NIGERIA CENTRE FOR DISEASE CONTROL

Guideline For Antimicrobial Resistance Laboratory Surveillance

FOR LABORATORIES IN NIGERIA

2018

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Abiodun Egwuenu

Foreword

Antimicrobial resistance (AMR) has gained worldwide recognition, as the emergence of multi-drug resistant organisms has led to increased morbidity, mortality and economic burden. Nigeria is no exception to the challenges faced due to AMR. Thus, the prudent use of antimicrobial drugs and the prevention and control of AMR cannot be more emphasized now.

In May 2015, the World Health Assembly (WHA) resolution 68.7 requested that its Member States participate in an integrated global program for surveillance of AMR and adopt a country-specific action plan in-line with the Global Action Plan on AMR. As part of Nigeria's commitment to AMR prevention and control, the Honourable Minister of Health approved the establishment of an AMR National Surveillance Coordinating Body within the Nigeria Centre for Disease Control (NCDC) and convened a “One-Health” National AMR Technical Working Group (AMR-TWG). A situation analysis of AMR was conducted, and a National Action Plan on AMR was developed. Both documents were presented by the Honourable Minister of Health in May 2017 at the 71st WHA.

AMR surveillance data can inform standard treatment guidelines to support best practices for patient care. Monitoring AMR trends can be used for the assessment of interventions to reduce AMR, early detection of the emergence of new resistant strains, and rapid identification and control of outbreaks of bacterial pathogens.

In 2017, Nigeria enrolled in the WHO Global Antimicrobial Resistance Surveillance System (GLASS). Using globally set criteria, two laboratories were selected as national reference laboratories: University College Hospital Ibadan and National Reference Laboratory Gaduwa. There are 10 sentinel laboratories that prequalified to report antibiotic resistant organisms in humans to the National AMR surveillance.

In October 2017, NCDC engaged 10 laboratories to participate in the National Antimicrobial Resistance Surveillance System in Nigeria. Discussions were held to determine the roles and responsibilities of laboratories in the network, priority pathogens and antibiotics, External Quality Assurance (EQA) processes, data

needs, collection, reporting, monitoring and feedback for GLASS. One of the common challenges identified was the variation in inter-laboratory practice throughout the country, including a lack of agreement/concordance in standardised operating procedures used for antimicrobial susceptibility testing, quality assurance, data management, and reporting. This has an important effect on the quality of data being reported for local and global use. It has been largely due to the absence of national guidelines for AMR surveillance in the country.

As a result, a range of laboratory stakeholders within the AMR surveillance network including the University College Hospital Ibadan (UCH), Lagos University Teaching Hospital (LUTH), Aminu Kano University Teaching Hospital (AKTH), National Hospital Abuja (NHA), Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), University Teaching Hospital Ilorin (UITH), Babcock University Teaching Hospital, University Teaching Hospital Enugu (UNTH), Federal Medical Centre (FMC) Jalingo and NCDC were consulted to develop national guidelines for AMR laboratory-based surveillance in Nigeria. These guidelines aim to set a framework for surveillance implementation and promote the use of standardised operating procedures (SOPs) for laboratories participating in the AMR surveillance in the country. Participating laboratories in the network are expected to be trained on the use of SOPs for detection, characterisation and reporting of priority bacterial organisms as agreed for Nigeria and in line with the WHO GLASS reporting guideline. The implementation of these guidelines will be a primary strategy for tracking prevalent and emerging drug resistance in the population to support early and appropriate action.

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A Acronyms and Definitions

Acronyms	Definitions
AMR	Antimicrobial Resistance
API	American Petroleum Institute Agar
ASLM	African Society for Laboratory Medicine
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal Fluid
DSNO	Disease Surveillance and Notification Officer
EQA	External Quality Assurance
FMARD	Federal Ministry of Agriculture and Rural Development
FME	Federal Ministry of Environment
FMOH	Federal Ministry of Health
GLASS	Global Antimicrobial Resistance Surveillance System
HOD	Head of Department
IDSR	Integrated Disease Surveillance and Response
IQA	Internal Quality Assurance
IQC	Internal Quality Control
ISO	International Organization for Standardization
LGA	Local Government Area
NCC	National AMR Coordinating Centre
NCDC	Nigeria Centre for Disease Control
NCTC	National Collection of Type Cultures
NICD	National Institute for Communicable Diseases
NPHCDA	National Primary Health Care Development Agency
NRL	National Reference Laboratory
PCSI	Program Collaboration and Service Integration
PT	Proficiency Test
SOP	Standard Operating Procedures
TWG	Technical Working Group
UCH	University College Hospital
WHA	World Health Assembly
WHO	World Health Organization

D Definition of Terms

Antimicrobial Stewardship	A coordinated program that promotes the appropriate use of antimicrobials (including antibiotics), improves patient outcomes, reduces microbial resistance, and decreases the spread of infections caused by multidrug-resistant organisms.[1]
Integrated Disease Surveillance and Response (IDSR)	A strategy and tool to integrate and streamline common disease surveillance activities to promote rational use of resources; The present IDSR strategy in Nigeria is well coordinated and combines available resources to collect information on notifiable diseases from a single focal point at each level (Community, health facilities, LGA, State and Federal).[2]
Modified Kirby Bauer	The modified Kirby-Bauer disc diffusion test method is a reference method which can be used as a routine technique to test the sensitivity of an isolate in the clinical laboratory. The disc diffusion method was originally described in 1966, is well standardized and has been widely evaluated.[3]
Mannitol Salt Agar (MSA)	Mannitol salt agar is a commonly used selective and differential growth medium in microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. This medium is important in medical laboratories as one method of distinguishing pathogenic microbes in a short period of time. It contains a high concentration (about 7.5%-10%) of salt (NaCl), making it selective for gram-positive bacteria (Staphylococcus and Micrococcaceae) since this level of salt is inhibitory to most other bacteria.[4]
Tryptic Soy Broth (TSB)	Used in microbiology laboratories as a culture broth to grow aerobic bacteria. It is a complex, general purpose medium that is routinely used to grow certain pathogenic bacteria, which tend to have high nutritional requirements. Its agar counterpart is tryptic soy agar.[5]
VITEK	A fully automated system that performs bacterial identification and antibiotic susceptibility testing.[6]
WHONET	A free windows-based database software developed for the management and analysis of microbiology laboratory data with a special focus on the analysis of antimicrobial susceptibility test results.[7]

1 Introduction and Orientation

Background

There are, as of yet, no available national study outlining the full burden of antimicrobial resistance (AMR) and its health and economic impact on Nigerians[8]. In 2013, a situation analysis of Nigerian Medical Laboratories was conducted to obtain baseline information on the state of the laboratories and their capacity in the country. Only 5.7% of private and 7.3% of public laboratories participated in surveillance networks of epidemic-prone and other communicable diseases, respectively[8].

In 2015, the World Health Organization (WHO) launched the Global Antimicrobial Resistance Surveillance System (GLASS) to support the implementation of the global action plan on AMR. GLASS promotes and supports a standardized approach to the collection, analysis and sharing of AMR data globally by encouraging the establishment of capable national AMR surveillance systems[9,10].

Effective and efficient national AMR surveillance system is important in planning and implementing the National Action Plan on AMR in Nigeria. AMR surveillance data should be interpreted in the context of local clinical practice. This is particularly relevant in the Nigeria's context which uses syndromic management strategies to clinically diagnose and treat patients empirically[10,11].

Justification for Laboratory-based AMR Surveillance

There are currently no available national standard guidelines for the identification, characterisation, and reporting of AMR organisms in Nigeria. We need to create a systematic system to collect, analyse, and evaluate data in an accurate and reliable manner. The guidelines encourage discussion on a step-by-step approach for involving multidisciplinary teams from hospitals, laboratories, universities and national surveillance units in the containment of AMR.
AMR surveillance should aim to:

a. Provide standardised surveillance practice
b. Collect robust and quality-assured data that can inform prescribing policies and treatment guidelines based on local susceptibility patterns and ultimately improve the quality of care and patient safety
c. Provide an additional training platform for laboratory personnel
d. Detect emerging antimicrobial resistance in Nigeria
e. Ensure integration and harmonization of AMR surveillance in humans with surveillance of AMR and antibiotic residues in food-producing animals and the food chain towards the one health agenda.

AMR surveillance is crucial for detecting the emergence of new resistance patterns and for monitoring the impact of interventions towards minimizing the spread and burden of AMR[11]. An efficient surveillance system for AMR is also part of Integrated Disease Surveillance and Response (IDSR) implementation and health systems strengthening to reduce mortality and morbidity due to infectious diseases.

Goals and Objectives

The overall goal of these guidelines is to provide a standardised approach to guide the implementation of AMR surveillance in Nigeria. It will outline the required procedures, testing, specimens and analysis. It will facilitate the development of systems that are capable of monitoring AMR trends and producing reliable and comparable data on a regular basis and contribute to national and global monitoring data. The specific objectives include guidance to:

a. Identify priority pathogens based on GLASS recommendations
b. Perform AST for priority pathogens
c. Develop a system for internal and external quality assurance
d. Manage the AMR data at the facility level
e. Adopt and implement a standard reporting system including a national database and the sharing of feedback to all stakeholders to inform practice and policy
f. Evaluate the AMR surveillance system including standardised indicators.

Governance, Structure and Flow of Information

The NCDC is the coordinating body in the AMR surveillance, working with the Federal Ministry of Agriculture, Federal Ministry of Environment and the Federal Ministry of Health. Information flows to and from NCDC through the AMR Technical Working Group (TWG). Two-way communication between NCDC and sentinel laboratories through the national reference laboratories (UCH and NRL Gaduwa) will enable the flow of AMR surveillance data to the NCDC, TWG and all partners, who will be actively involved. Below is an illustrated summary of the governance and flow of information.

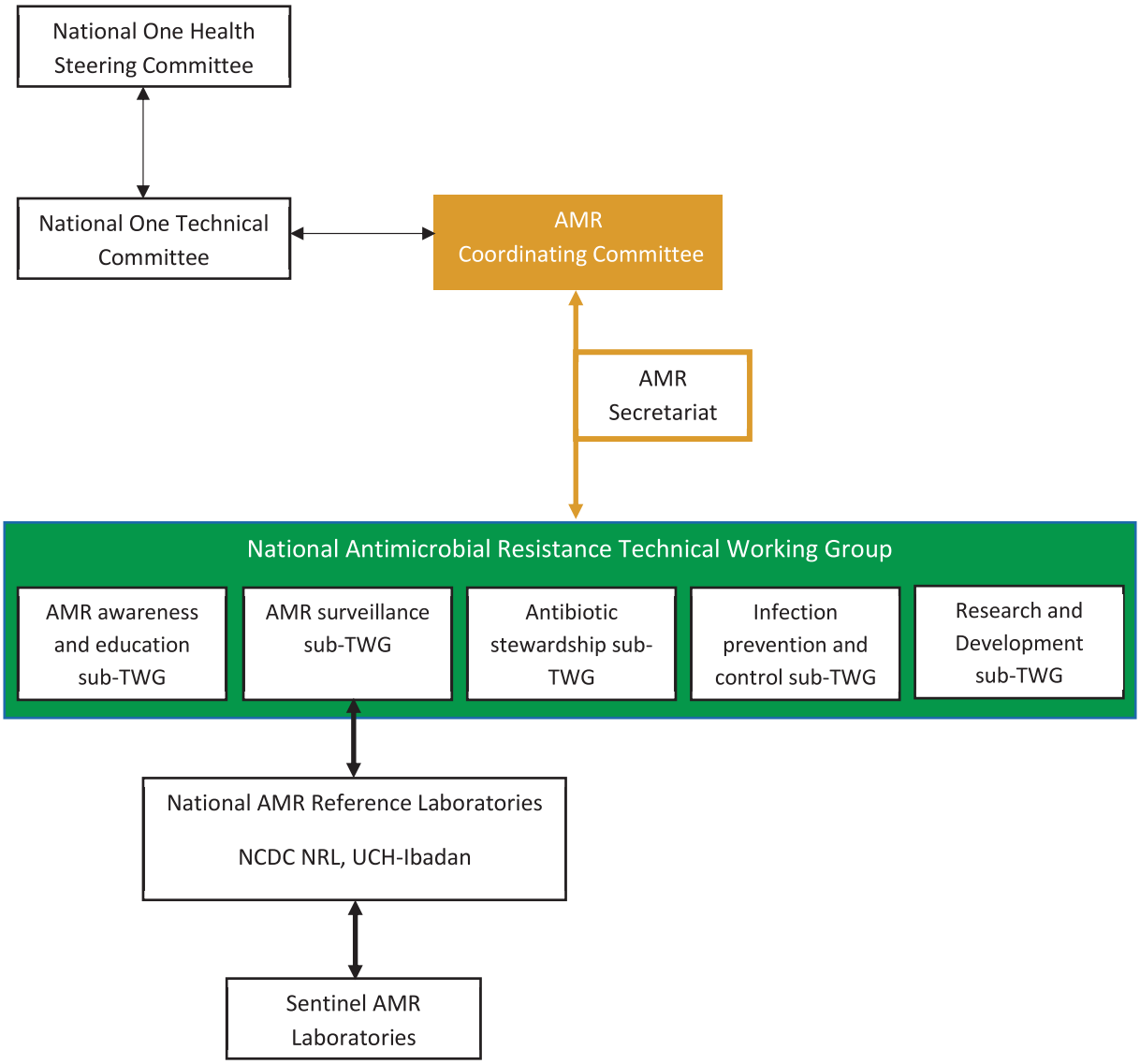

Figure 1 : Structure and responsibilities of network and participating laboratories

