FEDERAL MINISTRY OF LIVESTOCK DEVELOPMENT (FMLD)

NATIONAL ANTIMICROBIAL RESISTANCE SURVEILLANCE STRATEGY AND PROTOCOLS FOR TERRESTRIAL AND AQUATIC ANIMALS

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National Antimicrobial Resistance Surveillance Strategy
and Protocols for Terrestrial and Aquatic Animals
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About the Ministry

The Federal Ministry of Livestock Development (FMLD) was established on July 9, 2024, by President Bola Ahmed Tinubu to transform Nigeria's livestock sector into a sustainable and globally competitive industry. With a mandate to enhance livestock productivity, ensure food security, and drive economic growth, the Ministry is committed to addressing key challenges such as poor infrastructure and conflicts between farmers and herders. FMLD is structured into 17 departments, focusing on areas like livestock breeding, ranch development, pest control, and veterinary public health. Through strategic policies, research, and private-sector engagement, the Ministry aims to modernize livestock farming, create jobs, and boost Nigeria's economy.

Our Mandate

According to a circular issued by the Secretary to the Government of the Federation (SGF), Sen. George Akume, CON, on September 12, 2024, with Ref. No. SGF/OP//S3/X11/218, the Ministry is mandated to "develop the policies and programmes needed to transform the livestock sector into a vibrant, sustainable, as well as globally competitive industry, ensuring food security, economic growth, improved livelihoods for farmers, and maintenance of social harmony."

Our Vision

To build a resilient, sustainable, and inclusive livestock sector that drives economic growth, ensures food security, and improves livelihoods.

Our Structure

The Federal Ministry of Livestock Development is responsible for formulating and implementing policies to enhance livestock productivity, sustainability, and economic growth. The Ministry operates through 13 specialized departments, including seven (7) technical departments and six (6) common services departments, alongside a Special Duties Office, three (3) units, and seven (7) parastatals/agencies.

I. **Ruminants and Monogastric Department:** The Department functions in the formation and implementation of policy that enhances the productivity and sustainability of dairy, beef, sheep, cattle, goats, pig, poultry, and other micro livestock sectors. It focuses on improving breeding, managing, practicing, processing, and marketing livestock products for economic growth.

II. **Ranch and Pastoral Resources Development:** The Department formulates and implements policies for the development and sustainable management of grazing reserves, ranches, and stock routes. It also develops strategies to promote efficient land use within grazing reserves, improving livestock productivity and reducing conflicts between farmers and pastoralists.

III. **Pest Control Services:** This Department formulates and implements policies for preventing and controlling transboundary pests and vectors of economic and public health importance. It is also responsible for sensitization, advocacy, and monitoring of pesticides used in livestock production.

IV. **Quality Assurance & Certification:** The Department initiates policy direction on safety guidelines for livestock products to protect animal and public health. It also issues International Veterinary Certificates (export and import permits) in compliance with the World Organization for Animal Health (WOAH).

V. **Veterinary Public Health and Epidemiology:** This Department formulates policies and regulations on veterinary public health, ensuring good hygienic animal processing practices and safe delivery of animal products to consumers. It aims to protect public health from animal-borne diseases.

VI. **Livestock Extension & Business Development:** This Department develops policies and strategies to promote livestock extension services and business development for farmers, contributing to national food security and economic growth.

VII. **Human Resources Management:** Responsible for providing administrative guidelines to maximize productivity by optimizing employee effectiveness. It handles recruitment, training, staff performance evaluation, workplace culture, dispute resolution, and wellness programs.

VIII. **Planning, Research & Statistics:** This Department oversees corporate and strategic planning, research activities, and statistical data collection. It supports livestock sector development through research on new technologies and breeding processes.

IX. **Finance & Accounts:** Responsible for financial planning, budgeting, and fund management. It ensures financial stability, transparency, and fraud prevention while supporting management in financial decision-making.

X. **General Services:** Manages the Ministry's facilities, transportation, logistics, and workplace safety, ensuring smooth operational services.

XI. **Procurement:** Oversees negotiation, purchasing, and stock management of goods and services. It ensures quality, cost efficiency, and adherence to legal and ethical procurement standards.

XII. **Special Duties Office:** Handles urgent issues, unforeseen situations, and strategic tasks requiring special attention. It also manages special projects and acts as a bridge between departments.

XIII. **Reform Coordination & Innovation Service:** Drives policy reforms and innovative initiatives, ensuring continuous improvement, modernization, and efficiency within the Ministry.

XIV. **Press and Public Relations Unit:** Disseminates accurate information about government policies and programs related to the livestock sector. It handles media relations, press releases, social media engagement, and public awareness campaigns.

XV. **Internal Audit:** Ensures accountability, financial discipline, and compliance with regulations by identifying risks, detecting irregularities, and recommending corrective actions to safeguard public funds.

XVI. **Legal Unit:** Provides expert legal guidance, ensuring all Ministry activities align with national laws. It advises on contracts, agreements, and legislation affecting the livestock sector while ensuring regulatory compliance.

Our Function

The core responsibilities of the Ministry include the following:

i. Facilitate the expeditious resolution of farmers/herders conflicts.

ii. Increase the productivity and resilience of livestock systems to ensure long-term sustainability.

iii. Improve animal health systems to minimize the impact of livestock diseases on productivity and public health.

iv. Promote climate-smart livestock production to enhance sustainability in the face of

climate change.

v. Encourage innovation, technology adoption, and entrepreneurship among livestock value chain actors.

vi. Create enabling conditions and incentives to attract private-sector investments across the livestock value chain.

vii. Enhance market access and value addition through strategic marketing initiatives, infrastructure development, and compliance with global standards.

viii. Provide incentives for youth and women participation in the livestock sector as a means of job creation, improved livelihoods, and wealth generation.

Foreword

Antimicrobial resistance (AMR) presents a formidable global health challenge, impacting not only human health but also animal health, plant health and the environment. The interconnected nature of AMR demands a coordinated, multi-sectoral response guided by the One Health approach. Recognizing this, the **National Antimicrobial Resistance Surveillance Strategy and Protocols for Terrestrial and Aquatic Animals** has been developed to provide a structured framework for monitoring, data collection, evidence generation and mitigation of the spread of resistant pathogens in Nigeria's livestock and aquaculture sectors.

Additionally, this strategy underscores Nigeria's commitment to strengthening AMR surveillance by fostering intersectoral collaboration among key stakeholders, including government agencies, research institutions, private sector actors, and international partners. Surveillance plays a pivotal role in tracking resistance patterns, understanding the drivers of AMR, and implementing targeted interventions that safeguard both animal and public health.

Through this document, we aim to enhance our national capacity for AMR surveillance by establishing standardized protocols, improving data collection and reporting mechanisms, and ensuring that surveillance activities align with global best practices. By investing in comprehensive surveillance and data-driven policies, Nigeria is taking a critical step toward combating AMR and securing the health and productivity of its livestock and aquaculture industries.

Importantly, this strategy would not have been possible without the support of the UKAID's Fleming fund Phase II country grant which is being implemented by Management Sciences for Health (MSH) with support from the Food and Agriculture Organization (FAO), and the collective efforts of national and international stakeholders, technical experts, policymakers, and partners committed to tackling AMR through evidence-based interventions.

