National AMR surveillance guideline

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NATIONAL AMR PROGRAM

DEPARTMENT OF MEDICAL SERVICES, MOH

DEPARTMENT OF LIVESTOCK, MOAF

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1 Table of Content

2 ACRONYMS

AH	Animal Health
AMC	Antimicrobial consumption
AMR	Antimicrobial resistance
AMU	Antimicrobial usage
BAFRA	Bhutan Agriculture and Food Regulatory Authority
CLSI	Clinical and Laboratory Standards Institute
CRRH	Central Regional Referral Hospital, Gelephu
DoL	Department of Livestock
DRA	Drug Regulatory Authority
DVH	District Veterinary Hospital
EIDL	Enteric & Invasive Disease Laboratory
EML	Essential Medicines List
ERRH	Eastern Regional Referral Hospital, Mongar
ESBL	Extended spectrum beta-lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization
GLASS	Global Antimicrobial Surveillance System
HH	Human health
JDWNRH	Jigme Dorji Wangchuck National Referral Hospital
MoAF	Ministry of Agriculture and Forests
MoH	Ministry of Health
NBCH	National Biorepository Center for Human Health
NCC	National Coordinating Center
NCAH	National Center for Animal Health
NFTL	National Food Testing Laboratory
NRL	National Reference Laboratory
OH	One Health
OIE	World Organization for Animal Health
PGH	Phuntsholing General Hospital, Phuntsholing
RCDC	Royal Center for Disease Control
RLDC	Regional Livestock Development Center
RVL	Regional Veterinary Laboratory
WHO	World Health Organization

3 CHAPTER 1: AMR SURVEILLANCE SYSTEM AND GOVERNANCE

3.1

1. Antimicrobial Resistance in Bhutan

The discovery of antimicrobials has transformed the practice of medicine in human and animal health. The availability and use of antibiotics also contributed to progress in public health, animal health, food safety and security. However, these immeasurable benefits are under enormous threat from the emerging and increasing antimicrobial resistance (AMR) which have spread worldwide. Multidrug resistance in organisms most commonly result from the overuse and misuse of antimicrobial agents. Due to these, there is an urgent global call to prevent, control and manage AMR.

AMR is a global problem and Bhutan is equally affected by its emergence and spread. In Bhutan, primary healthcare, including medicines, is provided free of cost by the state as outlined in the country's constitution. The national essential medicines list (EML) outlines the categories of medicines (including antibiotics) that are supplied to different levels of hospitals. As a result of this, there are very limited antibiotics on the EML, especially in lower level health centers. In addition, there are comparatively fewer private pharmacy shops in the country, and they deal mostly with medicines that are not included in the EML. The Drug Regulatory Authority (DRA) of Bhutan monitors drug quality, controls private pharmacies and legally antibiotics can only be sold against a medical prescription. The livestock department also have antibiotics control mechanisms in place such as; restricting over the counter sale of antibiotics to few listed antibiotics, allowing antibiotic dispensing only against a prescription and banning antibiotics in animal feeds.

Despite these mechanisms put in place to limit irrational use of antibiotics, there are reports indicating inappropriate use of antibiotics including higher generation antibiotics to as high as 56% in clinical practice. A review of uro-pathogens from the national referral hospital found that resistance against commonly used antibiotics was as high as 70%. Another study in *Neisseria gonorrhea* isolates revealed resistance against nalidixic acid, ciprofloxacin, penicillin, and tetracycline of >70%. In the livestock sector, two ESBL (CTX-M-15) producing E. coli strains were isolated amongst 83 fecal samples from breeding pigs. In Salmonella isolates from imported chicken carcasses, resistance was highest for nalidixic acid (96%) followed by amoxicillin (12%), cephalexin (6%), ciprofloxacin (2%) and sulphamethoxazole-trimethoprim (2%). About 2% of the isolates showed intermediate resistance against chloramphenicol While no resistance was detected against gentamicin.

These reports, although limited, reveal increasing concern of antibiotic resistance in the country. Further, the use of antibiotics in Bhutan is increasing with at least 30% of the total annual medicines budget spent on procurement of antibiotics although they constitute only 10% of the total medicines on EML. With the increasing use of antibiotics in both human and livestock sector and inappropriate use contributing towards antibiotic resistance, a formal AMR surveillance guideline is the need of the hour as part of a joint global effort to combat AMR.

2. Current gaps in AMR surveillance of AMR in Bhutan

In the current system, microbiology laboratories in HH and AH conduct routine isolation and

antibiotic susceptibility testing of clinical samples and issue clinical reports to the respective clinicians and veterinarians. Rarely, some research related to AMR are carried out in both the sectors. However, there have been very limited studies and data related to AMR and no systems of routine AMR surveillance with centralized data collation and analysis. In addition, there is no system of collecting antimicrobial use and antimicrobial consumption in Bhutan. There is also limited number of skilled human resource and no formal national AMR surveillance guidelines.

3. Implementation of AMR Surveillance

This guideline will facilitate AMR surveillance in Bhutan both in the human and livestock sectors. All human health (HH) and animal health (AH) microbiology laboratories will become surveillance sites for national AMR surveillance coordinated by the National Coordinating Center (NCC) for AMR, based in the Ministry of Health.

With this guideline, AMR surveillance including antibiotics usage and consumption, will be conducted through a coordinated mechanism in both the HH and AH health centers. There will be extensive collaboration, resource and expertise sharing, and integrated and harmonized protocols (where relevant) between all laboratories in the country. All data on AMR should be uniform, reliable and a true representation of the respective institution and form an integral part of the comprehensive national AMR data. The data may be partially analyzed at institutional level for local/clinical use and wholly submitted to the National disease surveillance and epidemiology (NDSE) unit at the RCDC for final analysis to be shared, archived and used in national strategies and plans.

In addition, the national reference laboratories (NRLs) will participate in the WHO Global Antimicrobial Surveillance System (GLASS) for LMICs at the least and actively pursue higher and comprehensive resistance detection through continuous capacity building in terms of technology and human resources. The NRLs will also support the other surveillance site laboratories to develop core microbiological standards and take part in the GLASS reporting system by building a comprehensive network of surveillance sites for a high-quality representative national AMR data in the long run.

4. Legal and ethical considerations

Legally, this guideline takes authority from several government documents endorsed by the cabinet such as the Bhutan One Health Strategy Plan (2018-2023), National Action Plan on AMR (2018-2022), Livestock Act (2001), Medicines Act of the Kingdom of Bhutan (2005) and Bhutan Medicine Rules and Regulations (2012). In addition, committees (IMCOH, NATC), working groups (HH and AH TWGs) and designated laboratories with their endorsed ToRs are responsible to execute the tasks of AMR surveillance.

Surveillance activities in public health are usually mandated by the national government where use of individual patient data without individual consent are justified when the probability and the magnitude of harm to the population arising from not reporting surveillance data are moderate to major. In AMR surveillance, reporting the characteristics of resistant pathogens rarely presents a threat to patient confidentiality. However, the inclusion of simple clinical data such as age, sex, collection date, sources of samples, specimen type and syndromic diagnosis adds considerable value without to the information obtained from the laboratory, and there are clear benefits from AMR surveillance at patient, animal, food, pathogen and community levels. Therefore, the activities prescribed in this guideline does not breach any legal and ethical considerations of the human patients, animal subjects, food and

environmental samples.

However, given the need to integrate data from different sources (human, animal, food and environmental samples), it is essential that there are data governance agreements and procedures in place, especially when AMR surveillances activities are carried out as specific projects and data needs to be shared with multiple parties including external agents. Having such agreements should protect the confidentiality of individual patients but also facilitate the effective sharing of AMR surveillance data to inform policy locally, nationally and internationally. To meet ethical obligations, all technical, legal and/or political barriers to data sharing must be overcome through proper data management plan.

5. Scope of the document

This guideline encompasses all aspects of AMR in HH and AH including antibiotic usage (AMU) and antibiotic consumption (AMC). It will guide all the AMR surveillance site engaged in public health, animal health and food safety.

While recognizing that AMR also occur in viruses, fungi and parasites, this guideline shall focus only on bacterial pathogens in humans, animals and food products for now. In the long term, as part of a One Health approach and as AMR surveillance systems progresses, environmental aspects of AMR should become an integral part of the continuing surveillance activities.

AMR surveillance will be carried out in the WHO/OIE/FAO prioritized bacterial pathogens and other bacterial isolates encountered in the clinical/surveillance samples. The antibiotic panels to be tested against each bacterial isolate will be specific to the HH or AH sectors.

6. Objectives of this guideline

This guideline has the primary objective of supporting microbiology capacity development in a standardized manner, promoting good laboratory practices and generation of a uniform, useful and reliable national AMR data in the country.

Specifically, this guideline is intended to:

6.1 Establish a uniform, reliable and effective AMR, AMU and AMC surveillance system in all HH and AH microbiology facilities in the country;
6.2 Provide a roadmap for improving laboratory capacity for detection of AMR, data collection and surveillance for AMR, AMC and AMU with an effective One Health approach;
6.3 Promote cross-policy collaboration and enhance surveillance activities adopting a One Health approach to understand the emergence, transmission and dissemination of pathogens at the human-animal interface;

7. AMR Surveillance System in Bhutan

7.1 National Action Plan

Understanding the seriousness and urgency of the threat of AMR, Bhutan has the National Action Plan (NAP) (2018 – 2022) to combat AMR in the country. The NAP has clear strategies incorporating all aspects of AMR control, prevention, monitoring and research through a One Health approach. The Ministry of Health (MoH) and the Ministry of

Agriculture and Forests (MoAF) are the joint custodians and the relevant departments/programs under the two ministries are the implementing agencies of the NAP.