Role of National Reference Laboratory and Sentinel Laboratories

National AMR Coordinating Centre

The National AMR Coordinating Centre (NCC) is an arm of the NCDC. It is responsible for establishing and overseeing the national AMR surveillance program, collecting national AMR data, evaluating the system and sharing information with the WHO-GLASS programme.

National Reference Laboratory

The primary function of the NRL within the AMR surveillance system is to promote good microbiological laboratory practices, including adapting and disseminating microbiological methods, standards and protocols, to serve as a resource and coordination point for quality assessment and improvement in laboratories, and to facilitate collaboration with AMR surveillance sites (see appendix 1).[12]

Sentinel Sites

The primary function of sentinel sites will be to implement AMR surveillance at the facility-based level. They should have access to epidemiological support and a microbiology laboratory and should be actively promoting diagnostic stewardship. The commitment and active support of clinical staff, administration and management at the AMR surveillance sites is essential in order to conduct AMR surveillance according to the requirements and standards of the national system and GLASS, and to encourage the long-term sustainability of the system. A key function of these sites will be to apply their clinical expertise and use the quality-assured microbiological findings and AMR surveillance data to select and prescribe the most appropriate treatment (see appendix 1)[9,13].

Criteria for participation in Laboratory-based AMR Surveillance

Participating sites are selected by the National Coordinating Centre. When selecting a potential AMR surveillance sentinel site, the following criteria will be considered:

Laboratory infrastructure and capacity

a. Availability of outpatient and emergency out-of-hours service for different levels of health care at the facility
b. Access to enough and diversity of patients
c. High volume of laboratory diagnostic activity (at least 200 isolates per year) to allow a meaningful analysis of surveillance data
d. Ability to conduct bacterial and fungal cultures
e. Availability of appropriate laboratory equipment and biosafety equipment more than one autoclave, and one -20 °C freezer
f. Access to the appropriate reagents and supplies for bacteriologic testing
g. Availability of guidelines for equipment calibration and maintenance
h. Keeps records of sources of reagents and supplies for microbiological culture

Staffing capacity and training

a. Has appropriate cadre and adequate number of staff stationed at the microbiology section of the laboratory: at least one consultant pathologist and two medical lab scientists with bachelor's degree or higher
b. Ensures staff has access to annual professional/competency training

Antimicrobial Susceptibility Testing (AST) practices

a. Availability of SOPs for specimen collection, rejection and processing, culture media preparation and specimen processing and quality control and identification of bacterial isolates, conducting AST including current breakpoint guidance *(Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST))*
b. Access to distilled water for media and reagent preparation, potable water for general sanitation, appropriate media for AST and quality-controlled antibiotic disks, quality control (QC) strains and availability of SOPs for internal quality control, McFarland standards, simple turbidimetric devices or spectrophotometers to read density, rulers or callipers to read zone diameters
c. Participates in External Quality Assessment (EQA) for culture and AST
d. Maintains database of AST results including isolated putative contaminants
e. Availability of functional computers and internet
f. Availability of standard operating procedure for AST data recording, analysis

and reporting (zone diameters measured and recorded)

Utilization of AMR information

a. Ability to manage and report surveillance data, including denominator data to the national AMR surveillance system (e.g. specimens submitted for testing)
b. Ability to share isolates with national reference laboratory for quality control
c. Ability to support clinicians at the health facility to utilize the AST results in making treatment decisions
d. Ability to use AST data to update list of the antimicrobial agents used by the laboratory for testing and dispensed to patients at the pharmacy
e. Ability to mentor and support antimicrobial stewardship at associated sites[14,15].

The tool for annual assessment of laboratories participating in the national AMR surveillance system is also available on the NCDC website[16].

Priority Specimens and Pathogens

Population under Surveillance

The population under surveillance is all patients seeking medical care in hospitals participating in AMR surveillance nationally, managed either as outpatients or as inpatients including:

a. Patients sampled for prioritized specimens with growth of priority species or positive samples
b. Negative samples

Priority Specimens

a. Blood *(priority specimen for GLASS)*
b. Cerebrospinal fluid (CSF) *(as clinically indicated)*
c. Stool from suspected cholera patients *(as clinically indicated)*

This list may be adapted in the future to include other specimens and antibiotic combinations, for improved representative antimicrobial susceptibility profiles as determined appropriate based on existing data.

Pathogens of interest

a. *Escherichia coli*
b. *Klebsiella pneumoniae*
c. *Acinetobacter baumannii*
d. *Staphylococcus aureus*
e. *Streptococcus pneumoniae*
f. *Salmonella species*
g. *Vibrio cholera (non-WHO Priority but locally relevant)*
h. *Neisseria meningitidis (non-WHO Priority but locally relevant)*

Patient Sampling

Samples will be taken from patients seeking care at the participating sentinel site hospitals to test for priority pathogens, as clinically indicated according to local practice. Relevant patient information should be captured on request forms that accompany samples to the laboratory and this should correspond to the variables collected via WHONET (appendix 4).

Data to be collected

The following data should be collected. This information is necessary for analysis and isolate re-testing:

a. Patient information: patient hospital identification number, age, date of birth, gender, date of admission (for inpatients), hospital department, level of care (outpatient versus inpatient), hospital ward (for inpatients), previous use of antimicrobial agents (yes/no), admitting diagnosis (or current diagnosis for outpatients), previous hospitalisation in past 14 days (yes/no).
    i. A unique patient identification number is essential to be able to de-duplicate the results of patients with multiple isolates; participating laboratories/ health facilities should take efforts to improve patient ID labelling on medical records and samples.
    ii. Date of admission is critical data to determine if the infection is community-acquired (i.e. sample taken from patient in an outpatient department or admission for two days or less) or hospital-acquired (i.e. sample was taken greater than two days after admission or transfer).
b. Specimen information: specimen number, specimen type, type of sample or isolate source, date of specimen collection, organism isolated, and resistance testing results including actual zone diameters and selected resistant mechanism testing.

Laboratory Protocols for Specimen Processing and Pathogen Identification

Currently, there are two recognized standards for antimicrobial susceptibility testing (AST); Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines[17,18]. An AMR surveillance system should work towards the use of a common set of guidelines across participating laboratories to ensure standardized methods, results and interpretation. As at the end of 2018, more laboratories reported the use of CLSI guidelines. Laboratories should coordinate with NCDC and NRL to determine that they are using the current and correct CLSI version. In parallel, the AMR TWG will consider the use of EUCAST guidelines, a pilot using these guidelines, and long-term planning for scale-up and standardization. General minimum standards for specimen processing and pathogen identification are listed below. Further details on specific standards will be outlined in a practical manual of all standard operating procedures to follow the guidelines.

Media preparation

Minimum standards in media preparation that all participating laboratories must adhere to are:

a. Dedicated media preparation room according to acceptable standards (see appendix 6)
b. Media preparation protocols according to acceptable standards (see appendix 6)
c. Systematic use of distilled H2O for preparation
d. Systematic use of sheep's blood to prepare Blood/Chocolate Agar media; use of 5% mechanically defibrinated horse blood for Mueller-Hinton agar for fastidious organisms if EUCAST guidelines are being used
e. Systematic use of plates with standard agar depth, e.g. 4.0 mm ± 0.5 mm
f. Must adhere to internal quality control, i.e. sterility and growth performance testing, using standard guideline strains
g. If media is being purchased, it must be procured from an accredited supplier
h. Storage of media plates according to acceptable standards, e.g. dried at 20-25 °C overnight or 35°C for 15 minutes without lids to reduce moisture.

Table 1: Recommended isolation media by specimen type

S/no	Specimen	Isolation media*
1.	BLOOD	Blood culture broth Subculture on blood agar Chocolate agar MacConkey agar…
2.	CSF	Blood agar Chocolate agar MacConkey agar Modified Thayer-Martin agar
3.	STOOL	MacConkey agar Thiosulfate-citrate-bile salts-sucrose agar Alkaline Peptone Water Salmonella-Shigella Agar Xylose Lysine Deoxycholate Agar Selenite F broth

Specimen culture

Based on the priority specimens above, the recommended primary isolation media shall be as tabled below.

*** Important information to note**

a. MacConkey agar: For enteric gram-negative rods;
b. Chocolate agar: For fastidious organisms such as *N. gonorrhoeae, N. meningitides,* and *Haemophilus influenza;*
c. Modified Thayer-Martin agar: For *N. gonorrhoeae or N. meningitides* from specimens containing normal flora
d. Selenite or GN enrichment broth: The use of enrichment broth is controversial; it increases the yield of Salmonella by approximately 10%. GN broth must be sub-cultured at 6 hours and Selenite in 6-8 hours, failure to do so will result in overgrowth of normal enteric flora.
e. Incubation in CO2: Chocolate and Thayer-Martin agar **must** be incubated in CO2. It is preferred that blood agar plates also be incubated in CO2 as most bacteria grow better in increased CO2. However, if you do not have a CO2 incubator, or enough room or candle jars, blood agar plates can be incubated in room air.

Table 2: Recommended media for antibiotic susceptibility testing

S/no	Organisms	Basic CLSI Media	Basic Media (EUCAST)
4.	Non-fastidious organisms (e.g. Enterobacteriaceae, *Acinetobacter* spp., *Staphylococcus* spp., *Enterococcus* spp.)	Mueller-Hinton agar	Mueller-Hinton agar
5.	Fastidious organisms	Mueller-Hinton agar with 5% sheep blood agar (*Streptococcus* spp., *Neisseria meningitidis*)	Mueller-Hinton agar with 5% mechanically defribinated horse blood + 20 mg/L β-NAD (*Streptococcus* spp.)

Pathogen identification

Recommended identification protocol followed by the participating laboratories shall be as tabled below.

