6

1. Colorimeter
2. Electrically powered water bath
3. -20°C Freezer
4. Hot air oven
5. Inverted microscope
6. Low speed centrifuge (hand or electrically powered)
7. Safety cabinet- level 1 (protects material from contamination)

The question frame, score and weighting value in category 3 applies to indicators 1-7. The response type ‘non-functional’ is not applicable for indicator 1.

Category 4 1. Coverslips 2. Multipoint inoculator 3. pH meter 4. pH paper 5. Fluorescent microscope	-Availability and function	Present and functional=1, present and non-functional=0.5, absent =0	0.4
The question frame, score and weighting value in category 4 applies to indicators 1-5.			
2.2 Staffing	- On staff	Medical supervisor, No=0, yes=1 Technical supervisor, No=0, yes=1 Lab technologist, No=0, yes=1 Lab assistant, No=0, yes=1 Epidemiologist, No=0, yes=1 Microbiologist, No=0, yes=1 Clerical staff, No=0, yes=1 Other e.g supportive staff, No=0, yes=1	0.5 1 1 0.5 0.45 1 0.25 0.2
2.3 Microbiology competency	- Level of microbiology training	Master's degree= 1, Bachelor's degree= 0.9, Diploma course=0.75, In-house training= 0.5, None=0	1
2.4 Safety training	-Frequency of training on laboratory safety	>twice a year =1, twice a year= 0.9, once a year =0.75, once in two years =0.5, never=0	1

2.5 Safe practices in safe environment	-Presence of personal protective items (e.g Lab coats, gloves, visors)	None =0 only visors = 0.2 only lab coat = 0.35, only gloves = 0.35, only other =0.15, only other and visors =0.25, only visors and lab coat = 0.6, only visors and gloves = 0.6, only gloves and lab coats = 0.7, only other and gloves = 0.5, only other and lab coat = 0.5, only other, gloves and visors =0.8, only other, lab coat and visors =0.8, only other, gloves and lab coat = 0.85, only visors, lab coat and gloves =0.9 Lab coat, gloves, visors and other =1	0 0.2 0.35 0.35 0.15 0.25 0.6 0.6 0.7 0.5 0.5 0.8 0.8 0.85 0.9 1
2.6 Certification	-Confirmation of accreditation	No accreditation=0, Accreditation in progress= 0.5, accredited= 1	1

AST, Antimicrobial Susceptibility Testing; GLASS, Global Antimicrobial Resistance and Use Surveillance System; LIMT, Laboratory information management technology; SOPs, Standard operating procedures; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing.