7.2 Governance and structure

AMR surveillance is an important activity of the NAP. This will be carried out with a concerted and organized manner through a proper governance structure **(figure).** Accordingly, the AMR program under the MoH will function as the National Coordinating Center (NCC). The NCC is technically supported by the AMR focal persons from HH and AH. The National Antimicrobial Technical Committee (NATC), with members from MoH, MoAF and other medical and health committees, was designated as the highest-level committee on AMR. However, the NATC reports to the Inter-Ministerial Committee for One Health (IMCOH), which is the highest committee between the MoH and the MoAF. The NATC will be technically supported by Technical Working Groups (TWGs) of human and animal health, that would have separate or joint sittings as relevant to the task in hand.

7.3 National Coordinating Center

The National AMR Program in the Ministry of Health will function as the National Coordinator Center (NCC) for AMR. The NCC will function as per the ToR.

7.4 External organizations

External organizations will include the World Health Organization (WHO), World Animal Health Organization (OIE), Food and Agriculture Organization (FAO) and any other international stakeholders and funding bodies such as the Fleming Fung Grant. The MoH and MoAF will collaborate with these external organizations independently or through the NCC when relevant to carry out activities related to AMR surveillance.

7.5 AMR surveillance sites

There will be designated AMR surveillance sites both in the AH and HH. These sites will have their mandates as per the ToR.

7.6 Training

To promote AMR surveillance, education and training should be integrated into national education programs across all the disciplines involved in AMR surveillance. These include clinical, laboratory, information technology and public health training. Teaching on AMR should be introduced into formal training pathways, including undergraduate and postgraduate curricula across these disciplines.

AMR awareness should be developed through continuing professional development (training, workshops) at site, regional, national, and international levels. Such training should incorporate e-learning options and specific training modules. To enhance motivation, surveillance sites should consider appointing individuals with specific roles to act as AMR surveillance champions in clinical (including infection prevention and control), laboratory and data services.

4 Chapter 2: AMR SURVEILLANCE IN HUMAN HEALTH

1. Introduction

This chapter will deal with laboratory surveillance of AMR in Human Health (HH) and clinical healthcare facilities. The activities will be carried out through the designated NRLs (JDWNRH and RCDC) and the surveillance site microbiology laboratories.

2. Rationale

AMR surveillance in humans is important to understand the pattern of resistance in bacterial pathogens causing infections in the country. Treatment and control of infectious are complicated by emergence of several drug resistant bacterial pathogens such as Extended spectrum beta-lactamases (ESBLs), Carbapenem resistant enterobacteria (CRE), Methicillin resistance *S. aureus* (MRSA), and fluoroquinolone resistant *Shigella sonnie*. AMR surveillance is also critically important to measure accurate resistance patterns as well as changes in those patterns over a period.

AMR surveillance in clinical cases is useful in detecting shifts in susceptibility of various organisms to antibacterial agents and guide local therapy in treating emerging resistant pathogens, develop policy and alert the scientific community of potential growing threats and to help steer antimicrobial research and development aimed reducing or preventing AMR.

3. Objectives

The overall objective of AMR surveillance in human health and clinical environment is to detect resistance in pathogens for adequate patient management and for public health activities. Specifically, surveillance have the following objectives:

3.1 Collect, process and report pathogens isolated from clinical/environmental samples by trained and competent laboratory staff
3.2 Generate adequate and uniform data on antibiotic resistant bacteria of interest
3.3 Perform analysis of clinical and laboratory surveillance data
3.4 Provide timely antibiograms to clinicians for patient management in hospitals and data to the AMR program for national planning and policy interventions
3.5 Participate and share national AMR data through global and regional surveillance system (e.g. GLASS)

4. Types of AMR surveillance to be conducted in human health sector

AMR surveillance in Human will comprise of three important components for the comprehensive understanding of the situation of AMR in the country.

4.1 Routine (Passive) Clinical surveillance of AMR
4.2 Active surveillance of AMR in clinical settings
4.3 Hospital environment surveillance

4.1 Routine (Passive) Clinical Surveillance of AMR

Routine clinical surveillance will involve the collection of clinical and epidemiological data with microbiological samples from patients through routine hospital services. Samples will be processed as per routine microbiological procedure and every bacterial isolate will be subjected to antibiotic susceptibility testing (AST). Data will be analyzed at the participating surveillance sites and at national level for its use for different purpose.

This routine surveillance will involve various agencies under the Ministry of Health including the RCDC, the referral hospitals and other hospitals with microbiology facilities.

4.1.1 Surveillance sites

All HH microbiology laboratories will be designated as surveillance sites for routine AMR surveillance in clinical settings. Currently, all the referral hospitals and hospitals with microbiology facility are designated as the surveillance sites. In addition, the Enteric and Invasive Diseases Laboratory (EIDL) of the RCDC with its sentinel sites for food-borne disease surveillance (designated hospitals) will also function as surveillance site for enteric pathogens. Any hospital that establishes microbiology culture and AST facility in the future shall also be designated as the new surveillance sites.

These laboratories will perform sample collection, pathogen isolation, identification and antibiotic susceptibility tests. Methods followed in all laboratories should be according to international guidelines and harmonized to enable uniform results, easier monitoring and interpretation of laboratory results. These sites have their respective ToRs for AMR surveillance as per the NAP and other AMR documents.

4.1.2 Reference laboratories

The Microbiology Unit, Department of Pathology and Laboratory Medicine of the JDWNRH will function as the reference laboratory for all bacterial pathogens except enteric pathogens. For enteric pathogens, the EIDL of the RCDC will serve as the national reference laboratories for AMR surveillance. These reference laboratories should provide reference service for all priority bacterial pathogens and provide technical assistance, including training of laboratory staffs on bacterial identification, antibiotic susceptibility testing and laboratory quality assurance and other tasks as listed in their ToR.

4.1.3 AMR data analysis and management

The National Disease Surveillance and Epidemiology (NADSAE) Unit at RCDC is identified as the AMR data management center for HH. The center should routinely collect and validate AMR data reported by surveillance sites and other responsibilities as listed in the ToR.

4.1.4 Technical components of routine AMR surveillance in hospitals

4.1.4.1 Sampling Frame

All HH microbiology laboratories under the Ministry of Health will take part in the AMR surveillance. The sampling frame can be expanded as and when new microbiology facility develops at a hospital.

4.1.4.2 Clinical Surveillance

Clinical information will be collected in the Microbiology sample request form that should be filled in by Physicians, nurses and other health professionals responsible for collection and transport of microbiological samples.

An extended surveillance data may be conducted by trained laboratory staff/data assistants for any patients when a multi-drug resistant bacterial pathogen is detected from a patient using standardized form for additional clinical and epidemiological information.

4.1.4.3 Clinical specimen collection, storage and Transport

The investigation for AMR surveillance includes various clinical specimen collected from patients for bacterial culture and sensitivity along with clinical information. Suggestive specimen to be collected for the surveillance include, but not limited to, blood, cerebrospinal fluid (CSF), sputum, urine, stool, urethral/cervical swabs, pus, wound swabs, other body fluids and discharge. All samples received, from both out and in patients, for bacterial culture and susceptibility testing should be included in the surveillance.

Microbiological samples should be collected, transported and processed as per the individual Standard Operating Procedures (SOPs).

4.1.4.4 Isolate identification

Identification of isolated pathogens should be carried out with the standard procedures. Each laboratory should be able to identify a minimum of the priority bacterial pathogen from clinical specimen received in the laboratories. Bacterial identification should be carried out using the standardized internationally acceptable SOPs. Isolates with unusual or uncertain identification should be referred to the NRLs for advanced identification using advanced technique, including automated identification system and molecular method.

4.1.4.5 Target organisms

AMR surveillance should ideally be carried out for every isolate. However, due to technical and resource challenges there will be a list of targeted organisms against which the laboratories should built their expertise. These targeted organisms are selected based on the national priority considering the recommendations of the WHO, OIE and FAO. The suggested list of bacterial pathogens for AMR surveillance in human in Bhutan is given below. (Table 1).

Table 1: Priority pathogens for AMR surveillance in human health

Specimen	Pathogens
Blood	*Staphylococcus aureus* *Escherichia coli* *Klebsiella pneumoniae* *Acinetobacter spp.* *Pseudomonas aeruginosa* *Streptococcus pneumoniae* *Salmonella spp.* *Hemophilus influenzae* *Other enterobacteria*
Sputum samples	*Staphylococcus aureus*

	Escherichia coli *Klebsiella pneumoniae* *Acinetobacter spp.* *Pseudomonas aeruginosa* *Streptococcus pneumoniae* *Hemophilus influenzae* *Other enterobacteria*
Cerebrospinal fluid (CSF)	*Staphylococcus aureus* *Escherichia coli* *Klebsiella pneumoniae* *Acinetobacter spp.* *Hemophilus influenzae* *Streptococcus pneumoniae* *Salmonella spp.* *Other enterobacteria*
Urethral discharge	*Neisseria gonorrhea*
Urine	*E. coli* *Klebsiella pneumonia* *Proteus vulgaris* *Staphylococcus saprophyticus*
Fecal sample	*Salmonella spp.* *Shigella spp.* *Diarrheagenic E. coli* *Campylobacter spp.*

In addition to above mentioned pathogens, laboratory should also identify other pathogenic organism from any samples submitted to the laboratory for diagnostic purpose.