Table 3: Recommended pathogen identification protocol

S/no	Pathogen	Microscopy	Recommended Assays/Platform	
			Sentinel	Reference
1.	*E. coli*	Gram, Motility	Indole, KIA/TSI, Citrate, Urease, Microbact/API	VITEK and/ or Whole genome Sequencing
2.	*K. pneumoniae*	Gram, Motility	Indole, KIA/TSI, Citrate, Urease, Microbact/API	VITEK and/ or Whole genome Sequencing
3.	*A. baumannii*	Gram, Motility	Oxidase, KIA/TSI, Glucose, Microbact/API	VITEK and/ or Whole genome Sequencing
4.	*S. aureus*	Gram	Catalase, Coagulase (Tube and Slide), MSA, Microbact/API	VITEK and/ or Whole genome Sequencing
5.	*S. pneumoniae*	Gram	Optochin, Bile solubility, API	VITEK +_ quelling or latex agglutination serotyping and/ or Whole genome Sequencing

6.	*N. meningitides*	Gram	Oxidase, Catalase, API	VITEK +_ slide agglutination serotyping and/ or Whole genome Sequencing
7.	*V. cholera*	Gram, Motility	Oxidase, String test, Serotyping, API	VITEK or Whole genome Sequencing
8.	*Salmonella* species	Gram, Motility	KIA/TSI - Glucose, urease, lysine decarboxylase, indole test, H2S production, and fermentation of dulcitol, API	VITEK and/ or Whole genome Sequencing

Susceptibility Testing AST - Pathogen/Antibiotic Panels

Based on the priority pathogens above, the following antimicrobial susceptibility testing protocol are recommended for disk diffusion by participating laboratories:

Table 4: Recommended antibiotic susceptibility protocol

Pathogen	Antibacterial class	Antibacterial agents	Recommended testing
E. coli	Fluoroquinolones	Ciprofloxacin, Ofloxacin, Levofloxacin	Disk diffusion testing; ESBL screening (e.g. cefotaxime/ceftriaxone, ceftazidime) and confirmation (e.g. combination disk or double- disk synergy tests); Screening for carbapenemase production (e.g. mereopenem) and confirmation (e.g. modified carbapenem inactivation method (mCIM))
	Third-generation cephalosporins	Cefotaxime, Ceftriaxone, Ceftazidime	
	Fourth-generation cephalosporins	Cefepime	
	Carbapenems	Meropenem, Imipenem, Ertapenem	
	Penicillins	Ampicillin, Amoxicillin-clavulanic acid, Piperacillin/Tazobactam	
	Tetra cyclines	Tigecycline	
	Aminoglycosides	Gentamycin, Tobramycin, Amikacin	

K. pneumoniae	Fluoroquinolones	Ciprofloxacin, Ofloxacin, Levofloxacin	Disk diffusion testing; ESBL screening (e.g. cefotaxime/ceftriaxone, ceftazidime) and confirmation (e.g. combination disk or double- disk synergy tests); Screening for carbapenemase production (e.g. mereopenem) and confirmation (e.g. modified carbapenem inactivation method (mCIM))
	Third-generation cephalosporins	Cefotaxime, Ceftriaxone, Ceftazidime	
	Fourth-generation cephalosporins	Cefepime	
	Carbapenems	Meropenem, Imipenem, Ertapenem	
	Penicillins	Ampicillin, Amoxicillin-clavulanic acid, Piperacillin/Tazobactam	
	Glycylcyclines	Tigecycline	
	Aminoglycosides	Gentamycin, Tobramycin, Amikacin	
	Other	Trimethoprim-sulfamethoxazole	
A. baumannii			Disk diffusion testing
	Fluoroquinolones	Ciprofloxacin, Levofloxacin	
	Aminoglycosides	Gentamycin, Tobramycin, Amikacin	
	Carbapenems	Meropenem, Imipenem	
S. aureus	Penicillins	Cefoxitin (screening for methicillin resistance, i.e. MRSA; Oxacillin disk testing is not reliable**)**, Penicillin	Disk diffusion testing; MRSA screening (e.g. cefoxitin); D-test for inducible clindamycin resistance (e.g. erythromycin and clindamycin)
	Fluoroquinolones	Ciprofloxacin, Norfloxacin, Ofloxacin or Levofloxacin	
	Macrolide	Erythromycin	
	Lincosamide	Clindamycin	
	Glycopeptide	Vancomycin	
	Aminoglycoside	Gentamycin	
	Tetracycline	Doxycycline	
S. pneumoniae	Penicillins	Oxacillin (screening for beta-lactam resistance), Penicillin G	Disk diffusion testing; Oxacillin screening for beta-lactamase resistance
	Third-generation cephalosporins	Ceftriaxone or Cefotaxime	
	Fluoroquinolones	Ciprofloxacin, Norfloxacin, Moxifloxacin, Levofloxacin	
	Macrolide	Erythromycin	
	Other	Trimethoprim-sulfamethoxazole	

Salmonella spp	Fluoroquinolones	Ciprofloxacin or Levofloxacin	Disk diffusion testing; ESBL screening (e.g. cefotaxime/ceftriaxone, ceftazidime) and confirmation (e.g. combination disk or double- disk synergy tests); Screening for carbapenemase production (e.g. mereopenem) and confirmation (e.g. modified carbapenem inactivation method (mCIM))
	Penicillins	Ampicillin, Amoxicillin-clavulanic acid	
	Third-generation cephalosporins	Ceftriaxone/Cefotaxime & Ceftazidime	
	Carbapenems	Imipenem/Meropenem/ Ertapenem	
	Other	Trimethoprim-sulfamethoxazole	
N. meningitidis	Penicillins	Ampicillin	Disk diffusion testing
	Third-generation cephalosporins	Ceftriaxone or Cefotaxime	
	Carbapenems	Meropenem	
	Fluoroquinolones	Ciprofloxacin	
	Other	Chloramphenicol	
		Rifampicin	
V. cholerae (No EUCAST breakpoints)	Penicillins	Ampicillin	Disk diffusion testing
	Sulfonamide and Trimethoprim	Trimethoprim-sulfamethoxazole	
	Tetracyclines	Tetracycline , Doxycycline	
	Fluoroquinolones	Ciprofloxacin, Ofloxacin	

Priority will be given to the antibiotics listed here (which include GLASS recommendations as well as national priorities), but sentinel sites may add others for their own purposes according to local prescribing practice.

The modified Kirby Bauer disk diffusion method will be employed for AST by the sentinel sites. As stated above, appropriate SOPs should be used according to the most current guidelines of CLSI or EUCAST as determined with the surveillance network. Internal and external quality control should also be regularly performed with these SOPs. A practical manual with more details will be follow these guidelines.

Testing by broth dilution and/ or identification of virulence genes will be done at the NRL irrespective of whether sites perform these tests for their own purposes.

R Reporting System/Data Management

AMR data submission and transmission

The preferred method of data submission to NCDC is in a WHONET data file.

Standard WHONET file

NCDC will provide a configured WHONET file to each of the participating surveillance network sites. This preconfigured file contains the necessary data fields, organisms, specimen types, and minimum antibiotic panels relevant to AMR surveillance (patient and specimen data are outlined in aforementioned "Data to be collected" section). Key identifying information required includes:

a. Laboratory code, to be assigned by NCDC
b. Patient identification number
c. Isolate number

Sites should use this pre-configured file for entering and reporting AMR surveillance data. For sites that are already utilizing WHONET for their laboratory, the existing site-level WHONET file should be adapted and configured according to the specifications of NCDC (organisms, specimen type, data field etc). The expected AST results should focus on blood of the specified pathogens identified in patients seeking care. Results for CSF or stool isolates, as clinically indicated, will also be collected. The appropriately configured WHONET files should be submitted to NCDC on a monthly basis for better monitoring at the initial phase and quarterly thereafter as to be determined by NCC. NCDC will provide technical support to sites to configure their files, provide feedback on data quality and use of data for action[19]. NCC is also responsible for collating the individual participating laboratory information and reporting the country level data to WHO GLASS. A practical manual with more details on these procedures will follow these guidelines.

Diagnostic stewardship can be defined as the promotion of appropriate and timely diagnostic testing to guide therapeutic decisions. Accordingly, not only should standard and quality-controlled approaches be used to guide specimen collection, handling and pathogen identification, but accurate and timely reporting of results to guide patient management should also be encouraged.

Full antimicrobial susceptibility reports should be provided to clinicians at the health facility in a timely manner, including results for all antimicrobial agents tested. The full report should be saved on the WHONET software and line listed for submission to NCDC. The full report is particularly important in compiling the overall AMR profile at the surveillance site and informing treatment guidelines at both the local and national levels, as well as in identifying resistance mechanisms and monitoring multidrug resistance profiles.

Some core testing results include:

a. Number of patients with negative cultures per specimen type
b. Number of patients with positive cultures per specimen type
c. Bacterial species results for patients with positive cultures
d. AST results for patients with positive cultures including priority minimal antibiotic panels

Additionally, sentinel sites should submit an annual denominator collection form (see appendix 9). This should include the following required information:

a. Number of patients seeking care per annum at sentinel sites (outpatients and inpatient departments: from the statistics/records department of the health facility)
b. Number of beds
c. Average occupancy %
d. Patient catchment area (by State) for the year

The reporting channels and methods can be summarized as seen in Figure 2.

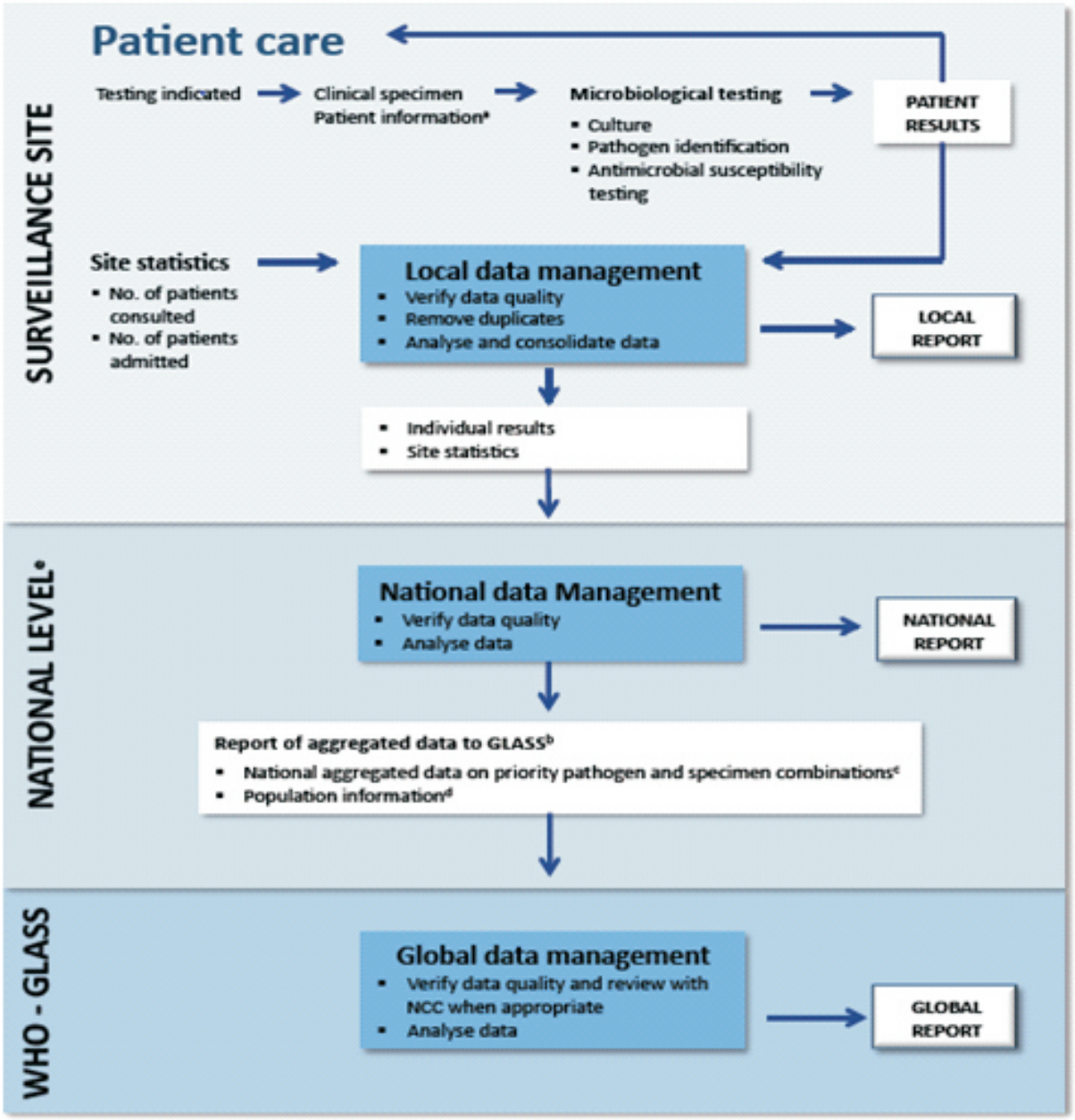

*Figure 2: Reporting channels and methods: feedback follows the arrow path backwards**

**Reference: Diagnostic stewardship: A guide to implementation in antimicrobial resistance surveillance sites* [9]

Quality Management System

A quality management system can be defined as “coordinated activities to direct and control an organization with regard to quality.” Laboratory quality can be defined in terms of the accuracy, reliability and timeliness of reported test results. The laboratory results and operations must be quality assured and reporting must be timely in order to be useful in a clinical or public health setting.[20]
Sentinel laboratories are strongly encouraged to participate and obtain certification under recognized laboratory quality assurance programmes, such as ISO and ASLM.