Finally, we encourage all key stakeholders, including veterinarians, researchers, farmers, policymakers, and private sector stakeholders, to actively engage in implementing this surveillance strategy.

Alh. Idi Mukhtar Maiha
Honourable Minister of Livestock Development

Acknowledgment

The development of the **National Antimicrobial Resistance Surveillance Strategy and Protocols for Terrestrial and Aquatic Animals** was made possible through the dedication, expertise, and collaborative efforts of multiple stakeholders across the One Health sectors.

Aptly, we acknowledge the leadership and teams of the **Federal Ministry of Agriculture and Food Security, Federal Ministry of Livestock Development,** for their commitment to conducting surveillance for AMR in the animal health sector. Also, we recognize the **Federal Ministry of Environment;** the **Federal Ministry of Health and Social Welfare,** and the **National AMR Coordinating Committee (AMRCC) domiciled in the Nigeria Centre for Disease Control and Prevention (NCDC)** for their collaborative support and one health partnership.

Importantly, we appreciate the teams at **Management Sciences for Health (MSH)**—the Country grantee for the **UKAID's Fleming fund Phase II grant**, and the Food and Agriculture Organization who supported the entire process from drafting to completion of the strategy.

We acknowledge the support provided by our national and international partners, including the **World Health Organization (WHO), Food and Agriculture Organization (FAO), World Organization for Animal Health (WOAH), and the United Nations Environment Programme (UNEP)**.

We acknowledge the efforts of our academic institutions, veterinary professionals, researchers, farmers, and private sector stakeholders who provided critical input during the development process. Their expertise and input were made through a team of consultants and subsequent expert reviewers who developed and improved the strategy. Their insights and field experience have enriched the strategy, ensuring its relevance and applicability to the realities of antimicrobial use and resistance in Nigeria's animal health sector.

Furthermore, we are thankful to the members of the **Veterinary Council of Nigeria (VCN), Nigerian Veterinary Medical Association (NVMA),** and **National Veterinary Research Institute (NVRI),** for their technical guidance and commitment to improving antimicrobial stewardship across Nigeria's animal health sector.

Finally, appreciation goes to the CVON's AMR team and all other individuals and organizations involved in this development and validation of the strategy; your unwavering commitment to tackling antimicrobial resistance through surveillance and evidence-based interventions will contribute significantly to safeguarding Nigeria's health system and food security.

Dr. Samuel Akawu Anzaku
Director/Chief Veterinary Officer, Veterinary Public Health and Epidemiology

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- **LIST OF ABBREVIATIONS AND ACRONYMS**

AAVN	Association of Aquatic Veterinarians of Nigeria
AIFP	Aquaculture and Inland Fisheries **Project**
AM	Antimicrobial
AMC	Antimicrobial consumption
AMR	Antimicrobial resistance
AMRIS	Antimicrobial Resistance Information System
AMR TWG	AMR Technical Working Group
AMS	Antimicrobial stewardship
AMRCC	Antimicrobial Resistance Coordinating Committee
AMU	Antimicrobial use
ARG	Antibiotic resistance genes
ARP	Antimicrobial resistance pattern
AST	Antimicrobial susceptibility testing
ATLASS	Assessment Tool for Laboratories and AMR Surveillance Systems
BHI	Brain Heart Infusion
BMD	Broth Micro Dilution
CAMHB	Cation Adjusted Mueller Hinton Broth
CAS	Chemical Abstract Service
CLSI	Clinical and Laboratory Standards Institute
CoA	Certificate of Analysis
CPD	Continuous Professional Development
CSO	Civil Society Organisation
ECV	Epidemiological Cut-off Value
EML	Essential medicines list
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FCT	Federal Capital Territory
FDVPCS	Federal Department of Veterinary and Pest Control Services
FMAFS	Federal Ministry of Agriculture and Food Security
FMARD	Federal Ministry of Agriculture and Rural Development
FMEnv	Federal Ministry of Environment
FMOH&SW	Federal Ministry of Health and Social Welfare
FMWR	Federal Ministry of Water Resources
FMLD	Federal Ministry of Livestock Development
GBRMC	Global Biorisk Management Curriculum
GLASS	Global Antimicrobial Resistance and Use Surveillance System
IATA	International Air Transport Association
IHR	International Health Regulations
ILRI	International Livestock Research Institute
IPC	Infection Prevention and Control
IQC	Internal Quality Control
KABP	Knowledge Attitude Beliefs and Perception
LIMS	Laboratory Information Management System
M&E	Monitoring and Evaluation

MAB	Mueller-Hinton Broth
MCT	Micro-centrifuge Tube Challenge Testing
MDRO	Multidrug-resistant organisms
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
MOU	Memorandum of Understanding
NADIS	National Animal Diseases Information and Surveillance
NAFDAC	National Agency for Food and Drug Administration and Control
NAP	National Action Plan
NCDC	Nigeria Centre for Disease Control and Prevention
NESREA	National Environmental Standard and Regulation Enforcement Agency
NGO	Non-Governmental Organization
NIFFR	National Institute for Freshwater Fisheries Research
NIOMR	Nigerian Institute for Oceanography and Marine Research
NRL	National Reference Laboratory
NVRI	National Veterinary Research Institute
PCN	Pharmacy Council of Nigeria
QC	Quality Control
SOP	Standard Operation Procedure
SBCC	Social Behavioral Change Communication
SWOT	Strengths, Weaknesses, Opportunities, and Threats
ToR	Terms of reference
TOT	Training of trainers
TWG	Technical Working Group
UNEP	United Nations Environment Programme
VCN	Veterinary Council of Nigeria
VTH	Veterinary Teaching Hospital
WASH	Water, Sanitation, and Hygiene
WAOH	World Organisation for Animal Health
WHO	World Health Organization

NATIONAL ANTIMICROBIAL RESISTANCE SURVEILLANCE STRATEGY AND PROTOCOLS FOR TERRESTRIAL AND AQUATIC ANIMALS

EXECUTIVE SUMMARY

Antimicrobial resistance (AMR) is the development of resistance in microorganisms (bacteria, viruses, fungi, and parasites) to an antimicrobial medication to which it was previously susceptible. Antimicrobial resistance (AMR) is a serious global, public health issue caused by the abuse/improper and excessive use of antimicrobial agents in human, agricultural, and veterinary settings. It is an important threat to human, animal, and environmental health. Addressing the problem requires robust strategies that can detect emergence and trends in microbial resistance in all the three sectors. Antimicrobial resistance is also a zoonotic health threat. This is because the use of antimicrobial agents in animals could result in the emergence and spread of resistant pathogenic and commensal microorganisms. Resistant bacteria, for instance, from animals may be passed to humans via the food chain, direct animal contact, or fecal contamination of the environment, as well as inadvertent discharge of antimicrobials into the environment, which may result in resistant pathogens. Surveillance of resistant bacteria in animal populations provides frontline information for detecting and quantifying zoonotic spread and environmental contamination. Surveillance in the terrestrial and aquatic animals, particularly food animal and food fish populations, is also essential in order to get a comprehensive picture of AMR problems in the Nigerian animal health sector. This document provides a framework for strategies, protocols and standard operating procedures (SOPs) for the surveillance of antimicrobial resistance in the Nigerian terrestrial and aquatic animal health sectors.