Appendix II: Prevalence, Distribution and Classification of the Bacteria Isolate

	Burkina Faso	Gabon	Ghana	Tanzania	All countries
Number of all samples, n	605	970	1708	767	4052
All bacteria, n (%)	38 (6)	35 (4)	129 (8)	17 (2)	219 (5)
Blood samples, n	482	593	1238	696	3009
Bacteria isolated from blood, n (%)	27 (6)	8 (1)	61 (5)	17 (2)	113 (4)
Enterococcus faecalis	4 (15)	0 (0)	0 (0)	0 (0)	4 (4)
Escherichia coli	7 (26)	1 (13)	1 (2)	2 (12)	11 (10)
Klebsiella oxytoca	0 (0)	0 (0)	1 (2)	0 (0)	1 (1)
Klebsiella pneumoniae	1 (4)	1 (13)	1 (2)	0 (0)	3 (3)
Nt *Salmonella*	10 (37)	4 (50)	55 (90)	0 (0)	69 (61)
Salmonella Typhi	1 (4)	0 (0)	0 (0)	10 (59)	11 (10)
Serratia marcescens	1 (4)	0 (0)	0 (0)	0 (0)	1 (1)
Staphylococcus aureus	3 (11)	2 (25)	3 (5)	5 (29)	13 (12)
Stool samples collected, n	18	91	232	71	412
Bacteria isolated from stool n (%)	1 (6)	3 (3)	5 (2)	0 (0)	9 (2)
Nt *Salmonella*	1 (100)	2 (67)	5 (100)	NA	8 (89)
Shigella sonnei	0 (0)	1 (33)	0 (0)	NA	1 (11)
Urine samples collected	105	286	238	0	629
Bacteria isolated from urine n (%)	10 (10)	24 (8)	63 (26)	NA	97 (15)
Acinetobacter baumannii complex	0 (0)	1 (4)	1 (2)	NA	2 (2)
Enterobacter cloacae	0 (0)	0 (0)	1 (2)	NA	1 (1)
Enterococcus faecium	0 (0)	0 (0)	3 (5)	NA	3 (3)
Escherichia coli	8 (80)	10 (42)	43 (68)	NA	61 (63)
Klebsiella pneumoniae	1 (10)	7 (29)	11 (17)	NA	19 (20)
Proteus mirabilis	0 (0)	2 (8)	1 (2)	NA	3 (3)
Proteus penneri	0 (0)	1 (4)	0 (0)	NA	1 (1)
Proteus vulgaris	0 (0)	1 (4)	0 (0)	NA	1 (1)
Pseudomonas aeruginosa	0 (0)	0 (0)	1 (2)	NA	1 (1)
Nt *Salmonella*	1 (10)	1 (4)	0 (0)	NA	2 (2)
Staphylococcus aureus	0 (0)	1 (4)	2 (3)	NA	3 (3)

Abbreviations: n, sample size; nt *Salmonella,* non-typhoid *Salmonella*

Appendix III: Prevalence of Resistances among the Isolated Bacteria to Commonly used Antibiotics

		Burkina Faso n (%)	Gabon n (%)	Ghana n (%)	Tanzania n (%)	All countries n (%)
Enterobacterales		N=31	N=31	N=119	N=12	N=193
	Ampicillin	28(90)	19(61)	72(61)	11(92)	130(67)
	Cefotaxime	14(45)	8(26)	18(15)	0(0)	40(21)
	Ceftazidime	14(45)	8(26)	18(15)	0(0)	40(21)
	Meropenem	0(0)	0(0)	0(0)	3(25)	3(2)
	Gentamicin	13(42)	8(26)	75(63)	0(0)	96(50)
	Ciprofloxacin	16(52)	4(13)	16(13)	1(8)	37(19)
	Tigecycline	0(0)	7(23)	8(7)	0(0)	15(8)
	SXT	31(100)	13(42)	66(55)	9(75)	119(62)
	Confirmed ESBL	14(45)	8(26)	18(15)	0(0)	40(21)
Staphylococcus aureus		N=3	N=3	N=5	N=5	N=16
	Gentamicin	0(0)	0(0)	0(0)	0(0)	0(0)
	Ciprofloxacin	0(0)	0(0)	0(0)	0(0)	0(0)
	Tigecycline	0(0)	0(0)	0(0)	0(0)	0(0)
	SXT	2(67)	3(100)	2(40)	5(100)	12(75)
	Penicillin	3(100)	3(100)	5(100)	5(100)	16(100)
	Cefoxitin	0(0)	0(0)	2(40)	0(0)	2(13)
	Erythromycin	1(33)	0(0)	1(20)	0(0)	2(13)
	Clindamycin	0(0)	0(0)	1(20)	0(0)	1(6)
	Teicoplanin	0(0)	0(0)	0(0)	0(0)	0(0)
	Vancomycin	0(0)	0(0)	0(0)	0(0)	0(0)
	Linezolid	0(0)	0(0)	0(0)	0(0)	0(0)
	Rifampicin	0(0)	0(0)	0(0)	0(0)	0(0)
	Tetracyclin	3(100)	1(33)	4(80)	0(0)	8(50)
Pseudomonas aeruginosa		N=0	N=0	N=1	N=0	N=1
	Meropenem	0(0)	0(0)	0(0)	0(0)	0(0)