For the purpose of AMR Surveillance, the following aspects of quality assurance and control can be grouped according to: Pre- analytical phase (i.e. sample collection and handling), Analytical Phase (i.e. laboratory testing) and Post-Analytical phase (i.e. results and feedback). A practical manual with more details on procedures that enable quality assurance and control will follow these guidelines.

The key points for each phase can be summarized as follows:

Pre-analytical Phase QA

Microbiology laboratories should regularly educate clinicians on adequate patients' preparation for proper specimen collection and handling. This includes appropriate timing of blood cultures (e.g. prior to antimicrobial therapy), disinfection of blood culture bottles, antisepsis of skin, enough blood volumes, and timely specimen transport. Additionally, laboratory request forms must be properly filled and completed including an appropriate patient identification number that can be used to effectively link results to patient management.

Analytical Phase QA

Internal quality control (IQC)
Internal quality control (IQC) is a routine procedure undertaken by laboratories to ensure quality of testing procedures. Sentinel sites are encouraged to make individualized quality control plans (IQCP) to help guide regular IQC procedures.

One example could be United States Centres for Disease Control and Prevention (CDC) guide to IQCP development[20]. The World Health Organization (WHO) Stepwise Laboratory Improvement Process towards Accreditation (SLIPTA) programme could also offer useful resources.

IQC procedures should be performed on regular basis (e.g. initially daily to several times a week depending on workload of specimens) for each testing method used, the results should be recorded and discussed with all staff members. Quality control data sheets and summaries of corrective actions should be retained for documentation. Typically, a test kit has a set of positive and negative controls that are to be included in each test run. The laboratory should select the appropriate QC strains to be used for each organism based on standard guidelines. For antimicrobial susceptibility testing, information on quality control strains and test result ranges can found in the CLSI document M100[21]. and EUCAST quality control tables[17]. Quality control strains may be purchased from official collections such as the American Type Culture Collection (ATCC) and the National Collection of Type Cultures (NCTC).

Laboratory equipment and materials should be assessed on a regular basis to ensure maintenance and quality. This includes sterility testing, quality control and performance testing of media, reagents, and antibiotic disks. For equipment, this includes validation, verification, routine and preventive maintenance, calibration, and temperature charting (e.g. ambient, fridges, freezers and incubators). Periodic competency assessment of personnel should also be conducted.

External Quality Assessment

All sentinel sites and the reference laboratory must participate regularly and successfully in EQA programmes (EQAP) such as WHO/NICD external quality assessment surveys and/or as determined by the NCDC. The reference laboratory should score 70% and above on the proficiency test (PT) panels each time. PT results must be reported according to instructions and submitted within required deadlines. Each laboratory manager or head of laboratory must deal with unacceptable PT results, and corrective action must be taken and documented. The microbiology WHO/NICD EQA programme includes challenges

for bacterial meningitis, diarrheal diseases and other significant bacterial infections. As noted, a practical manual with more details on these procedures will be follow these guidelines.

Post-analytical Data QA

The sentinel sites will collect data and send these to the National Reference laboratory to validate. Accordingly, NCDC will provide feedback to stakeholders on a regular basis.

Isolate Transport and Storage Procedures

Isolates from the sentinel sites will be sent to the National Reference Laboratory every third week of the month using the TRANEX transport system. The isolates must be accompanied by the appropriate epidemiological, demographic, clinical data and patient identification number or isolate number.

Important steps

a. Properly package & transport sample from sentinel lab every third week of the month
b. Provide appropriate holding for samples
c. Initiate sample courier process
d. Ensure total quality management in the system
e. Documentation for data
f. Documentation for non-conformance/occurrence
g. Provision of needed supplies

Isolate Transportation

a. All isolates of prioritized pathogens should be stored in Tryptic soy broth (TSB) with 15% glycerol and kept frozen to achieve viability. However, for fastidious organisms *(S. pneumoniae and N. meningitidis),* sheep blood or skimmed milk broth with 15% glycerol
b. All isolates of priority pathogens should be stored at 35-37 C in a -70 freezer or in liquid nitrogen (at -196°C)
c. All frozen isolates should be sent triple-packaged to the NRL at 1 monthly-intervals for confirmatory tests for the first year. A determined subset of isolates will be sent to the NRL for confirmatory testing to reflect representativeness
d. As soon as the NRL in Abuja is operational, all stored isolates from all sentinel sites and the interim NRL are to be transported to the NRL in Abuja where they will be stored permanently
e. Further details on preparation, transport, and storage as well as the confirmatory testing strategy will be outlined in a practical manual of all standard operating procedures to follow the guidelines

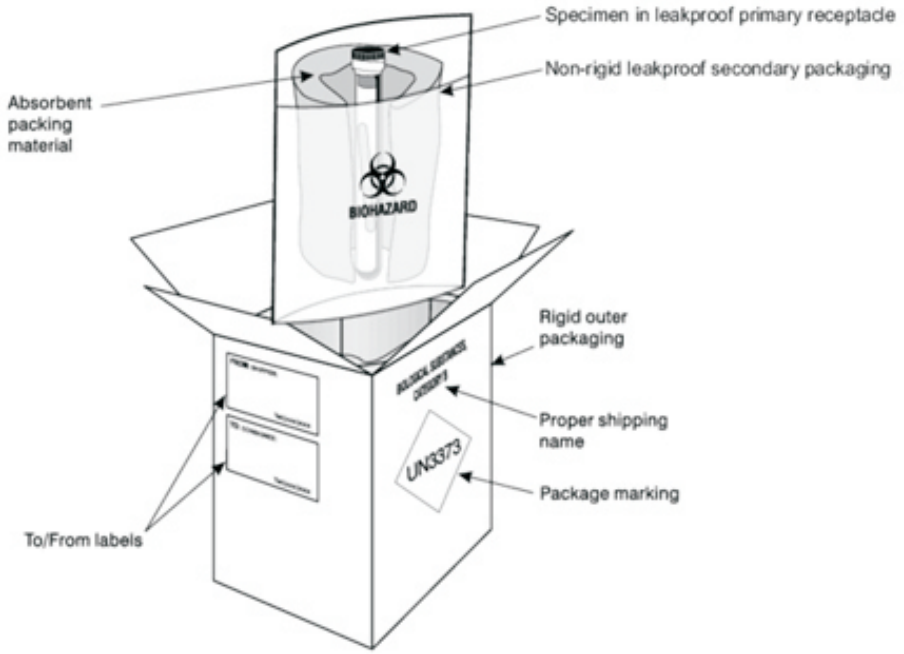

*Figure 3: Sample of triple packaged isolate**

*Reference: Guidance on good data and record management practices. WHO technical report series[22]

D Data Management System

Data quality control

Quality control should be ensured at every stage of the reporting and data management process. The WHONET file will have some integrated data entry quality checks that will alert the user to some data quality issues. The WHONET training and SOP will provide a reference to these functions. When a WHONET file is received at NCDC, the appointed data manager will review the file and provide a standardised monthly feedback report to the site, highlighting any data quality issues that need to be addressed by the sites. In particular, the data manager will assess the completeness of the data, possible duplications (only primary isolates per patient per year are requested), and microbiology consistency according to pre-defined pathogen-antibiotic combinations and plausible disk inhibition zones. Once the points in the feedback reports have been addressed and the data manager approves the submission, the data will be added to the national database. NCDC will also help support capacity-building and trainings for improved quality of surveillance data.

The confidentiality of all data collected should be protected and the following data security measures should be observed:

a. Ensure that the computers used in data storage have up-to-date virus protection
b. Trained staff should follow good data security practices (e.g. not clicking on unknown links) and make use of passwords to secure files
c. Only designated and trained individuals should have access to data
d. Make use of encrypted and password protected flash drives to move data to another device if need be
e. Back-up your data regularly in google drive
f. Maintain a data security policy[22].

Patient data should be stored with protective software i.e., software that controls data storage, removal, and use. Prior to electronic transfer of patient data, verify that all personal identifiers have been removed[22]. Discard hard copies and hard drives that are no longer in use. Head of Departments (HOD)

and data managers should ensure that de-identified line listed databases are submitted to the national AMR coordinator within the health facility.

Use of AMR data for clinical management and policy making

It is recommended that a multidisciplinary team be established nationally, comprising a range of disciplines e.g. epidemiologists, microbiologists, medical laboratory scientists, clinicians, pharmacists, infectious disease experts, data managers and other relevant stakeholders, with one designated focal point for AMR surveillance.

At health facilities participating in AMR surveillance, there should be robust diagnostic stewardship programs. The quality of AMR surveillance which is based on routine data can be improved by diagnostic stewardship, an integral part of both patient management and standardized surveillance. According to WHO, diagnostic stewardship can be defined as:

"Diagnostic stewardship is the coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions. This will promote appropriate, timely diagnostic testing, including specimen collection, and pathogen identification and accurate, timely reporting of results to guide patient treatment[(9)]*.*

The antimicrobial stewardship program (including the diagnostic stewardship program) guides therapeutic decision-making and informs local empiric treatment recommendations and AMR control strategies. An example of antimicrobial stewardship core components and committee terms of reference can be found here[(23)].
Furthermore, the AMR data should be used to inform relevant policy-making such as AMR communication messages to promote the adoption of positive behavioural practices (e.g. appropriate antimicrobial use, cross-sectoral antimicrobial stewardship), regulatory systems for AMR prevention, control and priority setting for AMR research funding.

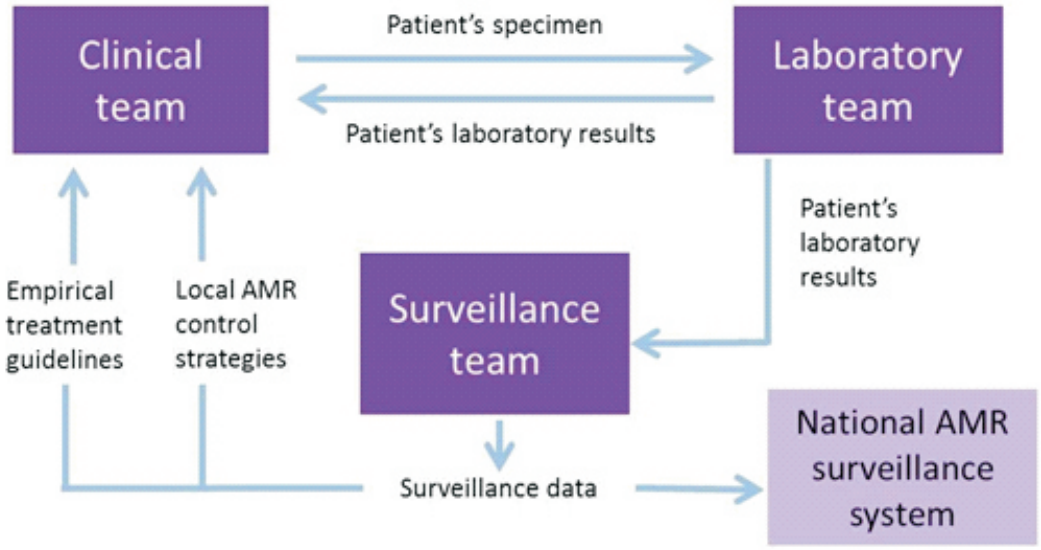

Figure : Diagnostic stewardship feedback mechanism

*Reference: Diagnostic stewardship: A guide to implementation in antimicrobial resistance surveillance sites[9]

Optimal patient care depends on good communication between clinical staff at point-of-care, microbiology laboratories and surveillance staff. Clear procedures should be in place for communication between clinical, laboratory and surveillance staff. Laboratories must also report all results from specimens. Managing clinicians should receive reports in a timely manner, including interpretive statements that enable the results to be easily and effectively applied for patient management. Procedures should specify the turn-around time to send the laboratory results back to clinicians. Ideally, the laboratory should contact the attending physician within 72 hours with the preliminary results. To encourage improved feedback, a standardised feedback form could be developed, and clinical microbiologists or other laboratory staff could use these forms to guide their communication of the results. Regular meetings should also be held to discuss results for individual care and adaptation local treatment guidelines according to findings. An evaluation form of these weekly meetings could also be developed to guide communication of the results. The laboratory HOD at sentinel laboratories should provide feedback on data at least quarterly to the hospital administration, clinical and laboratory staff, to support continued engagement with AMR surveillance. A practical manual with more resources to enable the use of data and diagnostic stewardship will follow these guidelines.