Traditional methods of livestock production in Nigeria were characterized by majorly extensive practices. The production systems of these food animals in Nigeria are largely small-scale, extensive pastoral with a limited number of large-scale producers. The intensification of livestock production systems to meet up the growing demand from the rapidly expanding Nigerian population necessitates the use of antimicrobials. This in turn results in overuse, misuse, and abuse of antimicrobial agents in food animals. Similar scenario plays out in the aquaculture industry, where the mass adoption of the intensive production systems occasioned by the huge shortfall in fish supply has led to the need for massive use of antimicrobials against the increasing incidence of infectious diseases. The surveillance plan recognizes that all production systems make differing contributions to the AMR problem and require investigation and intervention. Surveillance in terrestrial and aquatic animals, and their environment will support the One Health AMR strategy, provide greater understanding of drivers of AMR within these systems, and enrich the national bacterial isolate repository with resistant bacterial species from both the livestock and the aquaculture industries in Nigeria.

The plan for active AMR surveillance in the animal health sector will target, in phases, slaughtered animals at abattoirs, live animals on farms and livestock/live bird markets, and products of food animal origin at retail markets, for terrestrial animals. In the aquaculture industry, surveillance will target live fish from fish farms, fish hatcheries and fish markets, feed from feed-mills. The samples that will be collected and presented for testing at the designated sentinel and the established national reference laboratories are faecal, caecal, milk and other samples of livestock products,

and whole-fry, skin swab and gill swab from fingerlings, juvenile and adult fish, fish feed as well as pond water and sediment samples. The surveys will detect and quantify resistance to critically important antimicrobial agents in human and veterinary medicine exhibited by selected zoonotic and commensal target bacteria. These include *Escherichia coli*, *Salmonella* species (non-typhoidal), *Enterococcus* species, Vibrio, Streptococcus species, Aeromonas and *Campylobacter* species from all the target animals and fish species. In addition, the surveys will also investigate resistance in *Staphylococcus aureus,* especially *Staphylococcus aureus and Listeria monocytogenes* from dairy cattle. Laboratory isolation, testing, and interpretation procedures will utilize standard methods, especially the criteria prescribed by the Clinical Laboratory Standards Institute (CLSI) for resistance interpretation. Reporting of results will be in accordance with the disease reporting channel of Nigeria through the National Animal Disease Information System (NADIS) AMRIS and INFAM to the Food and Agriculture Organization of the United Nations.

The AMR risk assessment in terrestrial and aquatic animals is a comprehensive process that involves identifying AMR hazards, assessing exposure and consequences, characterizing risks, and implementing risk management strategies. The overall objectives of this document are to provide guidance on data collection, analysis, and reporting on harmonized data on AMR in food animals, aggregated at the national level, following the standard definitions described in this document. The experience gained and the capacities built during the active surveillance activities will provide opportunity for establishing routine subsequent passive surveillance for AMR in the animal health sector.

● 1.0 INTRODUCTION

1.1 Background on AMR in Terrestrial and Aquatic Animals

Terrestrial animals, according to the Food and Agricultural Organization (FAO), are animals that primarily live on land (FAO, 2023). The World Organisation for Animal Health (WOAH) definition of terrestrial animals (mammals, including wildlife, birds, reptiles, and bees) (WOAH, 2024), includes domesticated animals such as livestock (cattle, sheep, goats, pigs, and poultry) that live on land (FAO, 2023). As Nigeria's population grows swiftly, the population is projected to double to almost 400 million between 2015 and 2050. Consequently, these population changes will result in an exponential rise in the demand for livestock products. FAO GPS (2018) suggests that meat, milk, and poultry meat consumption will grow by 117%, 577%, and 253%, respectively. Livestock production is also critical to livelihoods and the economy of Nigeria. Nigeria's livestock sub-sector of the agricultural sector of the economy plays a vital role in the country's economy as it contributes about 21% of the agricultural GDP and 9% of the national GDP (ILRI, 2023). Around 13 million Nigerian households keep farm animals, and the sector alone contributes 6 to 8 percent of the national GDP despite the low level of development of commercial livestock production (FAO, 2018).

Nigeria has an estimated 12,478,818 ha of inland water bodies made up of reservoirs, lakes, rivers, ponds and perennial swamps (Ita *et al*., 1985) and some 741,509 ha of brackish waters, most of which are suitable for aquaculture. However, only about 5,476 ha of these waters are presently utilized for fish culture. Hence the country is richly endowed with the prerequisite resources for fish culture. With the backdrop of dwindling catch from artisanal and industrial fishing occasioned by overfishing, climate change and anthropogenic activities in the coastal regions in the country, fish production by culture presents a sustainable production method to meet the yawning gap in fish supply in Nigeria. This informed the need to transform the aquaculture sector into the country's major source of fish and other aquatic animal products for domestic human consumption and regional trade. Nigeria is the second largest aquaculture producer in Africa after Egypt and the largest producer of the predominantly cultured African catfish, globally. The commercial culture of other fish species, most especially tilapia (*Oreochromis niloticus*) is also gaining significant economic importance. In 2004, the number of fish farms inventoried in Nigeria were 2,658 with major concentration in the southern part of the country under the AIFP Project in 2004, but by 2009, the figure rose to over 5000 farms (Miller *et al.*, 2006). However, the 2011 National Fish Frame Survey Report indicates that there were about 5,664 fish farms and 5,752 fish farms with hatcheries. By estimation, using the annual growth rate that occurred between 2004 and 2009, the current number of farms may be above 20,000.

1.1.1 Poultry production

Poultry is widely produced in Nigeria. Current estimates put the total poultry population in Nigeria, including domestic and commercial flocks, at about 180 to 200 million (Adene and Oguntade, 2006; FAO, 2018). Chickens are the predominant poultry bird type kept with very limited numbers of turkeys, pigeons, ducks, and other domestic bird types. Indigenous free-range chickens form the majority of the chicken types kept, with estimates ranging from 70 to 84% of all chickens in Nigeria (Alemayehu *et al.,* 2018; Fagbamila *et al.,* 2018); they are also the most

preferred chicken type for consumption. The annual poultry production amounts to 454 billion metric tons of meat and 3.8 million eggs per year. The distribution of chickens according to the system of production showed extensive systems accounted for about 80 million chickens; 60 million are raised in semi-intensive systems, while the remaining only 40 million are in intensive systems (FAO, 2018).

1.1.2 Cattle production

Cattle production in Nigeria largely remains a traditional activity carried out under pastoral and agropastoral systems in the northern part of the country and mixed farming systems in the south (ILCA, 1989). The Nigerian national cattle population was estimated at 20 million heads (FAO, 2018), with beef and milk being produced by largely pastoral, agro-pastoral, and few commercial production systems. Cattle milk production amounts to 585 000 tons of milk per year, which only covers 40 percent of the demand in Nigeria. With a rapidly growing population and a potential regional export market, beef and dairy production in Nigeria is bound to increase. The net volume of milk produced nationally must be grown steadily using innovative dairy production methods in order to meet the growing demand.