4.1.4.6 Antimicrobial susceptibility testing

Antimicrobial susceptibility tests will be undertaken to assist the clinician in selecting the most appropriate antimicrobial to use in the treatment of an individual patient suffering from infection. All the isolates should be subjected for antimicrobial susceptibility testing using internationally accepted technique such as CLSI or EUCAST guideline with regular quality assurance being incorporated into the system.

Report for AST must include zone of diameter, rather than just interpreting as susceptible, intermediate and resistant, and should be entered and stored into WHONET or other form of electronic database. The result should also be disseminated to the clinicians within the turnaround time (TAT) for treatment purpose.

Whenever uncertainties in the results of identification and AST is encountered, the isolates should be referred to the NRL for confirmation. At NRLs, these isolates should be subjected for automated susceptibility testing systems. In addition, NRLs may use molecular methods to detect resistant genes in priority pathogens. E.g. *mecA* genes in MRSA.

4.1.4.7 Isolate storage and referral

All Isolates should be retained so that they are available for comparison and should be dispatched to RCDC for storage, future reference and further studies. While referring the

isolates, appropriate medium should be used, and temperature should be maintained as per the SOP. All isolates referred should be deposited, after performing confirmatory tests, at National Biorepository Center for Health (NBCH), RCDC as per the existing SOPs and National Bio-banking Guideline and a using electronic inventory system.

4.2 Active surveillance of AMR

Active surveillance of AMR should be carried out in patients suspected of being colonized with a multi-drug resistant organism such as MRSA and VRE. This may be established as screening protocols in hospitals especially in patients who are scheduled to undergo major surgeries or admitted into intensive care units.

4.2.1 Surveillance sites

Active surveillance is recommended for screening all patients at the time of admission in hospitals where specialist clinical services are provided.

4.2.2 Sampling frame

The sampling frame for the active AMR surveillance are all the patients admitted at the surveillance sites designated by Ministry of Health.

4.2.3 Clinical and epidemiological surveillance

The clinical data on the clinical information form and laboratory request form should be used for data collection from every patient admitted in the hospital as per the protocol or SOP. Detailed (additional) information should be collected from all admitted patients in the surveillance sites.

4.2.4 Laboratory procedures

For active surveillance, similar strategy of routine surveillance and laboratory process should be utilized. Based on the target organisms for active surveillance, appropriate sample should be collected.

4.2.4.1 Specimen

Admitted and referred patients from other hospitals should be screened at time of admission by taking nasal, throat, groin, axillary swabs or other relevant samples as required. Additionally, fecal samples should be collected from all diarrheal patients admitted.

4.2.4.2 Isolate identification and susceptibility testing

Swabs should be cultured using standard microbiological methods or available standard protocol to assess presence of target organisms for active surveillance. Antimicrobial susceptibility testing should be performed on all isolated pathogens to determine resistance patterns using standard methods.

4.2.4.3 Target organism

Recommended multi-drug resistant bacteria surveillance are extended spectrum β-lactamase producers, methicillin-resistant *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii*, vancomycin-resistant enterococcus and carbapenem-resistant enterobacteriales.

4.2.4.4 Isolate referral and storage

Like routine AMR surveillance, isolates detected and identified as target organism for active surveillance should be stored and referred to NBC at RCDC on regular basis. Refer National Guideline for Biorepository of Human Health for detailed guidance.

4.3 AMR Surveillance in Hospital Environment

Hospital environment surveillance is essential for monitoring the presence of pathogenic organisms in the hospital environments. This is especially important in critical clinical awards such as patient wards, operation theaters and intensive care units. This is required since most admitted patients are vulnerable to hospital-acquired infections due compromised immune system from highly resistant pathogens. In Bhutan, MDR pathogens encountered in hospital environment may include MRSA, MDR *Pseudomonas* species, Acinetobacter, Enterobacter and *Klebsiella pneumoniae* of variable prevalence. Although, *Clostridium difficile* is not tested and documented, its presence in the hospital cannot be excluded and ignored.

Hospital environmental sampling for AMR should be undertaken as part of an outbreak investigations specific to different units of the hospital or carried out at intervals as a routine monitoring or research activities. The results obtained from such activities should be shared with the respective wards and units, the Infection control unit and the hospital administration for prevent and control intervention.

5 CHAPTER 3: AMR SURVEILLANCE IN LIVESTOCK, FOOD ANIMALS AND ANIMAL PRODUCTS

6 Introduction

AMR is a global threat and effect human and animal health equally. With the emergence and spread of antimicrobial-resistant pathogens, decades of progress in prevention and control of infectious diseases in animals and humans is under threat. Veterinary Services play a critical role in building awareness of AMR and encouraging the prudent use and management of antimicrobial medicines in animals and food products which have direct implications on human health and the environment.

This chapter will deal with laboratory surveillance of AMR in Animal Health (AH) including livestock, healthy food animals and food products. The activities will be carried out through the designated animal health laboratories under the Department of Livestock and the National Food Laboratory under the Bhutan Agriculture and Food Regulatory Authority (BAFRA), discussed below in two separate sections;

1) Clinical surveillance of AMR in livestock

2) AMR surveillance in healthy food animals and animal products

1. Clinical surveillance of AMR in livestock

The section will provide guidance on AMR surveillance in pathogens recovered from clinically or sub-clinically diseased livestock, poultry and companion animals. The guideline is aligned with the regional guidelines to ensure the comparability of the finding across the region. It focuses on providing harmonized AMR surveillance and antimicrobial susceptibility testing methods for common bacterial pathogens isolated from diseases terrestrial animals. This surveillance will provide an estimate of the prevalence of AMR in pathogen bacteria isolated from clinically or sub-clinically diseased animals at the animal health center level. The information generated through this surveillance will also help in developing treatment guidelines for the common pathogens and promote evidence-based treatments in lieu of unwarranted methylases and broad-spectrum preventive treatment.

6.1 Objectives

6.1.1 To detect resistant bacterial pathogen in animals reported to animal health facilities
6.1.2 To measure the prevalence of antimicrobial resistance at the point of animal health facilities
6.1.3 To monitor and predict AMR trends with respect to time, populations, geography and other risk factors.
6.1.4 To provide information for veterinarians to inform their treatment decisions;
6.1.5 To guide the planning, implementation and evaluation of programs and policies related to AMR

6.2 Target population

The target population is the entire set of units for which the surveillance data are to be used to make inferences. Target populations must be specifically defined, as the definition determines whether sampled cases are eligible or ineligible for the surveillance.

The target population for AMR surveillance is clinically or sub-clinically diseased livestock, poultry and companion animals that may or may not be reported to Livestock Extension Centers, Dzongkhag Veterinary Hospitals, Thromde Veterinary Hospitals and National Veterinary Hospital for availing veterinary services.

6.3 Surveillance sites/areas

The areas in which the samples are collected for surveillance are defined as the surveillance site. The surveillance sites are the areas from which the clinically or sub-clinically diseased livestock, poultry and companion animals may be presented to animal health centers included in study for availing veterinary services. The animal health centers include Livestock Extension Centers, Dzongkhag Veterinary Hospitals, Thromde Veterinary Hospitals and National Veterinary Hospital.

6.4 Source population

A source population is a subset of a target population. It is a smaller population within a larger target population from which a sample is drawn. Conceptually, all units in the source population should be listed and have a non-zero probability of being included in the study. The source population for clinical AMR surveillance is the clinically or sub-clinically

diseased livestock; poultry and companion animals reported to animal health facilities for availing veterinary services in surveillance sites.

6.5 Sampling frame

The sampling frame is defined as the list of all the sampling units in the surveillance area. It is the information about the source population that enables you to draw a sample. The sampling frame for clinical AMR surveillance are the list of all animal health care facilities, private animal clinics and animal welfare organization listed under the Department of Livestock and CSOA. After health care facilities are selected, a sampling strategy is devised for selecting the animals within those health care facilities.

6.6 Sample source

A source population is a subset of a target population. It is a smaller population within a larger target population from which a sample is drawn. Conceptually, all units in the source population should be listed and have a non-zero probability of being included in the study. The source population for clinical AMR surveillance is the clinically or sub-clinically diseased livestock; poultry and companion animals reported to animal health facilities for availing veterinary services in surveillance sites.

6.7

6.8 Sampling strategy

The objective of a study will influence the sampling strategy employed. The sampling strategy should ensure that the sample is representative of the population of interest and meets the objective of the surveillance. An appropriate sampling strategy needs to be developed prior to collection of samples specifying the number of animals or other sampling units to be sampled. The sampling plan will include the sampling methods and sample size including the method of sample collection.

6.8.1 Sample size

The sample size should be large enough to determine the prevalence or trends in existing and emerging antimicrobial resistance phenotypes. The sample should avoid bias and be representative of the animal population, process, product or other units of interest whilst taking into account the expected prevalence of the bacteria in the sample type, the expected prevalence of the resistance phenotype and the desired level of precision and confidence.

The sample size calculation should be based on independent samples. However, if there is any clustering at the establishment or animal level, the sample size should be adjusted accordingly. At low levels of expected prevalence, exact methods of sample size calculation should be preferred to approximate methods.

Since samples from which bacteria are not isolated cannot be used in the calculation of the prevalence of the resistance phenotype, a consideration should be given during sample size determination. The number of isolates required to estimate the prevalence of resistance amongst the isolates for a fixed level of confidence varies with the expected prevalence and the desired level of precision, as shown in Table below. Highest numbers of samples are required to estimate prevalence levels of 50% for a given precision; if a more precise estimate is required, the sample size increases.