Abbreviations: N,n, sample size; SXT, trimethoprim-sulphamethoxazole; ESBL, extended-spectrum beta-lactamase

Appendix IV: Drug Resistance Patterns of the Enterobacterales

	Ampicillin	Cefotaxime	Ceftazidime	Ciprofloxacin	Gentamicin	Meropenem	SXT	Tigecycline
	n(n/N%)	n(n/N%)	n(n/N%)	n(n/N%)	n(n/N%)	n(n/N%)	n(n/N%)	n(n/N%)
All Enterobacterales (N=193)	130(67)	40(21)	40(21)	37(19)	96(50)	3(2)	119(62)	15(8)
Nt Salmonella (N=79)	32(41)	3(4)	3(4)	8(10)	63(80)	0(0)	34(43)	2(3)
Escherichia coli (N=72)	59(82)	23(32)	23(32)	21(29)	19(26)	0(0)	58(81)	2(3)
Klebsiella pneumoniae (N=22)	22(100)	14(64)	14(64)	6(27)	13(59)	0(0)	13(59)	6(27)
Salmonella Typhi (N=11)	10(91)	0(0)	0(0)	2(18)	0(0)	3(27)	10(91)	0(0)
Proteus mirabilis (N=3)	2(67)	0(0)	0(0)	0(0)	0(0)	0(0)	2(67)	3(100)
Enterobacter cloacae (N=1)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Klebsiella oxytoca (N=1)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Proteus penneri (N=1)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)
Proteus vulgaris (N=1)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)
Serratia marcescens (N=1)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
Shigella sonnei (N=1)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)
ESBL-producing Enterobacterales (N=40)	40(100)	40(100)	40(100)	27(68)	31(78)	0(0)	36(90)	3(8)
Escherichia coli (N=23)	23(100)	23(100)	23(100)	18(78)	15(65)	0(0)	22(96)	0(0)
Klebsiella pneumoniae (N=14)	14(100)	14(100)	14(100)	6(43)	13(93)	0(0)	11(79)	3(21)
Nt *Salmonella* (N=3)	3(100)	3(100)	3(100)	3(100)	3(100)	0(0)	3(100)	0(0)

Abbreviations : N,n, sample size ;Nt *Salmonella*, non-typhoid *Salmonella* ; SXT, trimethoprim-sulfamethoxazole; ESBL, extended-spectrum beta-lactamase

Appendix V: Multilocus Sequence Types for ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates by Country

	Burkina Faso	Gabon	Ghana	Tanzania	All countries
ESBL *E. coli* sequence types (ST)	N=9	N=2	N=12	N=0	N=23
ST131	5	1	3	NA	9
ST410	0	0	3	NA	3
ST10	0	0	2	NA	2
ST167	1	0	0	NA	1
ST3052	0	1	0	NA	1
ST38	0	0	1	NA	1
ST410	1	0	0	NA	1
ST617	1	0	0	NA	1
ST617	0	0	1	NA	1
ST648	0	0	1	NA	1
ST93	0	0	1	NA	1
Non assigned ST	1	0	0	NA	1
ESBL *K. pneumoniae* sequence types (ST)	N=2	N=6	N=6	N=0	N=14
ST14	2	0	0	NA	2
ST1031	0	1	0	NA	1
ST1072	0	1	0	NA	1
ST1430	0	1	0	NA	1
ST147	0	1	0	NA	1
ST1891	0	1	0	NA	1
ST215	0	0	1	NA	1
ST2734	0	0	1	NA	1
ST307	0	0	1	NA	1
ST3248	0	1	0	NA	1
ST36	0	0	1	NA	1
ST39	0	0	1	NA	1
ST530	0	0	1	NA	1

Abbreviations: ST, sequence type; N, sample size.