1.1.3 Small ruminant production

Nigeria is believed to have the largest small ruminant herd in Africa, whereas Sudan, Chad, Ethiopia, and Kenya lag behind. The distribution of the small ruminant population in Nigeria is 73.8 million goats and 42.1 million sheep, mainly made of indigenous breeds (FMARD, 2017). Small ruminant livestock in Nigeria, as in sub-Saharan Africa, also share a similar sectoral distribution as cattle, predominantly extensive pastoral, agro-pastoral, and semi-intensive production systems (Wilson, 1991). Small ruminants constitute the major farm animals reared by smallholder farmers in developing countries, including Nigeria, largely for providing animal protein for home consumption, income generation**,** and livelihood (Sosina, 2020). Sheep and goat production have remained largely free range with mixed flocks of sheep and goats or raised together with cattle or in much fewer numbers in urban and peri-urban households (Iyayi *et al., 2003*; Yusuf *et al.,* 2018). Sheep and goats are important in the rural economy, readily disposable for income where cattle cannot be culled.

1.1.4 Piggery production

Pig production is one of the profitable enterprises of the livestock sub-sector of agriculture. While Nigeria has the highest pig population in Africa, production is a more limited and isolated activity (Adesehinwa *et al.,* 2024). They are mostly reared in rural areas by smallholder farmers, where the majority of the pig population are indigenous breeds and their few crosses. Pig plays important roles in the sustenance of livelihood in the rural communities and has cultural and social significance (Adesehinwa *et al.,* 2024*;* Chen *et al.,* 2022). Despite its rapid growth, pig production in Nigeria has not grown into popular commercial enterprises. The pig production system is predominantly extensive traditional scavenging, but with few rising modern systems (Adesehinwa,

2023). However, there is a steady growth in the number of smallholder producers and free-range rearing, where pigs serve to protect the livelihoods of poor families (Ezeibe, 2010; Chen *et al.,* 2022).

1.1.5 Aquaculture production

Aquaculture production in Nigeria increased from 25,718 tonnes in 2000 to 261,711 tonnes in 2020 (FAO, 2022). The 12.3 percent of annual growth was slightly lower than the sub-regional average, yet higher than regional and world averages. The major fish species cultured in Nigeria include the African catfish species (*Clarias gariepinus*, *Heterobranchus spp.,* and the hybrid of these two genera popularly called Heteroclarias) and the Tilapia species (*Oreochromis niloticus*). The aquaculture value-chain comprises hatchery production that propagates and supplies fish seeds to grow-out farmers who grow the fish for table-fish production and supply fresh-fish to fish mongers, restaurants and fish processors. There are aquaculture input suppliers that include outlets for drugs, biological, chemicals, feed and feed ingredients, implements and equipment. There are also local feed-millers that produce fish feed by toll-milling.

1.2 Emergence of AMR Pathogens in Livestock and Aquaculture

The exponential increase in Nigeria's population and urbanization exerts sustained demands for livestock and aquaculture products, which in turn necessitates intensification of production systems to meet up the growing demand. This consequently results in overuse, misuse, and abuse of antimicrobials in food animals. Antimicrobials are natural, semi-synthetic, and synthetic drug compounds used to treat bacterial, viral, fungal, and other unicellular parasitic infections in human and veterinary medicine (Giguère, 2013; Nankervis, 2016). Antimicrobials are used in terrestrial and aquatic animals, particularly livestock and fish production practices as growth promoters at a sub-therapeutic level and therapeutic as well as prophylactic purposes to preserve animal health and public health (for prevention and control of zoonoses) (FAO 2016; FAO, 2024). Antimicrobial agents are essential in the treatment of infectious diseases in both humans, animals, and plants; it is pertinent to note that about 73% of antimicrobials sold globally are used in food animal production (Van Boeckel *et al., 2017*). In Nigeria, like in most of the developing countries, the relevant legislation concerning appropriate conditions for the importation, manufacture, distribution, and use of veterinary products, including antimicrobials, is very often not properly applied. Terrestrial animals, such as livestock (e.g., cattle, poultry, pigs), aquatic animals such as fish and companion animals can harbor bacteria that are resistant to antibiotics. Antimicrobial resistance (AMR) in food animals is now considered a growing threat to animal health and potentially to human health (Zhao *et al.,* 2024*).* This resistance can arise due to various factors, including the misuse or overuse of antibiotics in veterinary medicine. Microorganisms exposed to low dosages (sub-lethal or sub-therapeutic dosages) will elicit selection pressure on genetic and phenotypic variability in the exposed microorganisms (Andersson & Hughes, 2014). Resistance to antimicrobial agents develops when microorganisms adapt and grow in the presence of the agents to which they are previously sensitive. Antimicrobial resistance development is linked to how

often antibiotics are used (Mancuso *et al., 2021*). Many antibiotics may belong to the same class of medicines; therefore, resistance to one specific antibiotic agent can lead to resistance to a whole related class (WHO, 2024a). The emergence of AMR in one organism or location can rapidly and unpredictably spread through exchange of genetic material between different bacteria, affecting antibiotic treatment of a wide range of infectious diseases (WHO, 2024a). There exists substantial evidence that supports the view that the emergence of antimicrobial resistance in bacterial populations in livestock systems is related to the emergence of AMR in bacterial populations that colonize and infect humans (Singer *et al., 2003*; O'Neill, 2015). Humans can be infected by both pathogenic and non-pathogenic resistant bacteria from livestock by consumption of foods of animal origin or through direct contact with infected animals or their waste in the environment (Marshall and Levy, 2011).

Also, increase in the incidence of infectious diseases in aquaculture correlates positively with the mass adoption of intensive rearing methods targeted toward optimizing stocking density and yield, without regard to the associated stress and disease susceptibility. This development has brought about massive mis-use of antimicrobials across the aquaculture value chain as a result of indiscriminate/extra-label use of antimicrobials, culminating in a rising incidence of antimicrobial resistance (AMR) which has been widely reported in Nigeria. Antimicrobial resistance has been widely reported in fish bacterial pathogens such as *Aeromonas* spp., *Vibrio* spp., *Flavobacterium columnare*, *Pseudomonas* spp., *Edwardsiella tarda* and *Streptococcus* spp., and also in bacteria associated with the aquatic environment in addition to those that are important in food safety, including members of the coliform group; *Salmonella* spp., *Yersinia* spp., *Campylobacter* spp., *Escherichia coli*, *Enterococcus* spp. as well as *Staphylococcus aureus*. Since similar antimicrobials are used to treat diseases caused by similar pathogens in both humans and animals, resistant pathogens and their resistant genes (ARG) are inevitably shared between fish, other animal production systems, humans and the environment. The aquaculture situation analysis corroborated the widespread mis-use of antimicrobials in commercial aquaculture systems, and showed potential impacts on fish health, food-fish safety and environmental health. This becomes even more complicated with respect to the mode of antibiotic application; by immersion, the affluent of which is discarded into the environment. Even when antimicrobials are administered orally via the diet, they are still passed to the environment unchanged in the fecal material as environmental contaminants. The use of animal manure in pond fertilization, and the feeding of poultry offals as supplementary diet to cultured fish, also provides avenues for exchange of antimicrobial resistant genes (ARG).