Table: Number of isolates required to estimate the prevalence of resistance to a specific antimicrobial in a particular bacterial species with a 95% confidence level, for two levels of precision (5% and 10%). (Extracted from OIE Terrestrial Animal Health Code10).

Expected AMR Prevalence	Number of bacterial isolates needed	
	Desired precision	
	10%	5%
10%	35	138
20%	61	246
30%	81	323
40%	92	369
50%	96	384
60%	92	369
70%	81	323
80%	61	246
90%	35	138

*Note that Table indicates the number of bacterial isolates required for the estimation of prevalence of resistance in that bacterial genus or species, not the number of animals sampled. Based on the surveillance objective and sampling method deployed, a study sample will be selected from the clinically or sub-clinically diseased livestock, poultry and companion animals visiting the selected animal health facilities.

6.8.2 Sampling timetable

A sampling timetable should be prepared showing the number of samples to collect from each surveillance site by date. The timetable needs to ensure that the days of sample collection, numbers of sample collected, and the frequency of sampling match the laboratory's capacity to process the samples.

6.9 Sample type

Based on the sampling strategy, sampling may be from individual animals, from a group of animals, or from the environment depending on the purpose and objective of the surveillance. To provide scientifically and statistically valid results the samples collected must be appropriate and representative for the intended purpose of testing and technologies to be used. The volume or quantity of samples must be sufficient to perform initial testing, to perform any subsequent confirmatory testing and to provide sufficient residual sample for a referral or archival purposes. In order to prevent contamination of the samples, appropriate biosafety and containment measures must be followed during sampling. For the clinical surveillance, based on the species and clinical signs the following samples should be preferred and should be collected as per the SOP:

- Faeces
- Milk
- Swabs from secretion and excretions

6.10 Sample processing, transport and storage

All the samples should be collected following the sample collection method outlined in the sampling strategy. All samples must be placed inappropriately secured containers and must be transported safely, timely, efficiently and legally from the surveillance sites to designated

laboratories. Considerations such as the speed at which a sample is frozen or chemically preserved, size and density of the material to be preserved, storage container and media, and also protocols for reconstitution, thawing, and reviving agents will be given depending on the type of samples.
The method of preserving samples at the collection sites will depend on the type of samples and their intended testing. Considerations for preserving the integrity of the samples must be given priority to protect from desiccation, frequent or extreme temperature fluctuations, humidity, contamination, and the potential for loss of identification and associated archival documentation.
Storage conditions should be managed to maintain the properties of the samples to the maximum extent possible.

6.11 Target organisms

The selection of target organism will be based on the surveillance objectives and country's need and preferences. Wherever possible, all four WHO priority bacteria should be included in the surveillance programme. However, if resources do not allow this, the following order of priority for inclusion is recommended:

6.11.1 Escherichia coli
6.11.2 Salmonella spp.
6.11.3 Enterococcus faecium and E. faecalis
6.11.4 Campylobacter spp

Further, all clinical isolate will be subjected to AST. The other isolates that are multidrug resistance will also be reported.

6.12

6.13 Antimicrobial Panel

(each sector to present on the antimicrobial panel during the joint TWG meeting and panels should be decided. Furthermore, it was agreed to keep it as common topic).

Given that the AMR surveillance is ultimately for the protection of public health, an expert committee consisting of clinicians, researchers and veterinarians should recommend the panel of antimicrobials for AST which should be approved by National AMR Technical Committee. The expert committee should be guided by the following:

- WHO Guidance on classification of critically important antimicrobials, 2016
- OIE guidance on list of antimicrobial agents of veterinary importance, 2019
- National Essential Medicine list, Ministry of Health
- Essential Veterinary Medicine List, Ministry of Agriculture
- Recent research & publications (national & International)
- Recommendations of other relevant International Organizations (CLSI, EUCAST, FAO, CAC)

6.14

2. AMR surveillance in healthy food animals and animal products

In this guideline, the chapter covers on the AMR surveillance from the major healthy food animal species including broilers, swine, cattle fish and their products (i.e. chicken, meat pork, beef, milk, fish etc.). Such surveillance provides an unbiased estimate of the national prevalence of AMR at the farm level (or at the retail level) for different bacterial/antimicrobial combinations. The information obtained from this type of surveillance will be important for understanding the epidemiology of AMR in the food chain and for monitoring the impact of antimicrobial usage in animals in addition to providing meaningful information that should guide evidence-based actions to address AMR. Furthermore, the data can be used for risk analyses for both human and animal populations for the evaluation of interventions and has the potential to transform policies and practices. With objectives primarily centered on protecting public health, surveillance of AMR in bacteria from healthy animals intended for food consumption involves active monitoring of AMR in apparently healthy food-producing animals and in animal food products.
This section will provide guidance on the design, planning, implementation, and data application relevant to monitoring AMR in bacteria from apparently healthy animals intended for human consumption. The AMR surveillance in food animal and their products will be prioritized based on the availability of resources.

6.15 Objectives

6.15.1 To assess and determine the source and presence of resistant bacteria in food animal and their products.
6.15.2 Evaluate the temporal and spatial pattern and the emergence of new antimicrobial resistance in food animals and their products
6.15.3 Institute active surveillance for antimicrobial resistance in animals and their products.
6.15.4 Create a national biorepository of bacterial isolates from the animal origin at the national reference laboratory.

6.16 Target population

The target population is the population to which it might be possible to extrapolate results from the study. The target population for AMR surveillance in food animals are semi commercials and commercials farms of cattle, pig, poultry and fish in the country.
Similarly, the target population for food of animal origins are domestically produced livestock products of dairy, piggery, poultry and aquaculture.
For imported products, the target population will be meat products such as beef, pork, chicken and fish. The AMR surveillance in major food animals and their products includes:

Food Animals

- Poultry – Layer, Broiler, Turkey.
- Dairy animals: Cattle
- Pigs
- Goats/ Sheep

Aquaculture

- Fish
- Crustaceans

Animal products

- Dairy products
- Eggs

- Meat and meat products

6.17 Surveillance sites

The areas in which the samples are collected for surveillance are defined as the surveillance site. The surveillance will target district which has semi-commercial and commercial farms with higher animal population, major livestock production and easy access to antimicrobials. When considering the selection of surveillance sites for AMR surveillance, criteria such as the transboundary movement of animals, food, people and antimicrobials across the border will be considered. For food of animal origin, the surveillance site will target district which has slaughterhouse, meat storage facility, and retail meat shop, milk processing unit and dairy outlets.

6.18 Source population

The source population is the population from which the study subjects are drawn. Conceptually, all units in the source population should be listed and have a non-zero probability of being included in the study. The source population for AMR surveillance in food animals are semi-commercial and commercial farms of major food animals in the surveillance sites.
The source population for food of animal origin are slaughterhouse, retail meat shop, meat storage facility, dairy processing unit outlets in the surveillance sites.

6.19 Sampling frame

The sampling frame is defined as the list of all the sampling units in the source population and it should contain the information about the source population that enables you to draw a sample. The sampling units are the basic elements of the population that is sampled (e.g. herds, animals). The sampling frame for food animals is a list of semi-commercials and commercial farms of dairy, piggery, poultry, and fishery farms registered with DoL or BAFRA in the source population.
For food of animal origin, the sampling frame includes list of slaughter-house, retail meat shop, and meat storage facility, dairy processing unit and dairy outlets registered with BAFRA in the source population.

6.20 Sampling strategy

The objective of a study will influence the sampling strategy employed. The sampling strategy should ensure that the sample is representative of the population of interest and meets the objective of the surveillance. An appropriate sampling strategy needs to be developed prior to collection of samples specifying the number of animals or other sampling units to be sampled. The sampling plan will include the sampling methods and sample size including the method of sample collection. When developing the sampling strategy for food of animal origin intended for consumption, different steps of the food chain, including processing, packing and retailing, should also be considered.

6.20.1 Sample size

The sample size should be large enough to determine the prevalence or trends in existing and emerging antimicrobial resistance phenotypes. The sample should avoid bias and be representative of the animal population, process, product or other units of interest whilst

considering the expected prevalence of the bacteria in the sample type, the expected prevalence of the resistance phenotype and the desired level of precision and confidence.
The sample size calculation should be based on independent samples. However, if there is any clustering at the establishment or animal level, the sample size should be adjusted accordingly. At low levels of expected prevalence, exact methods of sample size calculation should be preferred to approximate methods.
Since samples from which bacteria are not isolated cannot be used in the calculation of the prevalence of the resistance phenotype, a consideration should be given during sample size determination. The number of isolates required to estimate the prevalence of resistance amongst the isolates for a fixed level of confidence varies with the expected prevalence and the desired level of precision, as shown in Table below. Highest numbers of samples are required to estimate prevalence levels of 50% for a given precision; if a more precise estimate is required, the sample size increases.
Table: Number of isolates required to estimate the prevalence of resistance to a specific antimicrobial in a bacterial species with a 95% confidence level, for two levels of precision (5% and 10%). (Extracted from OIE Terrestrial Animal Health Code10).

Expected AMR Prevalence	Number of bacterial isolates needed	
	Desired precision	
	10%	5%
10%	35	138
20%	61	246
30%	81	323
40%	92	369
50%	96	384
60%	92	369
70%	81	323
80%	61	246
90%	35	138

*Note that Table indicates the number of bacterial isolates required for the estimation of prevalence of resistance in that bacterial genus or species, not the number of animals sampled.