Appendix VI: Distribution of Mutations and Genes Conferring Ciprofloxacin Resistance among *Salmonella enterica*

ID	country	*Salmonella enterica*	Sequence type	MIC (g/L)	Mutation in *gyrA*	Mutation in *gyrB*	plasmid mediated genes	ESBL
118	Tanzania	*S*. Typhi	ST1	0.19	E133G	S464Y	negative	negative
700613	Ghana	nt *Salmonella*	ST11	0.064	D87G	no	negative	negative
701845	Ghana	nt *Salmonella*	ST11	0.064	D87G	no	negative	negative
A00-Iso02141	Burkina Faso	nt *Salmonella*	ST313	0.38	no	no	*qnrB*	ESBL
A06-Iso02144	Burkina Faso	nt *Salmonella*	ST313	1	no	no	*qnrB*	negative
A56-Iso02149	Burkina Faso	nt *Salmonella*	ST313	0.75	no	no	*qnrB*	negative
C00-Iso02162	Burkina Faso	nt *Salmonella*	ST313	0.25	no	no	*qnrB*	ESBL
C04-Iso02164	Burkina Faso	nt *Salmonella*	ST313	0.25	no	no	*qnrB*	ESBL
D00-Iso02167	Burkina Faso	*S*. Typhi	ST2	0.064	D87G, E133G	no	negative	negative
D01-Iso02170	Burkina Faso	nt *Salmonella*	ST313	1	no	no	*qnr B*	negative

*** no mutations in *parC* and *parE* genes as well as *aac(6') Ib(-cr)*, *qnrA*, *qnrC*, *qnrD* and *qnrS* genes were found.**

Abbreviations: ID, patient identification number; *S.* Typhi, *Salmonella* Typhi; nt *Salmonella*, non-typhoid *Salmonella*; ST, sequence type; MIC, minimum inhibitory concentration; ESBL, extended-spectrum beta-lactamase

Appendix VII: Sequence Types and *Spa*Types of *Staphylococcus aureus* Isolates by Country

Country	Sequence Type (ST) (n)	spa types (n)	MRSA n (%)	*PVL* n (%)
Burkina Faso (N=3)	ST152 (3)	t314 (1), t355 (1), t4198 (1)	0(0)	3 (100)
Gabon (N=3)	ST152 (2), ST8 (1)	t355 (2), t1476 (1)	0(0)	2 (67)
Ghana (N=5)	ST121 (1), ST152 (1), ST15 (1), ST88 (2)	t314 (1), t4454 (1), t355 (1), t186 (2)	2(40)	3 (60)
Tanzania (N=5)	ST 152(4), ST88 (1)	t355 (4), t2526 (1)	0(0)	5 (100)

***No vancomycin resistance was observed**

Abbreviations: N,n, sample size; MRSA, methicillin-resistant *Staphylococcus aureus*; *PVL*, Panton-Valentine leukocidin

Appendix VIII: Proportion of Antimicrobial Testing Facilities in Regions of Kenya by Gap Identified, Affiliation, Level, Urbanicity

	LIMT availability (15;40.5%)	EQA participation (25;67.6%)	Regional level reporting (25;67.6%)	GLASS pathogen-antimicrobial combinations (19;51.4%)	All (N=37)
Affiliation (%)					
*Public[1]	7(35.0)	13(65.0)	14(70.0)	9(45.0)	20
*Private[2]	8(47.1)	12(70.6)	11(64.7)	10(58.8)	17
Level (%)					
National referral	5(100.0)	5(100.0)	4(80.0)	5(100.0)	5
Research	2(100.0)	1(50.0)	2(100.0)	2(100.0)	2
County referral	6(40.0)	8(53.3)	9(60.0)	5(33.3)	15
Sub-county	1(11.1)	8(88.9)	6(66.7)	3(33.3)	9
Health centres	0(0.0)	0(0.0)	1(100.0)	0(0.0)	1
*Other[3]	3(42.9)	5(83.3)	5(71.4)	6(85.7)	7
Urbanicity (%)					
Rural	2(25.0)	6(75.0)	5(62.5)	2(25.0)	8
Urban	13(58.6)	19(65.5)	20(69.0)	17(58.6)	29
Administrative region (%)					
Central	3(50.0)	5(83.3)	5(83.3)	1(16.7)	6
Coast	0(0.0)	3(75.0)	2(50.0)	2(50.0)	4
Eastern	1(20.0)	4(80.0)	2(40.0)	2(40.0)	5
Nairobi	13(92.9)	11(78.6)	10(71.4)	11(78.6)	14
Northeastern	NA	NA	NA	NA	0
Nyanza	2(33.3)	2(33.3)	6(100.0)	3(50.0)	6
Rift Valley	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1
Western	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1