The emergence and persistent rise in the occurrence of multidrug-resistant (MDR) bacteria has become a global public health concern due to the decline in the therapeutic options for the treatment of some bacteria infections in humans. Globally, the public health threat and costs of AMR are widely recognized, as the European Commission [EU] (2023) estimated that antibiotic-resistant pathogens cause approximately 33,000 deaths per year in Europe and 4.95 million total deaths worldwide. In addition to this, AMR accounts for about €1.5 billion (N264.5 billion) in healthcare expenses and productivity losses annually. The World Health Organization (WHO, 2024b) described AMR as "one of the ten global public health threats facing humanity" and further predicted that by the year 2050, AMR will be responsible for over 10 million deaths per year, with a loss of more than one hundred trillion dollars ($100 trillion) worldwide. The true extent of the AMR problem is still not known in most African countries, including Nigeria, due to unavailability or incomplete data. Recent estimates showed that the western sub-Saharan African region has the

highest AMR burden, with 27.3 deaths attributable to AMR per 100,000 overall deaths (Dadgostar, 2019).

Zhao *et al.* (2024) reported that there are significant ($p < 0.05$) temporal increases of resistance for all antimicrobials apart from tetracycline. The global mean prevalence of resistance in *E. coli* and nontyphoidal *Salmonella* were respectively reported to be 59% and 54% for tetracycline (TET), 57% and 46% for ampicillin (AMP), 45% and 36% for sulfamethoxazole-trimethoprim (SXT), 35% and 26% for chloramphenicol (CHL), 30% and 26% for ciprofloxacin (CIP), 28% and 23% for gentamicin (GEN), 33% and 19% for cefotaxime (CTX) (Zhao *et al., 2024*). Globally, the prevalence of resistance increased significantly for AMP, CHL, CIP, and CTX for poultry and for AMP, SXT, CHL, CIP, GEN, and CTX for pigs (Zhao *et al., 2024*).

The emergence of AMR in terrestrial animals is a global public health concern, but the situation in Africa is bedeviled by lack of access to appropriate antimicrobial therapy, weaknesses in regulations and legal framework in the use of antimicrobials for humans and animals, weak or complete absence of AMR surveillance systems, and lack of updated antimicrobial use and treatment guidelines (Kimera *et al.,* 2020). In a review on antimicrobial use and resistance in food-producing animals and the environment, Kimera *et al.* (2020) gave the African perspective on antimicrobial use (AMU) in animal production as ranging from 77.6% in Nigeria to 100% in Tanzania, Cameroon, Zambia, Ghana, and Egypt. Tetracycline, aminoglycoside, and penicillin groups are predominantly used, with the prevalence of multidrug-resistant isolates ranging from 20% in Nigeria to 100% in South Africa, Zimbabwe, and Tunisia. The prevalence of MDR in the African environmental isolates ranged from 33.3% to 100%. Emeje *et al.* (2022) observed that most antibiotics used in animal production are done with little or no expert consultation, and some of these antimicrobials are indiscriminately sold as over-the-counter drugs in many countries. These, coupled with lack of awareness, absence of agricultural extension services, inadequate manufacturer's instructions, and the need for profit by animal producers, result in the irrational use of antibiotics. Among the factors that militate against prudent use of antimicrobial agents among veterinarians, Adekanye *et al.* (2020) observe that only 20% of the respondents in a study conducted antimicrobial susceptibility testing (AST) frequently; 82% of the veterinarians reported unavailability of veterinary laboratory services, while 72% of the clients reported inability to pay for AST.

The Institute for Health Metrics and Evaluation (IHME, 2023) estimated that there were 64,500 deaths attributable to AMR and 263,400 deaths associated with AMR in Nigeria. Currently there are no dedicated AMR surveillance systems for terrestrial and aquatic animals in Nigeria, as most estimates emanated from isolated individual research and systematic reviews of those anecdotal data. High levels of resistance were observed among bacterial isolates of animals in Nigeria against antimicrobials such as aminoglycosides (gentamicin), macrolides (erythromycin), cephalosporin, quinolones, and tetracycline (NCDC, 2024). Studies estimated that 10.9% of bacteria reported were multidrug-resistant (MDR) and 2.3% were extensively drug-resistant (XDR). The resistance patterns in Nigeria, ranging from "high resistance" to "very high resistance" against some popular antimicrobials, among *Proteus*, spp. *Listeria* spp., *Enterococcus* spp., *Enterobacter* spp., *Vibrio* spp., *Clostridium* spp., *Bacillus* spp., *Aerobacter* spp., *Citrobacter* spp., *Streptococcus* spp., *Serratia* spp., and *Micrococcus* spp., were reported in food animals. The prevalence of AMR observed includes, among others, chloramphenicol (50%), amoxicillin-clavunanic acid (65%), ampicillin (82%), tetracycline (75%), co-trimoxazole (50%), trimethoprim (100%), vacomycin

(100%), and erythromycin (55%). Adesokan *et al.* (2015) reported that tetracyclines, fluoroquinolones, and beta-lactams/aminoglycosides constituted the majority of the antibiotics used over the 3 years in livestock in south-western Nigeria. They also demonstrated that antibiotic consumption increased by 40.4% between 2010 and 2012 in that region, and significantly higher consumption was recorded during the rainy (52.5%) than the dry (47.5%) seasons. Very high percentages 85% to 100% of MRSA isolates from mastitic cows from selected herds in Kaduna were resistant to penicillin, amoxicillin, ampicillin, tetracycline, and erythromycin (Umaru *et al.,* 2019).

In a study that investigated various qualitative and quantitative indices of AMU in companion animals (CAs) at a Veterinary Teaching Hospital and its annex in Nigeria, Adebowale *et al.* (2023) documented antimicrobial administrations of 98.8% and 1.2% in dogs and cats, respectively. They also found oxytetracycline and metronidazole are mostly administered in dogs and cats compared to enrofloxacin, ciprofloxacin, and azithromycin.

Tetracycline-, penicillin-, and sulfonamide-resistant bacterial isolates have been commonly observed among chicken and swine populations, while MDR isolates were reported to be significantly higher in these animals than those from cattle (FAO, 2016). The prevalence of *Escherichia coli* (34.2%), *Salmonella* spp. (57.5%), *Klebsiella* spp. (5.8%), and *Pseudomonas* spp. (2.5%) was reported in poultry in the Guinea Savanna agro-ecological zone of Nasarawa State, Nigeria. All four species are found to be multi-drug resistant, and 30% of the isolates had a multiple antibiotic resistance (MAR) index ≥ 0.2 (Bamidele *et al., 2022*). Highly antibiotic-resistant bacterial isolates were reported with prevalences of 58.0% and 44.0% from FUNAAB Alpha and Noiler chickens, respectively, while 22.6% of the isolates from local chickens were most resistant to aminoglycosides (Bamidele *et al., 2022)*.

Codex Alimentarius (2011) described AMR risk assessment as a science-based component of risk analysis, a process that also includes risk management and risk communication. It can provide a transparent, systematic evaluation of relevant scientific knowledge to inform decisions regarding risk management activities. Caffrey *et al.* (2019), in a review on risk assessments evaluating foodborne antimicrobial resistance in humans, identified the most frequent hazard of concern as the consumption of retail meat. The national surveillance strategy and protocols, designed to monitor the prevalence of AMR in microorganisms and the use of antimicrobial agents, can aid in establishing a baseline standard to evaluate the effectiveness of risk management measures.