Availability of results

The NCDC should disseminate non-identifiable summary data to stakeholders as soon as possible after data are collected. As described above, data quality will be assessed at the national level before disseminating any results. Similarly, the facility should take measures to assess data quality before sharing results. Data-release policies should define the purposes for which the data can be used and provisions to prevent public access to raw data or data tables that could contain indirectly identifying information.

Integration into IDSR

Until 2008, the diseases under the Integrated Disease Surveillance and Response (IDSR) were mainly those diseases that are targeted for eradication, elimination, epidemic prone diseases and some communicable diseases of public health importance. Periodically (i.e. weekly or monthly), the health facility summarizes the number of cases and deaths from priority diseases and report the total to the local government areas LGA and state levels. The health facility performs some analysis of the data such as keeping trend lines for selected priority diseases or conditions and observing whether certain thresholds are passed to alert staff to act. At the state level, the aggregated data is sent to NCDC (14). Feedback on surveillance performance indicators are shared to the lower levels. The emergence of multidrug-resistant organisms has led to exponentially increased mortality with huge economic burden. Therefore, there is a need to revise the IDSR guidelines and include AMR priority organisms[8]. The feasibility of this as it relates to the roll-out of these AMR surveillance guidelines will be assessed on an ongoing basis, and actions will be taken as appropriate.

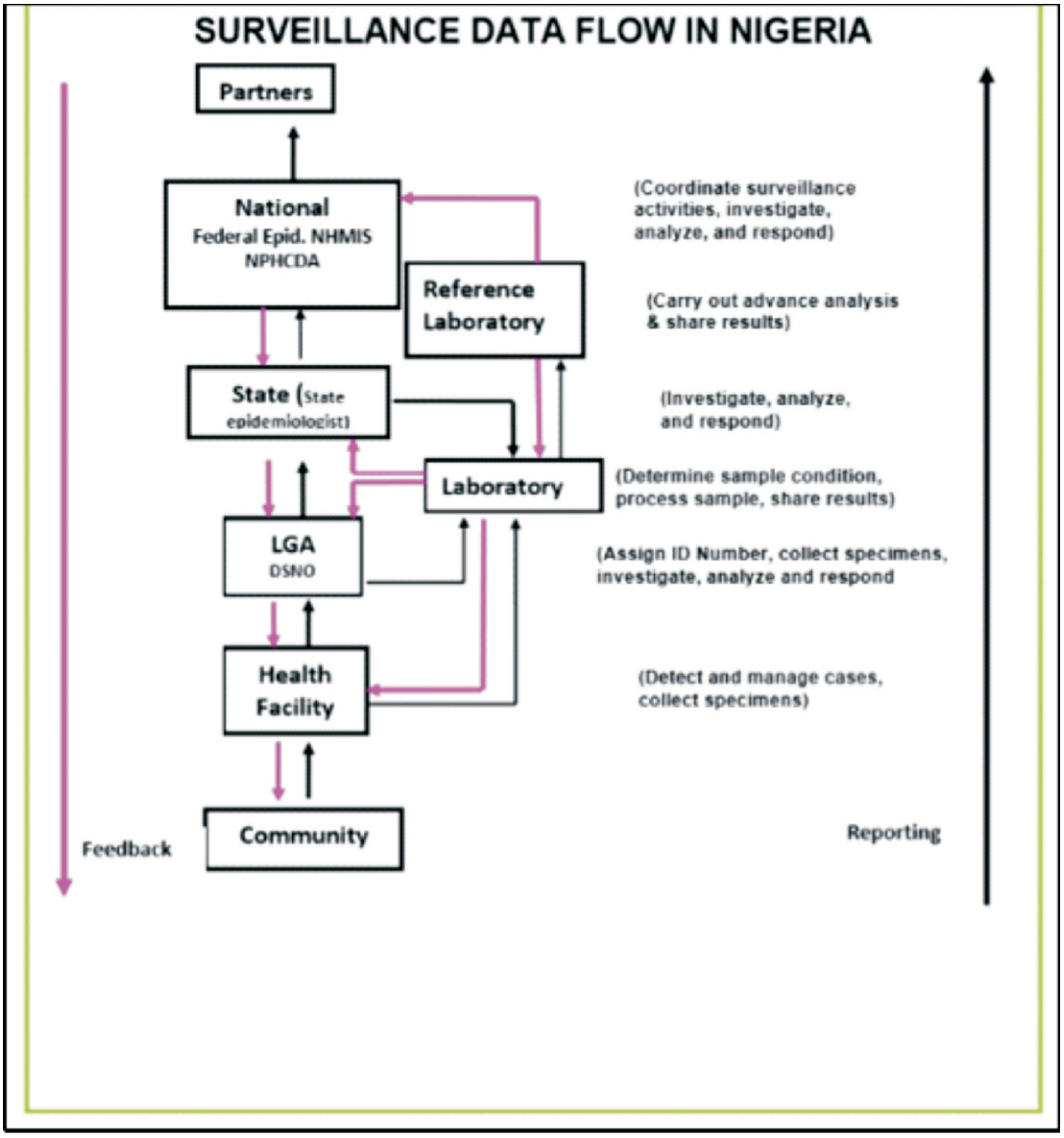

Figure 5: Integrated disease surveillance system information system flow in Nigeria
**Reference: Technical guidelines for Integrated Disease Surveillance and Response in Nigeria*[24]

Analysis & Reporting

Isolates from selected patients or settings (e.g. only severe patients, certain types of infections or certain departments) may give a biased picture of the national situation. There is a need to review the sources of the organisms that are tested and consider their representativeness. Also, it is important to ensure enough isolates are included in the database to allow for an adequate sample size to draw conclusions from. At a minimum, 30 isolates per pathogen should be available to consider the isolates of minimum quality. Clinical isolates that

give inconsistent or uncommon results are important for recognizing the emergence of a new resistance profile such as penicillin- or chloramphenicol-resistant or fluoroquinolone-resistant *Salmonella*. Such isolates should be sent for confirmation to a reference laboratory and for determination of minimum inhibitory concentration. Priorities and protocols for confirmatory testing at NRL will be assessed on an ongoing basis and communicated to the sentinel sites accordingly.

In coordination with NCDC and the data management team, the clinical bacteriologist at the NRL should coordinate the confirmation and validation of AMR test results. The bacteriologist will review atypical or unusual results to maintain the quality of the results and performance of the laboratory, which are requirements of IDSR. Data on overall levels of resistance should be shared regularly with stakeholders. Basic analysis can be done at the facility level for local usage while the main analysis will be done at the NCDC. In particular, the proportion of resistance will be calculated considering the antibiotic and the antibiotic group (i.e. antibiotic class or a subset such as third generation cephalosporins) for each pre-determined pathogen-antibiotic combination. As appropriate, both results for resistance (R) and resistance + intermediate (R+I) will be assessed. Bacterial species and AST results will also be described by hospital and patient characteristics. If feasible, temporal trends of AMR will be assessed by site.

The NCDC will manage the central AMR surveillance database and ensure provision of annual reports, newsletters, publications and updated guidelines at the national level. The NCDC also establishes linkages with other stakeholders including antimicrobial stewardship committees at the sentinel sites.

Monitoring, Evaluation and Analysis

Monitoring and Evaluation Logical Framework for AMR Surveillance

Quality Indicators

Quality indicators are objective measures of surveillance and laboratory practices. Indicators can be developed that look at timeliness, patient refusals, and lost or delayed laboratory reports and sample rejection among other measures. NCDC and the AMR TWG will review the logic framework and indicators listed in Tables 4 and 5 below biannually to evaluate the AMR surveillance and decide on appropriate actions. Such monitoring and evaluation will aim to improve the surveillance system in the long term[13,15].

Table 5: Monitoring and evaluation logical framework

Input (Resources)	Process (Activities)	Output (Results)	Outcome
1. Funding for personnel, equipment, consumables 2. Local guidelines and SOPs 3. Staff with expertise in the field of AMR surveillance including epidemiology and laboratory expertise 4. Communication protocols and facilities	1. Mobilization and management of funds 2. Development or adaptation of SOPs 3. Development and implementation of training materials 4. Agreed means and frequency of communication between clinical laboratory and surveillance staff 5. Implementation of network mentorship and quality assurance	1. Sustainable financing and resources available on regular basis 2. Common understanding of protocols 3. Trained staff with relevant competencies at surveillance sites 4. Quality-controlled implementation of AMR surveillance methods 5. Reliable information on defined AMR public health priorities available	National strategy informed by national AMR surveillance

Table : Monitoring Indicators for AMR surveillance in Nigeria

Type	Indicator	Definition
Input	Number of AMR surveillance sites	Number of surveillance sites fulfilling requirements to collect and report data on patients and AST that can be feed into the national system
	National Reference Laboratory (NRL)	At least one NRL is designated with agreed terms of reference to support national AMR surveillance system
	National plan for AMR surveillance	Presence of a strategic and operational plan for implementing and strengthening AMR surveillance, including participation in GLASS
Process	Existence of documented roles & responsibilities	Number of surveillance sites with roles and responsibilities well- documented # with surveillance sites with an AMR focal person or committee
	Routine validation of surveillance data	% of surveillance sites with established monthly feedback provided following validation of surveillance data **Or** % of surveillance sites visited at least once in a year for validation of surveillance data
	Internal QA at sentinel sites	% of laboratories with an internal quality control plan % of laboratories with active internal quality control programmes
	Participation of sentinel sites in external quality assurance programmes (EQAP)	% of laboratories participating in n EQAP
	EQA performance of laboratories contributing data to the national system	% of sites passing EQA Proficiency Testing
	Staff trained in AMR	# of scheduled training sessions in AMR surveillance including GLASS methodology conducted % of staff with appropriate training in AMR surveillance including GLASS methodology % of staff participating in a laboratory mentorship programme

Output	Priority specimen types included in the AMR surveillance	% of national target specimens that submit (We need to define a target goal, e.g 50%, 75% etc. based on the total number of priority specimens)
	Priority pathogens	% of surveillance sites reporting all pathogens listed with n out of N national targets included
	Completeness of data reported	% of surveillance sites submitting reports to surveillance system every month
	Timeliness of submission of surveillance reports	% of surveillance sites submitting reports to national level every month
Outcome	National strategy informed by AMR surveillance	Treatment and drug policy documents revised based on AMR surveillance data

A Appendices

Appendix 1: Terms of reference for National Coordinating Centre and the laboratories in the AMR surveillance network

National Coordinating Centre: Nigeria Centre for Disease Control

a. Define AMR surveillance objectives within the national AMR strategy
b. Facilitate linkages with AMR surveillance across human health, animal health and environmental sectors
c. Develop or adapt national AMR surveillance standards, protocols and tools and coordinate their dissemination
d. Provide guidance and information on data collection and reporting to the national reference laboratory and AMR surveillance sites
e. Monitor and evaluate the AMR surveillance system on an ongoing basis
f. Define strategy for participation in GLASS
g. Assure data management structure and format and IT solutions
h. Select and facilitate enrolment of surveillance sites
i. Coordinate collection and compilation of national AMR data
j. Conduct data analysis, validation and quality assurance including feedback to facilities
k. Analyse and feedback AMR surveillance results to AMR surveillance sites in collaboration with the National Reference Laboratory
l. Aggregate and report national AMR data and data on implementation status of national AMR surveillance system to GLASS[14] .