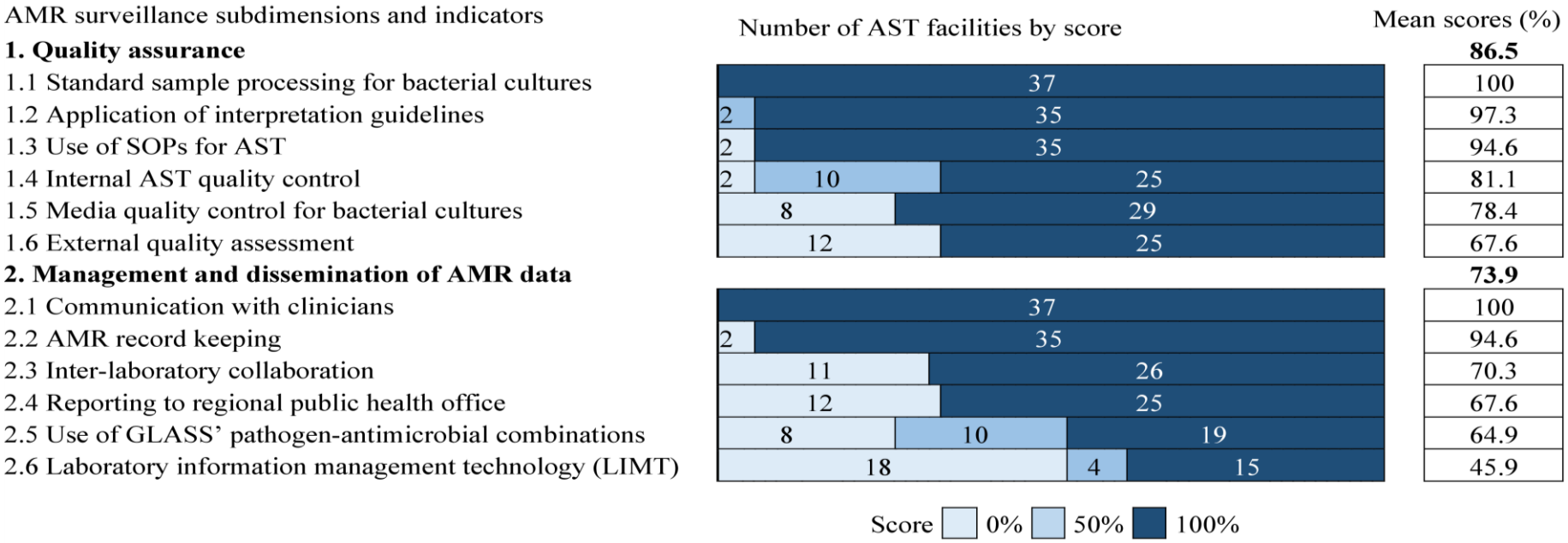
N, sample size; [1]*Public includes government facilities and academic institutions. [2]*Private includes entities supported by faith-based and non-government organizations as well as those run for profit by individuals or non-public companies. [3]*Other include facilities of non-public ownership that fall outside the indicated level categories. LIMT, Laboratory information management technology; EQA, External quality assessment; GLASS, Global Antimicrobial Resistance and Use Surveillance System