1.3 The Mission of AMR Surveillance in Terrestrial and Aquatic Animals

The proposed AMR surveillance is targeted towards achieving prudent use of antimicrobials and strengthening evidence-based policy making and action to protect livestock, poultry and aquaculture, human and environmental health.

1.4 The Vision of AMR Surveillance in Terrestrial and Aquatic Animals

To develop livestock, poultry and aquaculture industries where antimicrobial use is based on global best practices, and antimicrobial resistance is promptly mitigated.

1.5 The Goal of AMR Surveillance in Terrestrial and Aquatic Animals

The goal is to reduce, prevent, and slow the evolution of resistant organisms and their impact on food animals and both terrestrial and aquatic environments, while ensuring optimal use and improved access to effective, safe, and quality assured antimicrobials and diagnostics for continued successful management of infections.

1.6 The Scope of AMR Surveillance in Terrestrial and Aquatic Animals

There is no coordinated AMR surveillance (passive and active) yet in the animal health sector in Nigeria. Nigeria is not yet reporting AMR animal health surveillance data to GLASS. However, a lot of AMR work is carried out in the universities, research institutes and veterinary diagnostic laboratories but data generated therefrom are not pooled and reported as surveillance data. Reports from research publications in animal health include resistance profiles of bacteria in food animals notably, *E. coli, Salmonella* spp*., Pseudomonas* spp*., Aeromonas* spp*., Staphylococcus* spp*., Enterococcus* spp*.,* and *Campylobacter* spp. against a number of antimicrobials with high levels of resistance to aminoglycosides (gentamicin), macrolides (Erythromycin), Cephalosporins, quinolones and tetracyclines.

Therefore, active surveillance will be conducted to cover both terrestrial (cattle, sheep, goats, pigs, and poultry) and aquatic (fish) animals. Samples will be collected from these food animals in poultry farms, livestock farms, live birds markets, livestock markets, abattoirs/slaughterhouses, meat processing plants for terrestrial animals, and from fish farms, fish hatcheries, feed-mills and fish processing plants for aquatic animals. The sampling will involve healthy animals intended for human consumption. On the other hand, passive AMR surveillance will be conducted by generating data from AST of clinical cases presented to the sentinel laboratories. Both the active and the passive surveillance will span a period of five years (2024 to 2028).

The surveillance will cover the six geo-political zones of the country, with two states having the highest terrestrial animal / fish production selected per zone, based on standard methods (WOAH, 2018).The Stakeholders to be involved in the surveillance are: Federal Department of Veterinary and Pest Control Services, Federal Department of Fisheries, State Veterinary Departments, National Veterinary Research Institute, Vom, Veterinary Faculties/Colleges/VTHs of Universities, Private Veterinarians, FAO, WOAH, AfCDC, WAHO, NCDC, VCN, Farmers Associations (PAN, MACBAN, Sheep and goats Association of Nigeria, Catfish and Allied Fish Farmers Association of Nigeria, Tilapia Producers Association of Nigeria etc)

The following guiding principles underpin the foundation of this AMR surveillance strategy, protocols and Standard Operating Procedures (SOPs) and its implementation.

1. Inclusive governance

2. Private sector collaboration

3. Community engagement

4. Prevention first

5. Evidence-based decision making

6. Sustainable interventions

7. Incremental targets for implementation

2.0 STRENGTHS, WEAKNESS, OPPORTUNITIES AND THREAT (SWOT) ANALYSIS FOR AMR SURVEILLANCE IN TERRESTRIAL AND AQUATIC ANIMALS IN NIGERIA

In order to be able to assess all internal and external factors that might affect the smooth execution of the AMR surveillance in the poultry, livestock and aquaculture industry, it becomes important to put the strengths, weaknesses, opportunities and the threats associated with it into perspective.

STRENGTH	WEAKNESS
● Availability of laboratory infrastructure at the NRL and the sentinel laboratories ● Established unified protocols for active AMR surveillance ● Capacity for early detection of emerging resistance trends to enable timely interventions. ● Good Laboratory networks ● Trained laboratory focal points on AMR in WHONET ● Laboratories are enrolled for external quality assurance ● Effective collaboration among the Regional Tripartite (FAO, WOAH and WHO), UNEP, AU, RECs, CSOs, funding institutions and other partners on AMR issues ● Increased political commitments toward AMR mitigation ● Revised AMR national Action Plans (NAPs)/ policies by Nigeria in the context of the One Health approach ● NRL is ISO 17025 certified ● Support from donor agencies for AMR surveillance activities ● The NRL and sentinel labs have been renovated and equipped for AMR surveillance ● There is a technical working group in the animal health sector ● There is guidelines on biosafety for laboratory ● Presence of SOPs for laboratories ● Availability of AMR animal health Fleming Funds Fellowship Fellows. ● Capacity for training of AMR surveillance agents	● Inadequate sentinel laboratories ● Inadequate funding for national AMR surveillance ● Inadequate laboratory equipment, reagents and consumables at national reference and sentinel laboratories ● Inadequate skilled laboratory personnel for AMR surveillance ● Inconsistent data collection methods and lack of standardization ● Limited surveillance coverage ● Poor awareness on implication of AMR ● Weak national collaboration mechanisms between federal and sub-nationals ● Limited domestication of AMR governance structure at the sub-national levels

OPPORTUNITIES	THREAT
• Inter-operatable surveillance network across sectors • Leveraging on existing LIMS in the animal health sector laboratories • Enhanced capacity in the animal health sector for AMR surveillance, data generation • Enrolment and strengthening of additional veterinary sentinel laboratories for AMR surveillance • Leveraging on cutting edge technologies, such as genomic sequencing and data analysis • Enhanced data sharing and coordinated efforts to combat AMR • Improved public awareness • Improved networks of experts between the reference laboratory and collaborating sentinel centres • Availability of AMR data for global frameworks and initiatives (One Health approach, IHR) • Integrating AMR activities into existing programmes • Engaging the private sector CSOs and NGOs on AMR awareness • Enhance relevance of AMR to One Health approach	• Emerging and re-emerging pathogens can outpace surveillance efforts • Limited or inconsistent funding can hinder the sustainability of surveillance programs. • Political will and economic instability can disrupt surveillance activities and data collection • In security in some part of the country • Poor staff motivation • Brain drain • Weak regulatory framework and enforcement • Poor accessibility of quality assured AM drugs • Low public understanding of AMR • Lack of emphasis on the development of affordable alternatives to AMs • Non involvement of trade, industries and the private sector in AMR surveillance activities • Inter agency rivalry • Inconsistency in government policies • Climate change and natural disaster challenges

3.0 Objectives

3.1 General Objective for AMR Surveillance in Terrestrial and Aquatic Animals

The general objective of AMR surveillance in terrestrial and aquatic animals is targeted towards monitoring and controlling AMR in bacterial organisms associated with livestock and aquaculture production and the environment. The specific objectives related to data collection, analysis, and management are to:

1. Monitor the prevalence and trends of AMR among terrestrial and aquatic animals for the protection of public health under the one health approach.
2. Identify animal groups, production systems associated with higher occurrence of antimicrobial resistance among priority zoonotic bacterial pathogens.
3. Enrich the biorepository with antimicrobial resistant bacterial isolates from livestock and aquatic animals in Nigeria.
4. Facilitates AMR risk assessments and mitigation strategies to control AMR in livestock and aquatic animals.
5. Educate and raise awareness among stakeholders about AMR existence in livestock and aquatic animals.
6. Facilitate international collaboration and data sharing.