National Reference Laboratory

a. Serve as a resource and coordination point for laboratory expertise and share information and advice with relevant stakeholders
b. Liaise with the National Coordinating Centre
c. Develop, maintain, and share relevant SOPs and reference material
d. Promote good laboratory practice by providing guidance, mentorship and technical support for quality management, pathogen isolation and identification and antimicrobial susceptibility testing (AST) methodology
e. Support capacity building of laboratories serving AMR surveillance sites through oversight, mentorship and training

f. Organise or facilitate participation in external quality assurance (EQA) schemes for laboratories serving AMR surveillance sites, review EQA performance of participating laboratories and provide feedback on EQA results to laboratories
g. Perform confirmatory testing and provide feedback on isolates sent for confirmatory testing by sentinel laboratories; reporting unusual resistance, new strains and outbreaks, rare resistance mechanisms
h. Collaborate and conduct research in the field of microbiology
i. Negotiate procurement and tendering process to improve access to reagents and consumables
j. All isolates from sentinel laboratories to be sent to the NRL for the first 1 year of reporting, for confirmation and archiving.

Sentinel Laboratories

a. Promote diagnostic stewardship activities on-site
b. Collect clinical specimens and clinical, demographic and epidemiological data according to standard protocols
c. The microbiology laboratory providing support to the surveillance site should:
    i. Isolate and identify pathogens, perform AST according to standards and report microbiological information derived from the tested clinical specimen
    ii. Conduct internal quality control and participate in a proficiency testing scheme
d. Compile and manage basic clinical, demographic and epidemiological information derived from tested clinical specimens
e. Feedback/discuss locally generated surveillance data to inform local treatment guidelines and AMR control strategies
f. Report quality-assured AST results and relevant core patient data to NCC.

Appendix 2: Patient selection for specimen collection (case definition)

(Adapted from University College Hospital Bacteriology Laboratory Manual)

Blood culture

Blood cultures are obtained when a patient has a fever, particularly if another cause of the fever is not evident. Also, if a patient has an infection in another location, such as meningitis or pneumonia, the same organism can also be in the bloodstream. An infection in the blood stream is known as sepsis and is a serious, life-threatening infection. Blood cultures are also performed to diagnose bacterial endocarditis. This is an infection of the endocardium of the heart, primarily the heart valves.

Cerebrospinal fluid culture (CSF)

CSF cultures are performed to diagnose (or rule out) meningitis. Any person with sudden onset of fever (>38.5ºC rectal or 38.0ºC axillary) and one of the following signs: neck stiffness, altered consciousness or other meningeal signs; should be suspected as having meningitis[26].

Stool culture

Stool cultures are performed to find the causative agent of infectious diarrhea. Stool samples should be collected from any patient with watery diarrhoea (may or may not contain blood). Diarrhoea is defined as having 3 or more loose faeces in 24 hours.

Appendix 3: Specimen collection procedure

How to collect blood culture[27,28]

a. Positively identify the patient by checking the name and hospital number and comparing this with the request form
b. Put on appropriate PPE
c. Set up equipment near where you will be drawing. Move a bedside table, if needed.
d. Select equipment needed for the blood culture and all other phlebotomy draws
e. Select gauge of needle based on the on the chosen site and vein characteristics
f. Wear latex gloves to find a vein, tie tourniquet, note selected site and release tourniquet. Clean the site with an alcohol pad, moving in a concentric circle. Allow the alcohol to dry[29]
g. Bottles are marked with an optimal 10 ml fill line. The rubber stoppers in the bottles are cleansed with alcohol prior to inoculation. Use 1 pad per bottle and allow the tops to dry for 1 minute before inoculating[27]
h. Check to ensure the top is dry before inoculating the bottle. Do NOT touch the tops of the bottles
I. Open the sterile glove package on a flat surface without touching the inside. This is your 'sterile surface'[30]. Open syringes, needles and transfer devices and drop them out of the packages onto the sterile surface without touching them
j. Retie tourniquet. Do not allow the tourniquet to touch the cleaned site. If the cleaned site is touched, the area must be re-prepped. You may palpate the vein with the sterile gloves, ONLY if you have not touched a non-sterile item or surface
k. Put on sterile gloves and clean the site with sterile alcohol swab, moving in a concentric circle. Do not touch the venepuncture site after it is cleaned
l. Assemble butterfly and syringe, place back on sterile surface. Keep second sterile syringe and transfer devices on sterile surface within easy reach. Do not touch anything that is not sterile
m. It is optimal to draw the specimen using a syringe or butterfly needle. Blood culture bottles should always be filled first. The anaerobic bottle should be

inoculated first, to prevent any air that might exist in the top of the syringe from entering and altering the anaerobic environment. It is recommended to use a safety needle and activate the safety device after obtaining blood, remove the needle, and attach a transfer device to transfer blood to blood culture bottles and tubes

n. Ten ml of blood is optimal in each blood culture bottle. Do not overfill the bottles as this can lead to false-positive results due to excessive WBC's. If less than 10 ml is obtained, 5 ml is placed into the aerobic blood culture bottle and the rest is placed into the anaerobic bottle. If less than 5 ml is obtained, such as for new-borns, all the blood is placed into the aerobic (blue) blood culture bottle. If less than the optimal volume is collected, a notation should be made on the label
o. After completing the venepuncture, untie tourniquet. The syringe, needle or butterfly needle should be disposed of in a sharps container
p. Mix the blood culture bottles by inverting several times to mix the broth with the blood to prevent clotting and possible trapping of bacteria in the fibrin
q. Label the bottles with patient's first and last name, site of venepuncture, amount of blood collected, type of specimen (ie. #1 of 2, etc.) date and time of collection and the phlebotomist's initials. Transport samples to laboratory for processing

Stool collection for cholera samples

a. Put on appropriate PPE
b. Assemble all necessary supplies for sample collection
c. Allow Cary Blair medium or alkaline peptone water (APW) to attain room temperature
d. Watery Stool
    i. Collect about 3mls of watery stool sample from patient in a clean sterile container (container MUST be disinfectant and detergent free)
    ii. Using a sterile swab stick pick adequate amount of the stool and dip in the Caryblair medium or APW
e. Rectal swab:
    i. Remove the wrapper from the end of the sterile swab and do not contaminate/touch the cotton end of the swab

ii. Dip the cotton tip of the swab stick in alkaline peptone water
iii. Carefully insert the moist/wet swab stick into the anus, rotate gently to obtain enough faecal material
iv. Carefully withdraw the swab stick from the anus and dip the swab stick in the Caryblair medium or APW.

f. The swab should be pushed to three quarter of the medium (without touching the container)
g. Using a clean pair of scissors, carefully cut off the top portion of the swab stick
h. Close tight the cover lid on Cary Blair tube to prevent leakage
i. Label the specimen container legibly, with patient's first and last name, type of specimen, date and time of collection and collector's initials
j. Seal all caps with Parafilm and transport samples to laboratory for processing[25]

How to collect cerebrospinal fluid[26].

a. Three tubes (1 ml each) of CSF should be collected for microbiology, chemistry, and cytology. If health facilities lack the ability to do chemistry, two tubes are sufficient. If only one tube of CSF is available, it should be sent for microbiology. Where specimen transport for microbiology will take over one hour away from collection point, the contents of the tube should be transferred to T-I medium
b. Standard bio-safety precautions apply to all steps in lumbar puncture procedure.
c. Gather all materials from the CSF collection kit and a sharps container for used needles. Wear surgical mask and sterile latex or nitrile gloves that are impermeable to liquids and change gloves between every patient
d. Positively identify the patient by checking the name and hospital number and comparing this with the request form
e. Ensure that the patient is kept motionless during the lumbar puncture procedure, either sitting up or lying on the side (for children, it is done preferably lying on the side with the body arched), with his or her back well arched so that the head almost touches the knees in order to separate the lumbar vertebrae during the procedure. Aim for maximum flexion of the

spine (curl into fetal position), but avoid over flexing the neck, as this may cause respiratory compromise

f. Disinfect the skin along a line drawn between the posterior superior iliac crests with 70% alcohol and povidone-iodine to clean the surface and remove debris and oils. Allow to dry completely

g. Imagine or draw an imaginary line between the top of the iliac crests. This intersects the spine at approximately the L3-4 inter-space. Position the needle in the midline, re-orientate such that the needle is parallel to the bed and perpendicular to the back slightly aiming towards umbilicus in direction, with the bevel pointing towards the ceiling (when in lateral decubitus position) or to the side (when sitting)

h. Advance the needle into the spinous ligament (increased resistance). Continue to advance the needle within the ligament until there is a fall in resistance. Remove the stylet. If CSF is not obtained replace the stylet and advance the needle slightly then recheck for CSF

i. Remove CSF (1 ml minimum, 3-4 ml if possible) and collect into sterile screw-cap tubes. If 3-4 ml CSF is available, use 3 separate tubes and place approximately 1ml into each tube. DO NOT COLLECT CSF INTO A SYRINGE, use screw cap bottles only

j. Withdraw the needle and cover the insertion site with sterile gauze and adhesive tape. Discard the needle in a sharps box

k. Remove mask and gloves and wash hands with antibacterial soap and water immediately after removing gloves

l. Inoculate CSF in T-I, a biphasic medium that is useful for the primary culture of etiological agents of bacterial meningitis from CSF. T-I media should be stored at 4°C and warmed to room temperature (25°C) before use. Label the T-I bottle with appropriate information: patient name, date and time of CSF inoculation, and Epid ID number

m. Use sterile forceps to pull the aluminum cover of a T-I bottle away from the rubber stopper (Do not completely remove the aluminum cover) and disinfect the stopper with 70% alcohol. Allow to dry. DO NOT use povidone-iodine as it may be carried into the medium by the passing needle and would inhibit growth of bacteria

n. Use a sterile syringe and needle to inoculate 0.5-1.0 ml of CSF into the T-I medium. The remaining CSF should be kept in the collection tube. It should

not be refrigerated but should be maintained at room temperature (20-25°C) before Gram staining and other tests (where available). Discard the needle and syringe in a sharps box

o. After inoculation, invert the T-I bottle several times to mix. If transport to the designated testing laboratory is expected to be delayed for more than a day, insert a venting needle (sterile cotton-plugged hypodermic needle) through the rubber stopper of the T-I bottle, which will encourage growth and survival of the bacteria. Be sure that the venting needle does not touch the broth. Incubate the inoculated T-I medium at 35-37°C in a candle-jar overnight or until transport is possible. If transportation is delayed for more the 4 days, remove the vented T-I bottle from the incubator or candle jar and place at room temperature until shipment. Remove the venting needle and wipe the rubber stopper with 70% alcohol before shipping

P. If the T-I bottle can be transported to the testing laboratory the same day, do not vent the bottle until it arrives in the receiving laboratory. If there is no T.I. media, incubate the specimens (with screwcap loosened) at 35-37°C in a candle-jar may improve bacterial survival

Appendix 4: Patient sample request form

Patient identification form sample

Hospital number ……

Names ……..

Sex: Male Female

Date of birth …….

Age …….. Months (if < 1 year) ……..……

Date of admission …….

Previous hospitalisation ……..

Hospital department……

Outpatient/inpatient …….

Hospital ward ……...

Previous use of antimicrobial agents ……..……

Diagnosis …….

Specimen information

Blood Stool CSF

Date of sample collection …….

Appendix 5: Specimen Processing

Specimen processing includes both microscopy and culture. Specimens should be processed in a timely manner. Microorganisms are labile, and improper handling of specimens prior to processing can result in death of fastidious pathogens or the overgrowth of contaminating bacteria present in the specimen.