Appendix IX: Health facilities completing the survey by affiliation, level and urbanicity in regions of Kenya, 2020

	No culture services (135; 61.6%)	*Cultures only[4] (47; 21.5%)	AST (37; 16.7%)	All (N=219)
Affiliation, n (%)				
*Public[1]	62(63.3)	16(16.3)	20(20.4)	98(44.7)
*Private[2]	73(60.3)	31(25.6)	17(14.0)	121(55.3)
Level, n (%)				
Level 6 (National)	0(0.0)	0(0.0)	5(100.0)	5(2.3)
Level 5 (County Referral)	6(22.2)	6(22.2)	15(55.6)	27(12.3)
Level 4 (Sub-County)	31(45.6)	28(41.2)	9(13.2)	68(31.1)
Level 3 (Health Centres)	26(70.3)	10(27.0)	1(2.7)	37(16.9)
Level 2 (Dispensaries)	36(97.3)	1(2.7)	0(0.0)	37(16.9)
Level 1 (Community)	30(93.8)	2(6.3)	0(0.0)	32(14.6)
Research	2(33.3)	2(33.3)	2(33.3)	6(2.7)
*Other[3]	3(25.0)	2(16.7)	7(58.3)	12(5.5)
Urbanicity n (%)				
Urban	55(50.9)	24(22.2)	29(26.9)	108(49.3)
Rural	80(72.1)	23(20.7)	8(7.2)	111(50.7)
Administrative region n (%)				
Central	16(59.3)	5(18.5)	6(22.2)	27(12.3)
Coast	10(58.8)	3(17.6)	4(23.5)	17(7.8)
Eastern	13(44.8)	11(37.9)	5(17.2)	29(13.2)
Nairobi	25(52.1)	9(18.8)	14(29.2)	48(21.9)
North Eastern	3(100.0)	0(0.0)	0(0.0)	3(1.4)
Nyanza	37(72.5)	8(15.7)	6(11.8)	51(23.3)
Rift Valley	12(54.5)	9(40.9)	1(4.5)	22(10.0)
Western	19(86.4)	2(9.1)	1(4.5)	22(10.0)