7. Facilitate capacity building in AMR surveillance and risk assessments to ensure safety of food of animal origin, and safeguard public health.

3.1.1. Strategic Objective 1: Improve evidence base through strengthening One Health AMR surveillance systems and operational research for decision making

Reliable data on antimicrobial use and resistance is central to shaping AMR policies, programs, and decisions. The data must be comprehensive, appropriate, correct and representative of the entire country, allowing for the close monitoring of emerging infections, identification of the region with higher prevalence, and informed decision-making for prevention and containment efforts. Strengthening laboratory capacities across the six-geopolitical regions of the country, including private sector participation, is crucial for a comprehensive approach to data collection.

National policies, developed within legal and ethical frameworks, should ensure consistent data collection and quality control through established guidelines and standard operating procedures (SOP), utilizing standard methodologies. Additionally, incorporating AMR-related indicators into existing surveillance systems will foster collaboration among various stakeholders, including government health departments, veterinary services, pharmaceutical industries, academic institutions, and international organizations. Comprehensive mapping of AMR surveillance across the country, covering clinical cases in the passive, and relevant samples in the active surveillance, is fundamental.

Building capacity for surveillance is also critical, addressing geographical disparities and involving private laboratories. Resource allocation, region-specific training programs, and investment in laboratory capacity based on identified needs in each geopolitical zone will be key to the success of surveillance efforts.

3.1.2 Strategic Objective 2: Strengthen a One Health approach to AMR surveillance by sharing AMR surveillance results from the animal health sector with those from the human health sector and working towards implementing more tightly integrated AMR surveillance that contributes to understanding possible links between resistance in animals and humans

The growing threat of antimicrobial resistance (AMR) poses a significant challenge to global public health, animal welfare, and environmental sustainability. This objective seeks to address this issue through a comprehensive "One Health" approach, which recognizes the interconnectedness of human, animal, and environmental health.

The core of this objective is to enhance AMR surveillance by fostering collaboration and data sharing between the animal health and human health sectors, thereby leading to a more integrated and effective surveillance system. A major aim of this objective is to move toward implementing a more tightly integrated AMR surveillance system that can track and compare data from both sectors in real time. This would involve harmonizing data collection methodologies, standardizing testing protocols, and developing shared databases where surveillance information can be easily accessed and analyzed by both human and animal health authorities.

The ultimate goal of this approach is to contribute to a deeper understanding of the possible links between resistance in animals and humans. Many studies have already shown that resistant bacteria can spread between animals and humans through direct contact, food consumption, or environmental pathways such as water and soil. However, the full extent of this transmission and its implications for human and animal health remain poorly understood. By aligning surveillance efforts, authorities will be better equipped to investigate how resistance in one sector may influence or drive resistance in the other, leading to more effective strategies to mitigate the spread of AMR.

3.2 Action Plan

The Nigerian AMR surveillance action plan outlines a coordinated series of activities and actions designed to achieve the goal of reducing, preventing, and slowing the development of resistant organisms and their effects on humans, aquatic, terrestrial animals, and the environment (terrestrial and aquatic). These activities cut across, and the AMR surveillance details the strategic intervention areas, quantities, lead implementers, timelines, and costs for each activity and sub-activity.

Strategic Objective 1: Improve evidence base through strengthening One Health AMR surveillance systems and operational research for decision making									
			Timeline						
Activity/Sub-activities	**Quantity**	**Responsible Agency**	**2024**	**2025**	**2026**	**2027**	**2028**	**Cost**	
Strategic intervention 1.1. Strengthen and expand AMR surveillance capacity to address geographical disparities within the six geopolitical zones in Nigeria									
Activity 1.1.1. Increase participation of private laboratories in the national surveillance networks (easily accessible to the community)									
1.1.1.1. Conduct stakeholder mapping analysis	1	FMAFS, FMLD							
1.1.1.1. Conduct stakeholders' workshops to identify and assess public and private facilities with high clientele which are approved and aligned with national requirements. WOAH/FAO	30	FMAFS, FMLD, NVRI, VTHs, MBE, Rep. of Private Laboratories							

Activity 1.1.2. Capacity building for existing and additional labs to join the surveillance network and consolidate a nationwide network of high-capacity labs with a quality assurance system									
1.1.2.1. Assess the capacity needs for food animal, surveillance laboratories and their personnel	1	FMAFS, FMLD, NVRI							
1.1.2.2. Upgrade the laboratory infrastructure	12	FMAFS, FMLD, NVRI							
1.1.2.3 Develop inclusion criteria and select focal point personel for sentinel laboratories in all veterinary faculties	12	FMAFS, FMLD							
1.1.2.4. Procure laboratory and data management equipment for AMR surveillance	6	FMAFS, FMLD, NVRI							
1.1.2.5. Train staff in AMR laboratory methods and data sharing,	10	FMAFS, FMLD, NVRI, VTH							
1.1.2.6. Install laboratory management information systems (LIMS, WHONET) at AMR surveillance sites with in-country training and onsite supervision	37	FMAFS, FMLD, NVRI, VTH							
Activity 1.1.4. Train surveillance and lab officers to understand their role in surveillance to strengthen the link between the labs and focal managers and surveillance officers									
1.1.4.1. Conduct national training workshop orientation workshop on AMR surveillance for food	1	FMAFS, Farmers Association, Feed millers							

animal sector									
Activity 1.1.5. Expand terrestrial and aquatic multidrug-resistant organisms (MDRO)/zoonosis surveillance system									
1.1.5.1. Identify priority pathogens for bacterial and zoonotic/MDRO	1	FMAFS, FMLD, NVRI, Academia							
Activity 1.1.6. Support procurement and distribution of laboratory consumables, and reagents for field and laboratories									
1.1.6.1. Procure sampling materials for AMR surveillance in terrestrial and aquatic animals	5	FMAFS, FMLD, NVRI, VTHs Public and Public laboratories							
1.1.6.2. Engage registered logistics service providers to transport isolates from the sentinel to NRL	5	FMAFS, FMLD							
1.1.6.3. Engage trained personnel to transport procured consumables and reagents to the AMR sentinel labs	5	FMAFS							
Strategic intervention 1.2 Strengthen all components of the AMR surveillance system for animals, including epidemiological skills (sample design, data analysis and data reporting), sample collection and processing, laboratory diagnostic capability and data management.									
Activity 1.2.1 Knowledge and skill gaps amongst field surveillance officers, laboratory and data management personnel filled based on needs assessment and training to fill the identified gaps									
1.2.1.1 Appoint a consultant to design and pretest a questionnaire for gap analysis	1	FMAFS, FMLD, AMRCC, Academia							
1.2.1.2 Design and Pretest a questionnaire	1	FMAFS, FMLD, AMRCC, Academia							
1.2.1.3. Engage	1	FMAFS,							

stakeholders to identify training needs		FMLD, AMRCC, Academia							
1.2.1.4. Administer a questionnaires for capacity gap analysis	1	FMAFS, FMLD, AMRCC							
1.2.1.5. Engage stakeholders to approve list of trainings needs following gap analysis	1	FMAFS, FMLD, AMRCC							
1.2.1.6. Conduct of approved trainings	12	FMAFS, FMLD, AMRCC							
Activity 1.2.2. Enhancing the capacities and readiness of veterinary services and laboratories devilment surveillance systems by installation of additional equipment in Sentinel Laboratories									
1.2.2.1.Audit of laboratories to identify existing equipment that require servicing and or recertification Audit laboratories to identify additional equipment required for effective surveillance activity	1	FMAFS, FMLD, AMRCC, NRL							
1.2.2.2. Procure and install additional equipment as need arises	1	FMAFS, FMLD, AMRCC, NRL							
1.2.2.3. Audit laboratories to identify additional equipment required for effective surveillance activity	1	FMAFS, FMLD, AMRCC, NRL							