6.20.2 Type of samples:

The most appropriate samples from the different species and environment will be collected. When selecting the type of samples for collection, the additional information such as age categories, types of production, and patterns of antimicrobial use over time must be considered. Accordingly, the following listed samples type's needs to be considered.

- ✓ Dairy: Feces and/or bulk milk samples/
- ✓ Swine: Feces/carcass
- ✓ Poultry: caeca for broiler, caeca, fecal and boot swab for layers
- ✓ Fish: whole Fish
- ✓ Food products: Fresh milk, beef, chicken, pork, and fish will be collected at different stages of food chain.

6.21 Target organisms

The surveillance will target at least the WHO priority pathogens including the main zoonotic and commensals organisms. The commensal organism such as *E. coli* and Enterococci should

be considered in surveillance as it serves as indicators organism in providing information on the potential reservoir of antimicrobial resistance genes, which may be transferred to pathogenic bacteria. All clinical isolate will be subjected to AST. The other isolates that are multidrug resistance will also be reported.
The list of target organism is not exclusive, and more animal species and bacteria can be added based on evidence and laboratory capacity.

Sl. no	**Animal species/ animal products**	**Target bacteria**
1	Poultry (and eggs)	*E. coli, Salmonella, Enterobacter, Campylobacter*
2	Cattle/ Buffalo (and milk)	*Pasteurella spp., E. coli, Staphylococci spp., Streptococcus spp., Salmonella spp.*
3	Pigs	*E. coli, Salmonella, Streptococcus suis, staphylococci*
4	Sheep/Goat	*E. coli, Staphylococci spp., Streptococcus spp., Salmonella spp.*
6	Aquaculture (Fish)	*Salmonella spp., Vibrio parahaemolyticus, Listeria monocytogenes.*

6.22 Sample processing, transport and storage

All the samples should be collected following sample collection method outlined in the sampling strategy. All samples must be placed in appropriately secured containers and must be transported safely, timely, efficiently and legally from the surveillance sites to designated laboratories. Considerations such as speed at which a sample is frozen or chemically preserved, size and density of the material to be preserved, storage container and media, and also protocols for reconstitution, thawing, and reviving agents will be given depending on the type of samples.
The method of preserving samples at the collection sites will depend on type of samples and their intended testing. Considerations for preserving the integrity of the samples must be given priority to protect from desiccation, frequent or extreme temperature fluctuations, humidity, contamination, and the potential for loss of identification and associated archival documentation.
Storage conditions should be managed to maintain properties of the samples to the maximum extent possible.

6.23 Antimicrobial Panel

Given that the AMR surveillance is ultimately for the protection of public health, an expert committee consisting of clinicians, researchers and veterinarians should recommend the panel of antimicrobials and approved by National AMR Technical Committee. The expert committee should be guided by the following:

- WHO Guidance on classification of critically important antimicrobials, 2016
- OIE guidance on list of antimicrobial agents of veterinary importance, 2019
- Essential drug list, Ministry of Health
- Essential Veterinary Medicine List, 2018
- Recent research & publications (national & International)
- Recommendations of other relevant International Organizations (CLSI, EUCAST, FAO, CAC)

6.24 Processing, transport and Storage

All the samples should be collected following sample collection method outlined in the sampling plan. Samples must be placed in appropriate secure containers and must be transported safely, timely, efficiently and legally from the surveillance sites to designation laboratories. Considerations such as speed at which a sample is frozen or chemically preserved, size and density of the material to be preserved, storage container and media, and protocols for reconstitution, thawing, and reviving agents should vary with different sample and agents.

The method of preserving samples at the collection sites should depend on type of samples and their intended testing. Considerations for preserving the integrity of the samples must include protection from desiccation, frequent or extreme temperature fluctuations, humidity, contamination, and the potential for loss of identification and associated archive documentation. Storage conditions should be managed to maintain viability, biochemical, and immunological properties of the samples to the maximum extent possible.

6.25 Laboratories

The AMR National reference laboratory is the apex laboratory that has capacity to promote and facilitate good laboratory practices and AMR surveillance at the country level. The National Center for Animal Health at Serbithang is identified as the AMR National reference laboratory.

The AMR national reference laboratory shall be supported by the surveillance laboratories located in different regions and districts (RLDCs and DVH).

Regional surveillance laboratories will coordinate and carryout AMR surveillance in liaising with surveillance sites within their region. The regional laboratories will conduct isolation, identification and AST and will be supported by district veterinary hospital for sample collection, processing, storage and transportation.

District veterinary hospital shall also supervise and monitor AMR at the district level.

National Food Testing Laboratory (NFTL) is surveillance laboratory for AMR surveillance in foods of animal origin in the country and has capacity to promote and facilitate good laboratory practices. NFTL will conduct identification, isolation and AST and will coordinate with the BAFRA field staff for sample collection and transportation to the lab. NFTL will liaise with AMR national reference laboratory for carrying out AMR surveillance in foods of animal origin

The AMR National reference laboratory is the apex laboratory that has capacity to promote and facilitate good laboratory practices and AMR surveillance at the country level. The National Center for Animal Health at Serbithang is identified as the AMR National reference laboratory for AH.

The AMR national reference laboratory shall be supported by the surveillance laboratories located in different regions and districts (RLDCs and DVHs).

Regional surveillance laboratories should coordinate and carryout AMR surveillance in liaison with surveillance sites within their region. The regional laboratories should conduct isolation, identification and AST and should be supported by district veterinary hospital for sample collection, processing, storage and transportation.

District veterinary hospital shall also supervise and monitor AMR at the district level.

National Food Testing Laboratory (NFTL) is surveillance laboratory for AMR surveillance in foods of animal origin in the country and has capacity to promote and facilitate good laboratory practices. NFTL should conduct identification, isolation and AST and should

coordinate with the BAFRA field staff for sample collection and transportation to the lab. NFTL should liaise with AMR national reference laboratory for carrying out AMR surveillance in foods of animal origin

6.26 Laboratory Methods

6.26.1 Sample Processing, Isolation, Identification and AST

All the samples should be collected following sample collection method outlined in the sampling plan. All samples must be placed in appropriate secure containers and must be transported safely, timely, efficiently and legally from the surveillance sites to designation laboratories. Considerations such as speed at which a sample is frozen or chemically preserved, size and density of the material to be preserved, storage container and media, and also protocols for reconstitution, thawing, and reviving agents will vary with different sample and agents.

The method of preserving samples at the collection sites will depend on type of samples and their intended testing. Considerations for preserving the integrity of the samples must include protection from desiccation, frequent or extreme temperature fluctuations, humidity, contamination, and the potential for loss of identification and associated archive documentation. Storage conditions should be managed to maintain viability, biochemical, and immunological properties of the samples to the maximum extent possThe samples are processed to isolate and identify target/WHO priority organisms based on standard bacterial isolation and identification methods. Depending on the capacity, laboratory may use automated systems including molecular methods. The isolates should be subjected to quality-controlled AST in line with international standards such as CLSI or EUCAST methodology and guidance. The results should be interpreted and reported as per the SOPs.

7

7.1.1 Isolates storage and referral

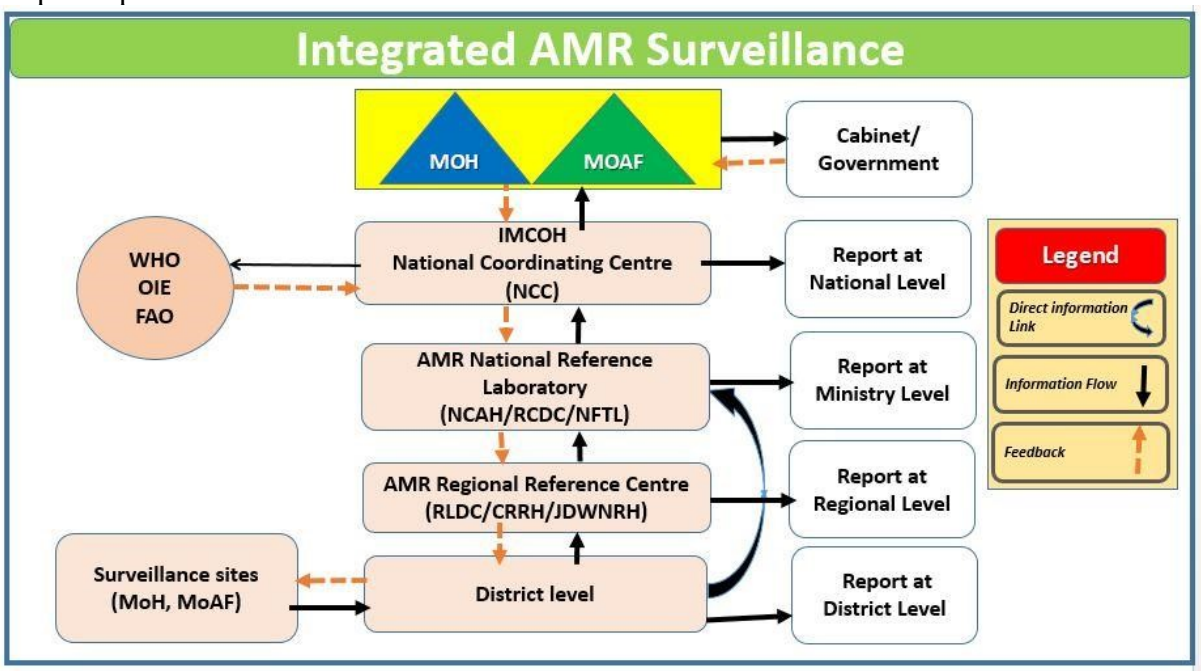
The bacterial isolates should be permanently preserved. The methods of storage must ensure viability, safety against loss because of contamination and cross-contamination, absence of changes in the strain properties and absence of phenotypic drift because of genetic instability. The surveillance laboratories should store all the isolates in overgrown agar slant or semi-solid medium at 4^{o}C degree and refer to AMR national reference laboratory at the earliest. The AMR national reference laboratory should store all the isolates as per the isolate storage protocol.