Materials

a. Culture media
b. Pre-cleaned slides
c. Sterile swabs, sterile inoculating loops, sterile applicator sticks, sterile transfer pipettes
d. Bunsen burner

Safety Precautions

Scientists should comply with the following policies for safety in specimen processing:

a. Wear laboratory gown and gloves when handling clinical samples
b. Process meningococcal and other samples where aerosols are likely to be produced inside the Biological Safety Cabinet (BSC)
c. Wipe spills immediately with disinfectant and disinfect the bench after each batch of specimens have been processed
d. Discard contaminated loops, sticks, swabs and other materials in bucket containing disinfectant (0.5% hypochlorite)
e. Discard slides used in direct smears in sharps container. Treat slides as infectious.
f. Autoclave processed specimens and other contaminated materials (swabs, loops, sticks, etc.) before discarding

Media and Slides

a. Use pre-cleaned slides with frosted end
b. Use pencil for marking slides
c. Use permanent marker for labeling media
d. Do not cover the name/batch number of the media when labeling the plates

e. Prior to use, check media expiry date. Do not use media agar that show these signs: hemolysis, excessive bubbles, contamination, cracked or damaged plates, and frozen or melted.

Specimen Processing Guidelines (an outline)

a. Process specimens as soon as they arrive
b. When multiple specimens arrive at the same time, priority should be given to those that are most critical: cerebrospinal fluid (CSF), blood, and sterile fluids
c. Stool, wound drainage and other non-sterile specimens can be processed last. Keep specimens in the refrigerator, at 4º to 8ºC when delay in processing is anticipated.
   Note: NEVER REFRIGERATE SPINAL FLUID, BLOOD, GENITAL, EYE, OR INTERNAL EAR SPECIMENS
d. When a specimen is received with multiple requests, but the amount of specimen is insufficient to do all of them, call the clinician to prioritize the testing
e. Prior to processing, check that the specimen and requisition match. Verify tests ordered. Record and initial on the laboratory book the date and time of sample collection and on the lab duplicate form the date and time of processing/plating.
f. Prepare required materials. Label slides and media with lab accession #, specimen, and date
g. Examine specimen and select portion to be taken for smear and inoculation to culture media. Prepare smear (if required). Heat-fix and Gram stain smears; read and report results
h. Invert inoculated plates; place broths in a tube rack. Arrange plates and tubes in numerical order
i. Incubate plates and broths (with loose caps) in appropriate condition
j. Store processed specimens appropriately. File requisitions in binder

Gross Examination of Specimen

Infection gives rise to purulence (abundant PMN), blood, necrosis, and mucus (mucucs membrane specimens.

a. Examine the specimen for presence of pus (yellow to tan purulence), blood

(red to rust-colored), mucus (clear and tenacious), and necrosis (brown to black discoloration of tissues). Use these areas of the specimen for smear preparation and for inoculation to culture media

b. For stool samples, describe if watery or rice-watery, and indicate presence of blood or mucus
c. Record these observations on the requisition so that all scientists working on the sample will know the results of the gross examination

Specimen Preparation

Many specimens require some form of initial treatment before inoculation onto primary plating media.

a. Centrifuge large volumes (>2ml) of sterile fluids such as CSF at 2,000 rpm for 10 minutes. Pipette all but 2 ml of supernatant from the original container. Gently mix sediment using sterile pipette. Use the suspension for inoculation
b. If fluid is clotted, grind the clotted material in 2-3ml Normal Sterile Saline (NSS); this will disperse the clots and release any trapped bacteria. Use the emulsion for inoculation
c. Keep all remaining emulsions and supernatant in sterile 15-ml screw-cap tubes. Store at 4oC to 8oC

Direct Microscopic Examination

Perform direct microscopic exam (e.g. Gram stain, potassium hydroxide (KOH), India ink) only on appropriate specimens.

Appendix 6: Antimicrobial susceptibility testing

Following examination of the stained smear and culture incubation, culture plates are interpreted for bacterial growth. Considerable judgement is required to decide what organism to look for and report. It is essential to recognize what constitutes indigenous (normal) flora and what constitutes a pathogen. Indiscriminate reporting of normal flora can contribute to unnecessary use of antibiotics and potential emergence of resistant organisms.

One of the most widely used approach to antimicrobial susceptibility testing in routine clinical laboratories remains disk diffusion. No special equipment is necessary, most bacterial organisms, including fastidious type can be tested and against a wide range of antibiotics. Use the **15-15-15 minute rule**: use the inoculum suspension within 15 minutes of preparation, apply disks within 15 minutes of inoculation and incubate plates within 15 minutes of disk application.

Preparation and storage of media

a. Prepare agar (as appropriate for organism) according to the manufacturer's instructions, with supplementation for fastidious organisms. The medium should have a level depth of 4±0.5 mm. Calculate the correct volume, based on the true dimensions of the Petri dish per manufacturer's guidelines
b. Ensure that the surface of the agar should be dry before use. No drops of water should be visible on the surface of the agar or inside the lid. If necessary, dry plates either at 20-25°C overnight, or at 35°C, with the lid removed, for 15 min. Do not over-dry plates. Store plates prepared in-house at 4-8°C
c. Determine plate drying, storage conditions and shelf life should be determined as part of the laboratory quality assurance programme for all agar plates. Label plates. Commercially prepared plates should be stored as recommended by the manufacturer and used within the labelled expiry date
d. Store all agar plates in plastic bags or sealed containers. This is to avoid excess moisture, which may result in problems with fuzzy zone edges and/or haze within zones

Preparation of inoculum

a. Use a sterile loop or a cotton swab to pick colonies from an overnight culture on nonselective media. Use several morphologically similar colonies (when possible) to avoid selecting an atypical variant. Suspend the colonies in saline and mix to an even turbidity
b. Adjust the density of the organism suspension to McFarland 0.5 by adding saline or more bacteria. A denser inoculum will result in reduced zones of inhibition and a decreased inoculum will have the opposite effect
c. Use the direct colony suspension method to make a suspension of the organism in saline to the density of a McFarland 0.5 turbidity standard. The direct colony suspension method is appropriate for all organisms, including fastidious organisms
d. Compare the density of the suspension visually to a McFarland 0.5 turbidity standard. To aid comparison, compare the test and standard against a white background with black lines
e. Suspend Streptococcus pneumoniae from a blood agar plate, preferably to the density of a McFarland 0.5 standard. When Streptococcus pneumoniae is suspended from a chocolate agar plate, the inoculum must be equivalent to a McFarland 1.0 standard
f. Use the suspension within 15 min and always within 60 min of preparation. A photometric device may be used to adjust the density of the suspension. The photometric device must be calibrated against a McFarland 0.5 standard according to the manufacturer's instruction.

Procedure for Streaking Plates for Isolation

The process described below is called streaking for isolation, because the microorganisms present in the specimen are successively diluted out as each quadrant is streaked until finally each morphotype is present as a single colony. Technique for streaking in 3 quadrants is employed:

a. Use sterile transfer pipettes (for fluids) or sterile swabs (for solids) for inoculation of media and preparation of smear
b. Always select the most purulent or blood-tinged portion of the specimen to inoculate media and prepare smear
c. Inoculate the least selective medium first to prevent carryover of inhibitory substances

d. Apply inoculum onto plates by swabbing a 2-cm area or placing a drop of liquid specimen onto one quadrant. Using the loop, cross-streak the original inoculum onto ¼ of the plate with a back and forth motion several times and without entering the area that was previously streaked (Q1)
e. Turn the plate a quarter turn. Flip over the loop. Pass the loop through the edge of the first quadrant approximately four times while streaking into the second quadrant. Continue streaking into the second quadrant without going back to the first quadrant (Q2)
f. Finally, rotate the plate another quarter turn and repeat the above procedure until one more additional quadrant is streaked (Q3). Spread the inoculum evenly over the entire agar surface ensuring that there are no gaps between streaks

Procedure for Culture Reading

a. Read all cultures on a daily basis, ideally early in the morning
b. Ensure that all reagents and supplies are available on the workbench before starting.
c. Record and initial all observations and workup on the specimen workcard

Day 1 (after overnight incubation)

a. Remove culture plates and broths from the incubator
b. Arrange cultures in numerical order. Read cultures in numerical order. Read new cultures (24-hour) before old cultures (48-, 72-hour)
c. Perform initial examination of growth on each media type. Record observations on the culture worksheet (work card)
d. Perform initial identification tests on potential pathogen (e.g., Gram stain, oxidase, indole, catalase, and coagulase). Record workup on worksheet. If there is insufficient growth for testing, subculture colony to plated media (label plate as purity plate or pp)
e. Set-up definite identification tests (e.g. Microbact, serotyping) and antimicrobial susceptibility testing (AST) from pure culture. Record workup on work card.
f. Compare direct Gram-stained smear and culture results
g. Reincubate all primary and subculture plates for an additional 24 or 48 hours, as appropriate

Day 2 and succeeding days

a. Follow-up identification and susceptibility testing results until all relevant isolates have been identified. Record all results on the work card
b. Issue final report including organism identification and AST results
c. Freeze isolates
d. Save culture plates

Initial Examination of Primary Plates

a. Observe growth on primary culture plates. Note different types of colonies on each plate
b. Differentiate normal flora from pathogens. Quantitate growth and record on culture worksheet. Normal flora, if present may be collectively quantified (e.g., Few normal flora)
c. Observe for isolated colonies of organism that do not appear to be part of the normal flora (potential pathogen). Quantitate and describe colony. Record observations on worksheet
    i. Describe gross morphology of colony (e.g., small, large colonies; lactose-fermenter, non-lactose fermenter, H2S+ (black), mucoid, swarming)
    ii. Note presence of hemolysis (alpha, beta, gamma)
    iii. Check for pigment production (e.g., green, yellow, red)
    iv. Describe characteristic odor (e.g., fruity, bleach)

Warning: Do not directly smell colonies; odor will be obvious just by opening the plate.

v. Perform Gram stain of colonies if needed to aid in identification.
d. If there are more than 1 potential pathogens, assign a number for each isolate (#1, #2); describe and workup separately, as appropriate

Media Inoculation for AST

a. Ensure that agar plates are at room temperature prior to inoculation. Optimally, use the adjusted inoculum suspension within 15 min of preparation. The suspension must always be used within 60 min of preparation. Sterilize the wire loop for 5-10 seconds. Allow to cool. Disposable loops may be used
b. Dip a sterile cotton swab into the suspension. Streak all plates with a four-way inoculation streak

c. Remove excess fluid by pressing and turning the swab against the inside of the tube for Gram-negative bacteria ONLY, to avoid over-inoculation. For Gram-positive bacteria, do not press or turn the swab against the inside of the tube.

N.B: Plates can be inoculated by using an automatic plate rotator.