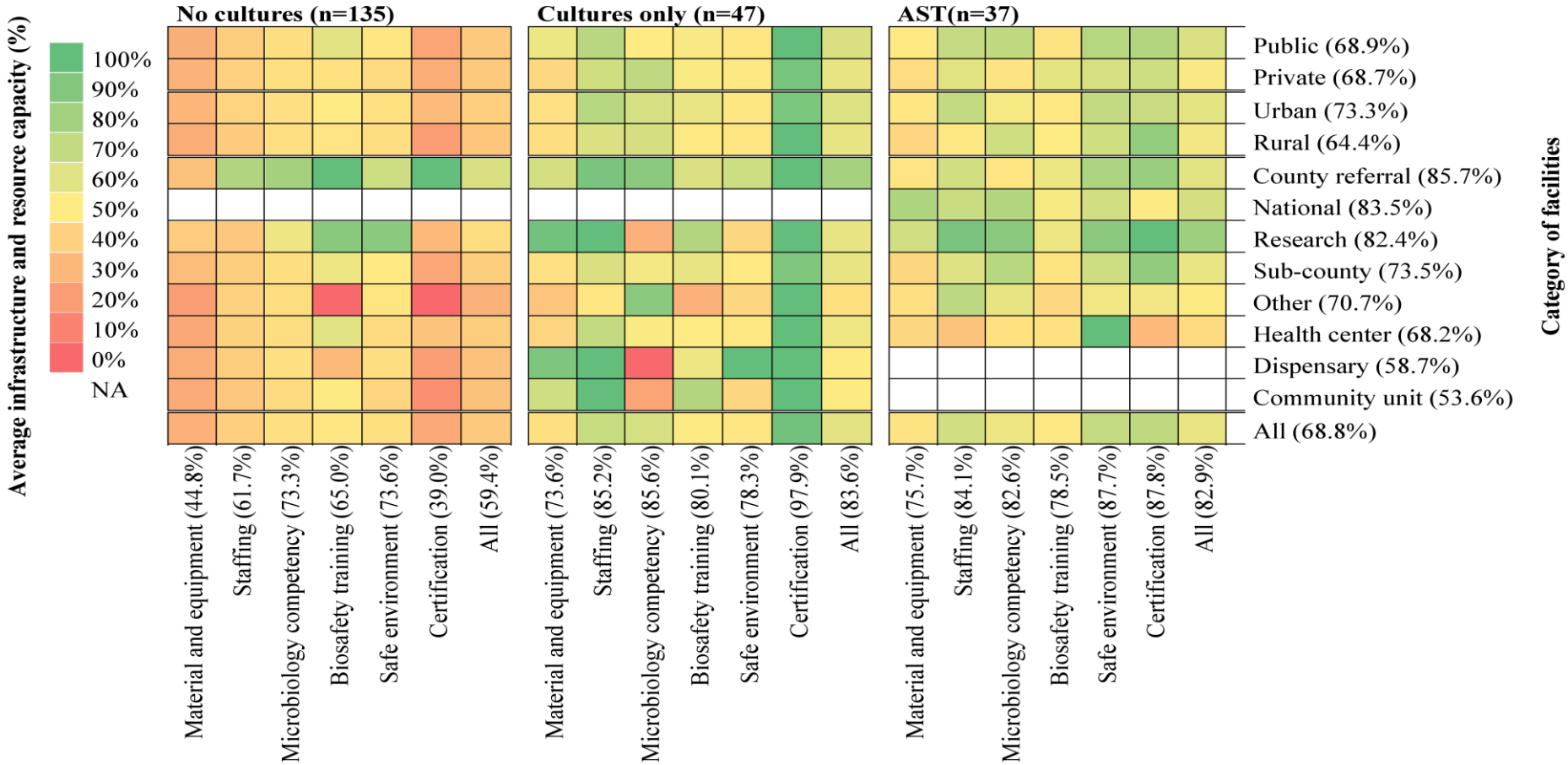
N, sample size; [1]*Public includes government facilities and academic institutions. [2]*Private includes entities supported by faith-based and non-government organizations as well as those run for profit by individuals or non-public companies. [3]*Other include facilities of non-public ownership that do not fall in the indicated level categories. [4]*Cultures only facilities offer bacterial culture services but no AST. AST, antimicrobial susceptibility testing

Appendix X: Distribution of Performance Scores for AMR Surveillance Practices



The figure provides information on the distribution of performance scores for AMR surveillance practices across facilities. The list of subdimensions and their component indicators is on the left. Shading and integers represent scores and facility count, respectively. The scores are ordered from left to right by increasing shade intensity. The number of facilities corresponds to column width. Percentage values on the right are the average score of all cells in each row. Average scores are ranked in descending order by subdimension and component indicator. AST, Antimicrobial Susceptibility Testing; SOPs, Standard operating procedures.

Appendix XI: Infrastructure and Resource Capacity Subdimesnions



The heat map details infrastructure and resource capacity scores of the study facilities. The list to the right of the map indicates category names for facility affiliation (Private, Public) urbanicity (Rural, Urban) and level (National referral, Research, County referral, County,

Health Centres, Dispensaries and Community units). The category ‘Other’ includes facilities of non-public ownership that fall outside the 6-level structure of the Kenyan health system. The list below indicates the subdimensions of assessment for infrastructure and resource capacity. Indices in parentheses after each category name is the average capacity score of all cells in each row for left list and all cells in each column for list below. Categories are ranked in descending order of average capacity score for affiliation, urbanicity and level, respectively. AST, Antimicrobial Susceptibility Testing; NA, Not Applicable