7.2 Data management & Reporting

The management of AMR surveillance data and analysis is crucial for the monitoring of AMR program in the country. The reporting of results requires efficient data management at all levels (i.e surveillance sites, districts, regional and national). All data related to AMR and AMU surveillance at all levels should be managed and reported.

Once the samples are received at the surveillance sites, the data entry should be done using the standard database. The data can be further collated and disseminated to the different levels as per the level of information required. Ideally, at every stage of data management and reporting, there should be quality control system in place with automated data validity checks for consistency, completeness and accuracy of data. A data confidentiality and security measures should be in place at all levels to generate authentic results, which can be used as basis for making science-based policy decision.

The information related to AMR and AMU data will flow from the surveillance sites to district, regional and national level which will be collated and submitted to National Coordinating Center (NCC) for the country level data aggregation and onward reporting to international levels as shown in the figure.
For verification and validation of the data at different levels, a feedback mechanism should be put in place at all levels.



The epidemiological data analyses of various antibiogram patterns will be managed using WHONET which is an analytical tool that facilitate understanding of the local epidemiology of microbial populations and selection of antimicrobial agents. The analysis of laboratory findings includes isolate line listings, antimicrobial susceptibility test statistics, and studies of multidrug resistance pattern and hospital & community outbreak detection. The data should be collected at surveillance sites and analyzed at regional and national level.

8 CHAPTER 4: SURVEILLANCE OF ANTIMICROBIAL USE AND CONSUMPTION

In the surveillance of AMR, accounting for antibiotic use by types and consumption by amount is important in addition to the microbiological laboratory monitoring of AMR. Increasing laboratory detection of AMR will result in the increased prescription of higher generation antibiotics.

As part of AMR surveillance, antimicrobial use (AMU) and antimicrobial consumption (AMC) will be assessed both in HH and AH. This chapter will describe AMU and AMC in two sections;

1) Surveillance of AMU and AMC in HH
2) Surveillance of AMU and AMC in AH

1. Surveillance of AMU and AMC in human health

1.1 Background & Scope

AMR is a complex problem with many interrelated causes. Inappropriate use of antimicrobials and lack of surveillance systems are core contributors to the spread of AMR. Other factors influencing AMR, such as poor infection prevention and control in healthcare facilities and lack of available, inexpensive and rapid diagnostic tests, are also important factors.

It is likely that inappropriate use of antibiotics is widespread; however, information on antibiotic use and consumption is scarce in low- and middle-income countries. In order to inform effective policies and interventions that optimize use and promote equitable access to medicines, it is essential to have surveillance on the current situation of antibiotic use/ consumption.

In the 2017 revision of the WHO Model List of Essential Medicines, antibiotics in the list were grouped into the AWaRe categories: Access, Watch and Reserve. The Access category includes first and second choice antibiotics for the empirical treatment of common infectious syndromes and they should be widely available in health care settings. Antibiotics in the Watch category have a higher potential for resistance to develop and their use as first and second choice treatment should be limited. Finally, the Reserve category includes “last resort” antibiotics whose use should be reserved for specialized settings and specific cases where alternative treatments have failed.

A common methodology to survey antibiotic use and consumption encourages standardization and facilitates comparisons of antibiotic use over time and between hospitals, districts, countries, and regions

1.2 Definition for Antimicrobial use and consumption

In this guideline, the term consumption refers to estimates of aggregated data or quantitative data, mainly derived from import, sales or reimbursement databases. Aggregated data on antimicrobial consumption, often collected for administrative purposes, are usually easily accessible and can serve as a proxy for actual use of antibiotics, for which data collection is often more laborious.

The term antibiotic use refers to data on antibiotics taken by the individual patients. Data are collected at the patient level, which allows a more comprehensive set of data to be gathered, such as information on indication, treatment schemes and patient characteristics. In general, the collection of data on antibiotic use requires more resources but provides additional information on prescribing practices, which is important for guiding antimicrobial stewardship activities.

Data on consumption and use each serve specific purposes and complement rather than replace each other.

1.3 Objectives

- Identify and provide an early warning of problems related to changes in antimicrobial exposure and use, and develop interventions to address the problems identified
- Monitor the outcomes of interventions
- Assess the quality of prescribing in terms of adherence to practice guidelines
- Raise awareness among health professionals, consumers and policy-makers about the problems of the inappropriate use of antimicrobials and its contribution to AMR
- Link antimicrobial exposure to the development of AMR.

1.4 Framework

The Point Prevalence survey can be used for single-center or multicenter surveys. The multicenter survey will be conducted in the selected AMU/AMC surveillance site to generate the national point prevalence report. The Antimicrobial consumption will be conducted in the selected AMU/AMC surveillance site to generate the consumption data at hospital level. For the nation data on antimicrobial consumption, the data from the annual distribution order will be analyzed and aggregated. For national use and consumption, the Antimicrobial stewardship center (AMSC) will be leading and coordinating the surveillance system.

Addition to the PPS and the AMC surveillance, post prescription audits of antimicrobial use will also be carried out in the selected AMU/AMC surveillance site.

1.5 Surveillance of antimicrobial use

In many countries worldwide, continuous data collection on antibiotic prescribing is not possible due to the high workload and level of resources needed for regular monitoring. A viable alternative is to collect data at a specific point in time, which can be done successfully using the point prevalence survey (PPS) methodology.

Point Prevalence survey will also be carried out in the surveillance sites using tools such as WHO, GLOBAL PPS and NAPS (National Antimicrobial prescription survey), the US Centers for Disease Control and Prevention, and the Medicines Utilization Research in Africa (MURIA) Tools. The targeted antimicrobials will be in line with the list of antimicrobials listed as critical by WHO (based on AWaRe classification).

The Point prevalence survey protocol will be developed and will be available as a separate document.

1.6 Surveillance of Antimicrobial Consumption

In 2016, WHO developed a global methodology for monitoring antimicrobial consumption, including antibiotics, and supports countries in implementing surveillance of antimicrobial consumption to obtain national estimates of antimicrobial consumption. However, one limitation of consumption data is the lack of information on how antibiotics are prescribed and used at the patient level.

In human health, the WHO Anatomical Therapeutic Chemical (ATC) classification/Defined Daily Dose (DDD) system will be used to measure the consumption of antimicrobials. A core set of antimicrobial classes recommended by the WHO global programme on surveillance of

antimicrobial consumption will be monitored mandatorily. Quantity of antibiotics as DDD per 1000 inhabitants per day for total consumption and by pharmacological subgroup (ATC3) will be used as the primary indicator for AMC surveillance.

1.7 Data flow

Data on antimicrobial use and consumption will be analyzed in the hospitals designated as the AMU/AMC surveillance site and submitted to the Antimicrobial Stewardship Center (AMSC). Consumption data from other hospitals will be analyzed by the AMSC. AMSC will compile and aggregate the national data on use and consumption and send it to the National Coordination Center (NCC) as shown in the figure below.

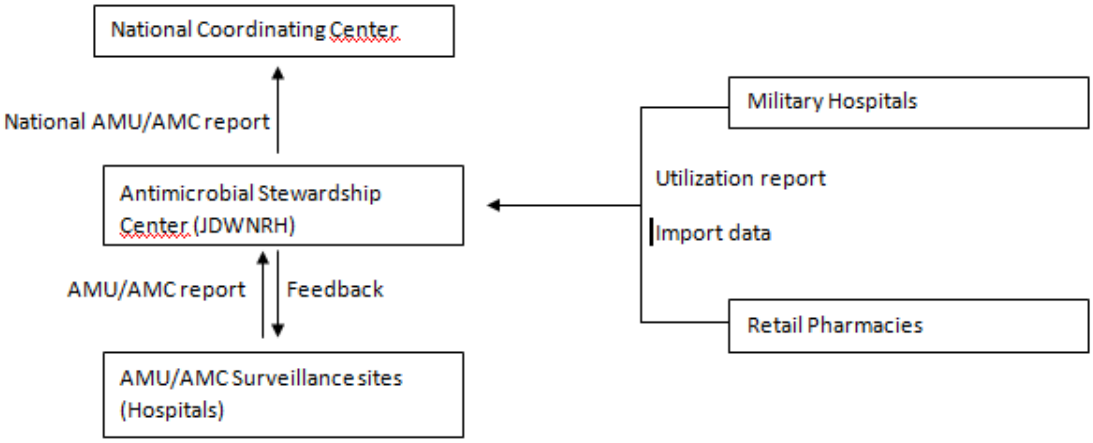


Fig: AMU & AMC Data flow in human health

9 Surveillance of AMU and AMC in animal health

9.1 Background & Scope

The use of antimicrobials in low and middle-income countries is increasing owing to the expansion of animal production to meet the growing demand for animal-source nutrition. It is now accepted that increased AMR in bacteria affecting humans and animals in recent decades is primarily influenced by increase in usage of antimicrobials for a variety of purposes, including therapeutic and non-therapeutic uses in animal production

There are numerous literatures providing evidence in support of the association between antibiotic use in food animals and antibiotic resistant bacteria in humans. This is a source of big concern since Bhutan is still importing 90 % of the meat from outside and thus is highly susceptible to the risks of acquiring AMR. Even within the country, there is a shift of subsistence farming to commercial enterprises. This generally means productivity is enhanced through increasing use of growth promotors in animal feeds or through preventive and prophylactic antimicrobial usage. The antimicrobial use in the country are probably increasing because there is no system for monitoring antimicrobial utilization. The estimates of the total annual global consumption of antimicrobials in animal production vary considerably. This is due to poor surveillance and data collection in many countries, including Bhutan. Without reliable evidence to estimate AMC/U in livestock, the links between AMC/U antimicrobial and resistance patterns are usually poorly quantified, and efforts and policies to optimize antibiotic use in animals are also poorly targeted.