Application of antimicrobial disks

a. Open antimicrobial disks and check expiry date on the container
b. Allow disks to reach room temperature before opening cartridges or containers used for disk storage. This is to prevent condensation, leading to rapid deterioration of some agents
c. Apply disks within 15 min of media inoculation. If inoculated plates are left at room temperature for prolonged periods of time before the disks are applied, the organism may begin to grow, resulting in erroneous reduction in zone of inhibition diameters
d. Apply disks firmly to the surface of the inoculated agar plate within 15 minutes of inoculation. Disks must be in close and even contact with the agar surface and must not be moved once they have been applied as the initial diffusion of antimicrobial agents from disks is very rapid
e. Limit the number of disks on a plate to avoid overlapping of zones and interference between agents. It is important that zone diameters can be reliably measured. The maximum number of disks possible on a 90mm agar disk is 6 and on a 150mm is 12 disks
f. To detect inducible clindamycin resistance in staphylococci and streptococci, the erythromycin and clindamycin disks must be placed at a distance of 12-20 mm from edge to edge for staphylococci and 12-16 mm from edge to edge for streptococci
g. Store disks, including those in dispensers, in sealed containers with a desiccant and protected from light and according the manufacturers' instructions. Once disk containers have been opened, use within the time limit specified by the manufacturer
h. Perform frequent quality control to ensure that antimicrobial disks have not lost potency during storage

Incubation of plates

a. Invert agar plates and make sure disks do not fall off the agar surface. Incubate plates within 15 min of disk application. If the plates are left at room temperature after disks have been applied, pre-diffusion may result in erroneously large zones of inhibition
b. Do not stack plates in the incubator as this may affect results due to uneven heating, a maximum of five plates per stack is appropriate for most incubators. Incubation beyond the recommended time limits should not be performed as this may result in growth within inhibition zones and reporting isolates as false resistant
c. Examine plates after incubation. A correct inoculum and satisfactorily streaked plates should result in a confluent lawn of growth. If individual colonies can be seen, the inoculum is too light, and the test must be repeated. The growth should be evenly distributed over the agar surface to achieve uniformly circular inhibition zones

Table 7: Incubation conditions for antibiotic susceptibility plates

Organism	Incubation conditions
Enterobacteriaceae, *Pseudomonas spp.* *Acinetobacter spp.* *Staphylococcus spp.* *Streptococcus pneumoniae* *Haemophilus influenzae*	35±1°C in air for 16-20 h
Other fastidious organisms	Pending

Most bacteria will grow at 35o to 37oC.

a. Aerobes grow in ambient air, which contains 21% O2 and small amount (0.03%) of CO2
b. Anaerobes grow in anaerobic jars, bags or chambers composed of 5% to 10% hydrogen (H2), 5% to 10% CO2, 80% to 90% nitrogen (N2), and 0% O2
c. Capnophiles (e.g., *H. influenzae, N. gonorrheae*) require increased concentration of CO2 (5% to 10%), and approximately 15% O2. This atmosphere can be achieved by using a candle jar (3% CO2), or a CO2 incubator, or jar with the appropriate Gas Pak
d. Microaerophiles (*C. jejuni, H. pylori*) grow under reduced O2 (5% to 10%) and increased CO2 (8% to 10%). This environment can be obtained using microaerophilic jars

Measurement of diameter zones and interpretation of susceptibility testing

a. For antimicrobial agents, the zone edge should be read at the point of complete inhibition as judged by the naked eye with the plate held about 30 cm from the eye
b. Read un-supplemented plates from the back with reflected light and the plate held above a dark background. Read supplemented plates from the front with the lid removed and with reflected light
c. Measure the inhibition zone diameters to the nearest millimetre with a ruler or a calliper. If an automated zone reader is used, it must be calibrated to manual reading
d. Interpret zone diameters into susceptibility categories according to the latest version of breakpoint tables (CLSI or EUCAST). If templates are used for interpreting zone diameters, the plate is placed over the template and zones interpreted accordingly to breakpoints marked on the template
e. If cultures are pure, colonies within zones should be considered when measuring the diameter

f. Do not use transmitted light (plate held up to light) or a magnifying glass, unless when reading linezolid susceptibility tests on staphylococci from the back with the plate

Quality control

a. Conduct quality control routinely using QC strains specified to monitor the performance AST in the laboratory. Generally typical susceptible strains are recommended, but resistant strains can also be used to confirm that the method will detect resistance mediated by known resistance mechanisms
b. Obtain QC strains from culture collections or purchase from commercial sources
c. Store control strains on glass beads at -70°C in glycerol broth (or commercial equivalent) is a convenient method. Non-fastidious organisms can be stored at-20°C.
d. Store two vials of each control strain should be stored, one as an in-use supply and the other as an archive for replenishment of the in-use vial when required
e. Subculture a bead from the in-use vial each week, onto appropriate non-selective media and check for purity. From this pure culture, prepare one subculture on each day of the week. Subculture fastidious organisms daily for no more than one week. When subculturing a control strain, use several colonies to avoid selecting a mutant
f. Check that results for control strains are within acceptable ranges in CLSI or EUCAST QC tables. Repeat testing of EUCAST quality control strains should yield zone diameter values randomly distributed within the recommended ranges
g. Use the recommended routine quality control strains to monitor test performance Control tests should be set up and checked daily, or at least four times per week for antibiotics which are part of routine panels
h. Examine the results of the last 20 consecutive tests for trends and for zones falling consistently above or below the target. If two or more of 20 tests are out of range, investigation is required
i. Test each new batch of Mueller-Hinton agar to ensure that all zones are within range
j. To control the inhibitor component of β-lactam-β-lactamase inhibitor combination disks, specific β-lactamase-producing strains are recommended

Table : Priority organisms quality control strains

Organism	Strain	Characteristics
Escherichia coli	ATCC 25922 NCTC 12241 CIP 7624 DSM 1103 CCUG 17620 CECT 434	Susceptible, wild-type
Escherichia coli	ATCC 35218 NCTC 11954 CIP 102181 DSM 5564 CCUG 30600 CECT 943	TEM -1 ß -lactamase, ampicillin resistant
Klebsiella pneumoniae	ATCC 700603 NCTC 13368 CCUG 45421 CECT 7787	ESBL-producing strain (SHV-18)
Pseudomonas aeruginosa	ATCC 27853 NCTC 12934 CIP 76110 DSM 1117 CCUG 17619 CECT 108	Susceptible, wild type
Staphylococcus aureus	ATCC 29213 NCTC 12973 CIP 103429 DSM 2569 CCUG 15915 CECT 794	Weak β-lactamase producer
Enterococcus faecalis	ATCC 29212 NCTC 12697 CIP 103214 DSM 2570 CCUG 9997 CECT 795	Susceptible, wild type
Streptococcus pneumoniae	ATCC 49619 NCTC 12977 CIP 104340 DSM 11967 CCUG 33638	Reduced susceptibility to benzylpenicillin
Haemophilus influenzae	ATCC 49766 NCTC 12975 CIP 103570 DSM 11970 CCUG 29539	Susceptible, wild type
Klebsiella pneumoniae	ATCC 700603 NCTC 13368 CCUG 45421 CECT 7787	ESBL-producing strain (SHV-18)
Staphylococcus aureus	NCTC 12493	*mecA* positive, hetero-resistant MRSA
Enterococcus faecalis	ATCC 51299 NCTC 13379	High-level aminoglycoside resistant (HLAR) and

Enterococcus faecalis	CIP 104676 DSM 12956 CCUG 34289	vancomycin resistant (*vanB* positive)
Haemophilus influenzae	ATCC 49247 NCTC 12699 CIP 104604 DSM 9999 CCUG 26214	Reduced susceptibility to β-lactam agents due to PBP mutations (ß-lactamase negative, ampicillin resistant, BLNAR)

Appendix 7: Specimen retention, isolate retention and reporting results

Specimen Retention

a. Keep all processed specimens, including fluid supernatants and tissue emulsions, at 4o to 8oC. Discard after 7 days
b. Keep all rejected specimens at 4o to 8oC. Discard after 7 days
c. Keep all gram stained smears in a slide box. Discard after 7 days
d. Discard specimens in biohazard bags; discard gram stain slides in sharps container
e. Specimens from non-sterile sites (e.g., urine, stool) are normally held only to resolve labelling problems; collect new specimens when re-testing is required

Retention of Culture Plates

It is useful to save the plates of completed cultures that grew pathogens for investigation of possible errors and allowing physicians the opportunity to call to request further identification or antimicrobial testing when clinically indicated.

a. For each culture, select a primary plate with the best growth of the identified pathogen. Also include the purity plate, if any
b. Keep plates in the refrigerator. Plan to subculture isolate for transfer to the national AMR-NRL for re-testing. Afterwards, store or discard plates as per laboratory's policy

Reporting of Results

a. All specimen workup details must be recorded chronologically using the worksheet. Once culture is completed, file worksheets in a designated binder
b. Record AST results using the WHONET software
c. Issue preliminary and final report on each specimen. Prepare the final report and submit to supervisor(s) for review and signature. The final report should be checked against the worksheet as accuracy check. Release reports to authorized personnel only

Result Feedback to Clinicians

a. Document all patient sample results in the laboratory report form
b. For positive samples, record the name of the bacteria and the appropriate

Antibiogram should be reported as sensitive, intermediate or resistant according to CLSI or EUCAST standards

c. Negative result should be reported if there is no growth after 5 days of incubation.

d. All results should be communicated immediately to the clinician on call with the laboratory report form

Appendix 8: Bacterial isolate transportation

Isolate storage

a. Short-term storage may be accomplished with transport media, freezing, or, in some cases (and for some pathogens) at room temperature on simple media plus mineral oil to prevent drying.
b. Long-term storage of bacterial isolates is best accomplished by either lyophilization or freezing. Bacterial cultures may be stored frozen or lyophilized in a variety of suspending media formulated for that purpose. Generally, serum-based media, skim milk, or polyvinylpyrrolidone (PVP) medium is used for lyophilization, and skim milk, blood, or a rich buffered tryptone soy broth (TSB) with 15%–20% reagent-grade glycerol is used for freezing.
c. Dorset Egg medium (DE) is useful for room temperature (i.e., approximately 25°C) storage of S. pneumonia. Use overnight growth from blood or chocolate agar, as appropriate, to inoculate a 4-ml DE slant in a 7-ml screw-top tube
d. Frozen cultures should be thawed at room temperature, and a Pasteur pipette should be used to remove a small amount of inoculum from the cryotube for culture.
e. Blood agar, tryptone soy agar (TSA), and heart infusion agar (HIA) are examples of good storage media for enteric organisms. Carbohydrate-containing media (e.g., Kligler iron agar [KIA] or triple sugar iron agar [TSI]) should not be used because acidic by-products of metabolism quickly reduce viability of the organisms
f. When preparing storage medium, place tubes of medium that are still hot after autoclaving in a slanted position to provide a short slant and deep butt (2–3 cm).
g. Other bacterial species can also be stored frozen in a -70°C freezer or in liquid nitrogen (at -196°C). To store frozen isolates, use a sterile swab to prepare dense suspensions of 18- to 24-hour pure cultures prepared in TSB containing 20% (vol/vol) glycerol.
h. Transportation / Temperature conditions:
   i. Isolate in Nutrient Agar Slant - Room Temperature
   ii. Isolate in Dorset Medium - Room Temperature
   iii. Isolate in Skimmed Milk Broth – Cold Chain

Transportation of Isolates to the National AMR-NRL

a. Package isolates, label using hospital ID and isolate ID
b. Fill necessary accompanying documents: chain of custody form, booking form in the and place in Ziploc bag/envelop, NOT INSIDE the giostyle
c. Record shipment info in sample referral register
d. Call TRANEX to confirm time of sample pick-up. TRANEX courier arrives with shipment seal. Properly and finally seal the package, after confirming giostyle content and accompanying document with Tranex staff
e. Hand over the packaged isolated to the Tranex staff at the pick-up for transportation to the designated National reference laboratory. Document time and date of pick up. TRANEX seals the shipment, fills the waybill, booking form and chain of custody
f. Request for copies of the waybill, booking form and chain of custody. Courier should transport isolates to AMR NRL(s) within 48 hours

Receipt of isolates at national AMR-NRL

a. Lab should be informed that sample is on the way, isolates should reach AMR NRL(s) within 48 hours
b. Sample is received at Lab reception area. Remember to use safety precautions. Confirm waybill, booking form and chain of custody are all available
c. Weigh package and record isolate in the register and confirm acceptance. Document any non-conformity
d. Collect copies of chain of custody form, booking form and waybill forms. Collect giostyle and packaging consumables
e. The NRL focal person should document on the referral register, Tranex staff should countersign entry
f. Fill the sample courier template on 1st-2nd of each month, commenting on the courier service for the month. Share with AMR-NRL

Appendix : Annual denomination form for GLASS implementation

Name of institution…….

Address of institution……..

Number of in-patient and out-patient departments available in the facility

……

Listing of departments…….

Minimum number patients received by institution annually

…….

Number of in-patient beds in the facility ……

Average occupancy per annum …….

Enrolment of laboratories in the Global Antimicrobial Surveillance System

Reporting AMR data by laboratory to GLASS during the national call

Participation in EQA programme by laboratory for bacterial identification

Participation in EQA programme by laboratory for AST

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