The surveillance data on AMU/C, in Bhutan, will be useful in knowing the trends and make comparisons with other countries. It will also provide reliable data in order to seek necessary policy interventions to institute a stewardship mechanism to combat the AMR issues in the country.

The measurement of AMU in human health and animal health and production settings is a central goal of the Global Action Plan on Antimicrobial Resistance and the complementary plans and strategies developed by the Food and Agriculture Organization of the United Nations (FAO), World Organization for Animal Health (OIE) and World Health organization (WHO)

9.2 Definition for Antimicrobial use and consumption

The antimicrobial use in animal health means “the administration of an antimicrobial agent to an individual or a group of animals to treat, control or prevent disease”- OIE. It is the actual use at the end user’s level. The AMU data can be availed from the prescription records maintained at the animal health centers and to some extent retail pharmacies. The AMU data involves detailed and specific information which is resource intensive and time consuming.

However, the antimicrobial consumption refers to the quantitative volume that is being imported for intended use in the animals. It often translates as sales of antimicrobial medicines. For Bhutan, all the antimicrobials are imported from outside.

9.3 Objectives

- Monitor AMU/C trends and benchmarking for optimization of antimicrobial AMU/C
- Compare AMU/C data across different areas, species, farms in the country
- Provide integrated AMU/C and AMR surveillance data
- Guide policy and targeted interventions optimizing antimicrobial use
- Surveillance and monitoring of AMU/C which are critically important in human health

9.4 Framework

The Point Prevalence survey can be used for single-center or multi-center surveys. The multi-center survey will be conducted in the selected AMU/AMC surveillance site to generate the national point prevalence report. The Antimicrobial consumption will be conducted in the selected AMU/AMC surveillance site to generate the national antimicrobial consumption report.

For animal health, antimicrobial consumption data shall be collected from direct sources (import data) maintained at the Drug Vaccine and Equipment Unit at National Center for Animal Health. The antimicrobial usage data will be collected from surveillance sites.

9.5 Antimicrobial Use Surveillance:

The retrospective AMU data collection from prescription/ records might be very challenging since there is no standard format for data entry/ prescription for animal health system. Convenience sampling from animal health centers known to be efficient in record keeping could be carried out to identify the gaps and challenges.

Point prevalence survey can also be conducted at the surveillance sites to get a baseline of AMU. The targeted antimicrobials will be in line with the list of antimicrobials listed as critical by WHO (based on AWaRe classification) and OIE list of antimicrobial agents of veterinary importance.

9.6 Antimicrobial Consumption Surveillance:

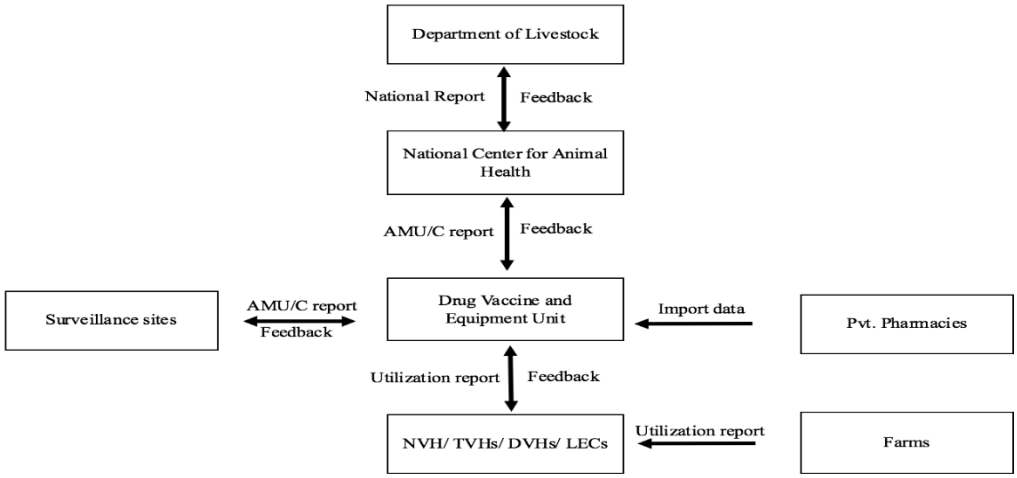
Multiple methodologies for quantifying consumption have been variously employed, hindering data comparability across countries and production sectors. The OIE terrestrial animal health code, the guiding framework for its 182 member nations, outlines the minimum standard as measuring gross consumption by weight of active ingredient per year. For animal health, the OIE AMU format will be adopted since the country reports the AMC to the OIE annually. The AMC use is calculated in kilograms against the biomass of animal population. Animal biomass is calculated as the total weight of the live domestic animals in a given population and year, used as a proxy to represent those likely exposed to the quantities of antimicrobial agents of interest.

Furthermore, a more detailed quantitative data of antimicrobial classes used in animals, with the possibility of separating amounts reported by type of use whether for Veterinary medical use (to treat, control or prevent disease) and Non-veterinary medical use (for growth promotion), animal groups (Terrestrial, Aquatic or Companion) and routes of administration (oral, injection and other routes) will be collected and analyzed. The data will be compared

across the national, regional/ district and farm level to make understand their trends.

9.7 Data flow

AMU/AMC data will be collected from surveillance sites and animal health centers (NVH/ TVHs/ DVHs/ LECs) including private retail pharmacies and farms. The reports generated from these centers will be collated and analyzed at DVEU, which will function as the National data repository. Initial data analysis will be conducted at surveillance sites or respective animal health centers, but verification and final analysis will be done by DVEU. DVEU will submit the final report to relevant Department through NCAH **(Fig).** The final reports will be shared with all the stakeholders and feedbacks provided to the points of data sources to further improve the data flow and/ or to disseminate key findings in the report**. ??? NCC**



NVH- National Veterinary Hospital; TVH- Thromde Veterinary Hospital; DVH- District Veterinary Hospital; LEC- Livestock Extension Centers

Fig ??: AMU & AMC Data flow in animal health

11 CHAPTER 5: AMR DATA MANAGEMENT

Data management encompasses data collection, collation, review, verification, validation and analysis. All designated surveillance sites, including the NRLs of both human and animal health sectors should report AMR data routinely. The HH laboratories will report to the NADSAE, RCDC and AH laboratories will report to the NCAH. The data on AMU and AMC will also be reported as agreed. Data provided should include for all priority bacterial pathogens mentioned in laboratory components of this surveillance guideline.

1. Data collection and management

1.1 Clinical and epidemiological data from participating surveillance sites

All clinical and epidemiological information of cases enrolled, including death, should be collected and collated on regular basis as per the established protocol. Information must include basic demographic information such as age, gender, current address, clinician's diagnosis, common clinical signs and symptoms, etc. Information to determine risk factors may be included as a part if extended surveillance acclivities. All relevant information shall be collected in active, passive or environmental surveillances.

1.2 Isolate and AST data from clinical and animal health laboratories and public health laboratories

Routine antimicrobial susceptibility testing (AST) results should be collected from clinical and animal laboratories by the participating surveillance sites. The data should be uploaded into WHONET or any electronic AMR surveillance system. However, online database system which collects all the data in a single system on regular basis is recommended. RCDC may also collect additional data on AMR from those surveillance sites which are not included as sentinel, such as diarrhea and acute undifferentiated fever illness (AUFI) surveillance.

1.3 AMC/AMU data

Data on AMC and AMU will be collected through the WHO tools adapted. These data will be collected from surveillance sites by the AMSU of the JDWNRH.

2. Frequency of data collection

Routine data will be compiled by surveillance sites every quarter. This quarterly data will be analyzed in brief for the local site use and then forwarded to the NASDE, AMSU or NACH as required.

3. Data review, validation and verification

Data review, verification and validation are techniques used to accept, reject or qualify data in an objective and consistent manner. As per the ISO 9000, verification is a process to confirm, through provision of objective evidence that specified requirements have been fulfilled. Validation on the other hand is defined as confirmation through provision of objective evidence that the requirements for a specific intended use are fulfilled. Data collected from each component of the AMR surveillance should be considered while conducting the data review/verification/validation processes.

Upon the submission of data from all the participating surveillance sites for both animal and

health centers, the designated data manager should review all the data at individual level. This review should help spot unusual value, indicating clerical errors, in the data collection system. If such errors or abnormal values are detected, such data should be flagged for verification. If data is collected using electronic system, procedure for data validation must be incorporated into the system which can erroneous data.

There should be a SOP or protocol in place at all the surveillance sites describing the process of verification and validation requirements and process for every data collected. The data verification process should ensure that the data collected from each component of surveillance and also incorporate operations such as data collection, collation, entry and reporting. Data validation ensures that reported values from the surveillance meet the quality goals of the AMR data operations. A progressive, systematic approach to data validation must be used to ensure and assess the quality of data.

4. Data analysis

Data should be analyzed on regular basis to understand the antimicrobial resistance patterns in pathogens from the participating sites in human, food and animals. Data analysis should also focus to determine the association of antibiotic resistance and usage in both animal and humans. Data should be analyzed at different levels of surveillance. Recommended analyses to be conducted at levels of surveillance are analysis of antimicrobial susceptibility data, individual surveillance site and national percentage (proportion) and trend analyses.

3.1 Susceptibility data categories

For analyzing susceptibility data, CLSI or EUCAST clinical breakpoint criteria should be used to determine the resistance status (i.e. Resistant (R), Intermediate(I) and Susceptible(S)) of a bacterial isolate

3.2 Surveillance site and national percentages

As a general rule, results from the surveillance sites should be reported as a surveillance resistance percentage, which can be done by the participating sites if they have the capacity to analyse the data, and at national level, NADSAE and NCAH should determine the resistance percentage after collating and analysing the data from all the participating sites, both in human and animal health sectors in the country.

b. Trend analyses

The statistical significance of temporal trends in resistance percentages by participating surveillance site and for the population-weighted mean and should be calculated every year. Due to lack of adequate data while developing this guideline, this guideline recommends performing trend analysis when adequate data is obtained, at least 3-4 years. To determine the significance of the trend, a chi square of trends analysis should be performed. Additional analysis may include sensitivity analysis to assess the significance of the trends.

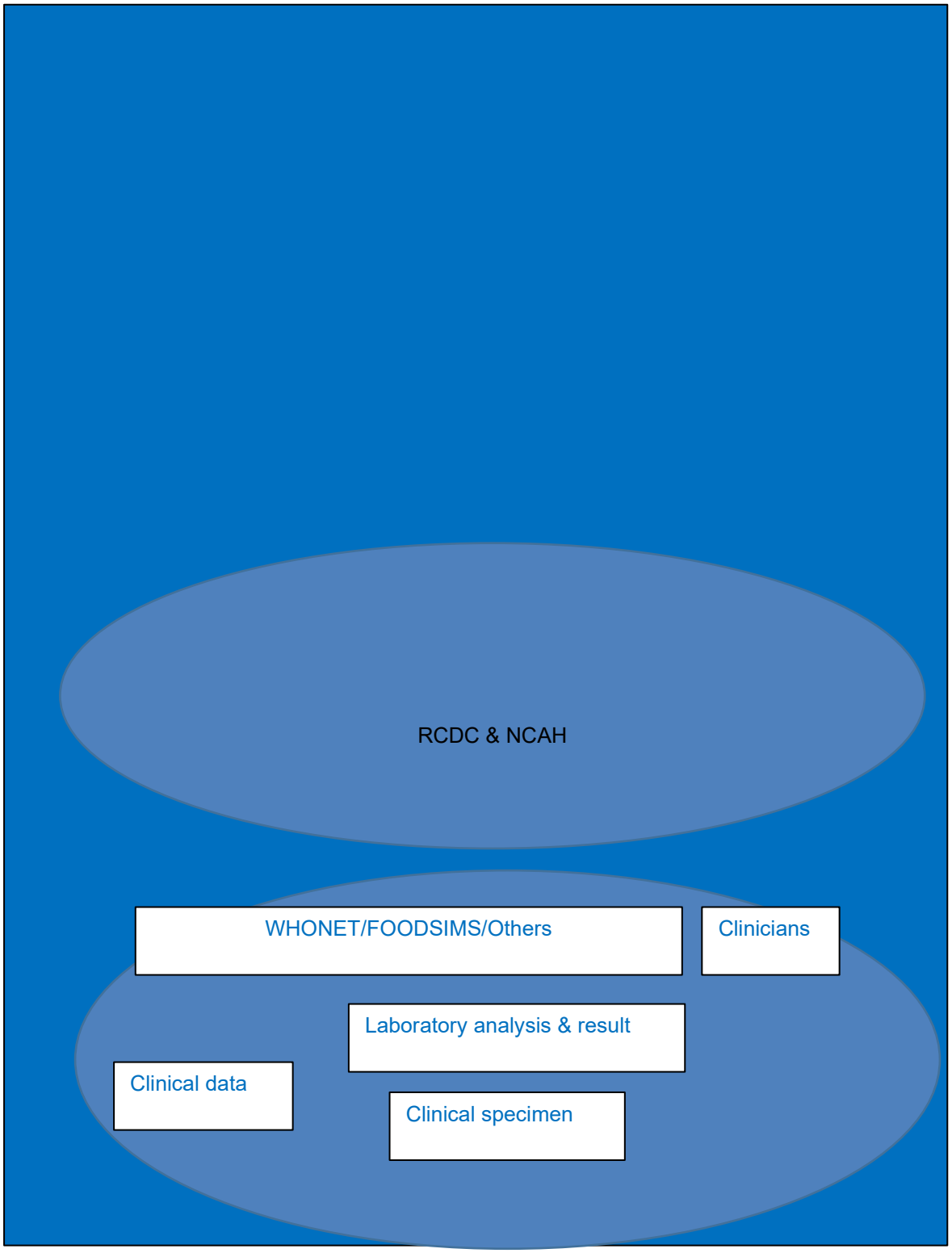
c. Publication of AMR Data, Reports and Feedback

To make AMR data widely available and to encourage local use of AMR data, NADSAE and NCAH should publish AMR surveillance data in the quarterly disease surveillance bulletin,

monthly disease bulletin, annual Surveillance Reports. Reports of analyses of overall susceptibility trends and prevalence of priority pathogen at surveillance site and national level should be included. These data should be shared with all the health and animal healthcare centers through formal publication for making informed decision by clinicians and veterinarians during the treatment. The same report should be shared through various web portals of Ministry of Health, Ministry of Agriculture and Forests, RCDC and NCAH as soon as it is available. The data may also be submitted to peer-reviewed journals for publication for wider reach.

Feedback mechanism is an important component of any surveillance. Timely and periodic feedback should be provided to surveillance sites by NADSAE, NCAH and national reference laboratories (NRL) to improve the surveillance system and to encourage staffs involved in the AMR surveillance and implementation of follow-up actions. Publication of AMR data and sharing with the sites should also encompass is a feedback system.

NADSAE, NCAH and NRL should conduct periodic supervisory visit to understand the problems of the surveillance system first-hand and help sort the issues at the site. Supervisory visit also presents an opportunity to provide constructive feedback and encourage the staffs involved to improve the AMR surveillance system.


RCDC & NCAH
WHONET/FOODSIMS/Others
Clinicians
Laboratory analysis & result
Clinical data
Clinical specimen

12 CHAPTER 6. QUALITY ASSURANCE, EVALUATION AND MONITORING

All aspects of AMR surveillance shall be carried out with good quality control and adequate monitoring and evaluation. It will be the responsibility of all the surveillance sites to conduct all the processes as per international and national guidelines and prescribed SOPs.

1. Quality Assurance

1.1 Quality assurance in Clinical and epidemiological data

For routine surveillance, data will be collected as per the data collection form in all the surveillance sites. The clinical staff of all health centers will be briefed on data collection and completeness.

For active surveillances, all data collection forms will be validated and tested for completeness before conducting the surveillance.

Where applicable, the data collection forms validated and used by organization such as the WHO, OIE and FAO will be adapted and used.

1.2 Quality assurance in Microbiological Procedures

For quality and uniformity of microbiological procedures, all surveillance laboratories will use the same international guideline such as the CLSI or EUCAST. Laboratories will follow the same procedures in testing and reporting, with a mostly harmonized SOP in all the procedures.

The NRLs will participate in international EQAS and keep their performances up to date. National EQA will be established and conducted twice each year by NRL (RCDC) and coordinated by RCDC as per the NEQAS guideline. Surveillance sites must be assessed for their ability to perform correct identification of bacterial pathogens and antimicrobial susceptibility testing using CLSI or EUCAST and NEQAS guideline. For this anonymously coded isolate representing priority pathogens for AMR surveillance may be utilized.

If the EQA results suggest problems in identification and antimicrobial testing, the NRL should identify and address these problems through supervisory visit, onsite training or at NRL and other remedial actions such as preparation of media and performing QC. Additional EQA panels may be dispatched depending on the extent of testing problems. Customized testing procedures may also be used to address specific testing difficulties.

1.3 Quality assurance in data collection and analysis

Data collected should be validated and corrective actions taken for subsequent quarters for continuous improvement.

2 Monitoring and Evaluation

Monitoring and evaluation is an important component of any surveillance activity. In order to scale up response, all the stakeholders need to constantly review their performance in detecting, reporting and responding to AMR.
The technical experts from the AMR program, the JDWNRH, the RCDC and the NCAH will be responsible for monitoring of the surveillance sites regularly (at least six monthly).

The clinical and epidemiological attributes of AMR surveillance system to be evaluated:

(i) sensitivity (proportion of the reported true cases to the total true cases),
(ii) positive predictive value (PPV) (proportion of the cases meeting case definition to the total reported cases),
(iii) completeness (percentage completion of variables entered in the reporting system),
(iv) validity (number of matched medical records among the reported true cases),
(v) timeliness (duration from date of diagnosis to date of reporting; using a one-day window as a cut-off)
(vi) representativeness (comparing the number of cases meeting case definition and reported cases);

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(vii) interviews for qualitative attributes