1.2.2.4. Award the Contract for procurement of identified equipment	12	FMAFS, FMLD, AMRCC, NRL							
1.2.2.5 Award a contract for servicing and recertification of the equipment	12	FMAFS, FMLD, AMRCC, NRL							
1.2.2.5. Engage a consultant to develop a list of surveillance items, consumables and reagents, computer software and hardware	1	FMAFS, FMLD, AMRCC, Consultant,							
1.2.2.6. Procure surveillance materials and other inputs	1	FMAFS, FMLD, AMRCC							
1.2.2.7. Distribute surveillance items to field offices and reagents to labs	1	FMAFS, FMLD, AMRCC							
Strategic intervention 1.3. Ensure standardisation and quality control for AMR surveillance data at national and state levels									
Activity 1.3.1. Develop and disseminate standard operating procedures for AMR data collection in food animal sector at national and state levels									
1.3.1.1. Train laboratory managers on quality management systems	2	FMAFS, FMLD, NVRI, VTHs							
1.3.1.2. Conduct quality assurance and periodic quality control assessment in the sentinel laboratories	37	FMAFS, FMLD, NVRI, VTH							
1.3.1.3. Conduct	10	FMAFS, FMLD,							

biannual quality assurance and control review meetings for feedback from AMR labs		NVRI, VTH						
Strategic intervention 1.4. Strengthen AMR surveillance within the food animal sector								
Activity 1.4.1. Improve laboratory capacity for AMR surveillance in food animal sector								
1.4.1.1. Map high risk area and assess current capacity for AMR surveillance in terrestrial and aquatic animal (review of existing laboratories)	1	FMAFS, FMLD						
1.4.1.2 Supervision of sentinel laboratories by NRL and to provide quality assurance	1	NRL						
1.4.1.2. Upgrade laboratory infrastructure	1	FMAFS, FMLD						
1.4.1.3. Procure laboratory and data management equipment and consumables for AMR surveillance	1	FMAFS, FMLD						
Activity 1.4.2. Develop SOPs on AMR surveillance for food animal sector								
1.4.2.1. Conduct a stakeholder workshop to develop SOPs on AMR surveillance	1	FMAFS, FMLD, NVRI, VTHs						
1.4.2.2. Conduct a workshop to review and validate the developed SOPs	1	FMAFS, FMLD, NVRI, VTHs, VCN						
1.4.2.3. Train Veterinarians and Veterinary paraprofessional on use of the SOPs	4	FMAFS, FMLD, NVRI, VTHs, VCN						

1.4.2.4. Print and disseminate validated AMR strategy and SOPs	1	FMAFS, FMLD							
Activity 1.4.3. Expand integrated surveillance for index bacterial organism(s) in the food animal sector									
1.4.3.1 Hold a stakeholder consultative meetings to streamline AMR surveillance to follow the One Health initiative by aligning analysis and results with human data (e.g. for *E. coli*, *Staphylococcus* and *Salmonella* species)	3	FMAFS, FMLD, FMOH&SW,							
1.4.3.2. Expand the number of sentinel sites	5	FMAFS, FMLD							
Strategic intervention 1.5. Estimate prevalence of resistance amongst priority zoonotic bacteria to antibiotics that have been specified by the WHO as critical for use in humans and other antibiotics of importance to animal health, in food animals produced across Nigeria									
Activity 1.5.1. Antimicrobial resistant priority bacterial pathogens isolated from terrestrial and aquatic food animals across Nigeria									
1.5.1.1. Conduct active AMR surveillance in poultry (Broilers and Layers)	4	FMAFS, FMLD, State Veterinary Department							
1.5.1.2. Conduct active AMR surveillance in cattle, sheep and goats on farms and slaughter locations	4	FMAFS, FMLD, State Veterinary Departments							
1.5.1.3. Conduct active AMR surveillance in pigs on farms and	4	FMAFS, FMLD, State Veterinary Departments							

slaughter locations									
1.5.1.4. Conduct active AMR surveillance in African catfish and Tilapia on farms, selling points and markets	4	FMAFS, FMLD, State Veterinary Departments							
1.5.1.5. Isolate and identify priority zoonotic bacteria using microbiological methods	4	FMAFS, FMLD, NVRI, VTH							
1.5.1.6. Antimicrobial susceptibility testing of isolated priority bacteria	4	FMAFS, FMLD, Heads of Sentinel laboratories							
Activity 1.5.2. Antimicrobial resistant bacteria from sentinel laboratories further characterized and resistant mechanisms determined in the central laboratory									
1.5.2.1. Characterise *Salmonella* isolates using molecular methods	4	NRL							
1.5.2.2. Confirm MDROs at NRL	4	NRL							
1.5.2.3. Conduct MIC for selected classes of antimicrobials	4	NRL							
1.5.2.4. Identify resistance profiles including MDR and XDR	4	NRL							
Strategic intervention 1.6. Obtain and continue to enrich bacterial isolates for the national biorepository for future investigation.									
Activity 1.6.1. Use of AMR surveillance data for decision making at national and state level									
1.6.1.1. Develop	1	FMAFS, FMLD,							

national/local antibiograms		VTH, NVRI, State Veterinary Hospitals							
1.6.1.2. Publish antibiograms and share with relevant stakeholders	10	FMAFS, FMLD, FMOH&SW,							
Activity 1.6.2 Expand and establish surveillance focal points in food animal production value chains									
1.6.2.1. Determine and Map AMR surveillance areas to determine high risk areas.	1	FMAFS, FMLD, Farmers, , Feed processors							
1.6.2.2. Designate and train state-level AMR surveillance focal points in all the terrestrial and aquatic animals	37	FMAFS, FMLD, State Veterinary Department							
1.6.2.3. Develop national antibiogram	5	FMAFS, FMLD, Consultants							
1.6.2.4. Publish and disseminate antibiogram to relevant stakeholders									
1.6.2.5. Map surveillance points to determine high risk points									
Strategic intervention 1.7. Enhance surveillance data with further studies and mapping exercises									
Activity 1.7.1. Develop or review and validate the national residue monitoring and control									
1.7.1.1. Conduct a workshop to review and validate national drug residue monitoring and control plans	2	FMAFS, FMLD							

Activity 1.7.2. Build capacity of field officers on residue monitoring and control										
1.7.2.1. Conduct TOT for national officers on drug residue monitoring and control	1	FMAFS, FMLD, AMRCC, ACADEMIA								
1.7.2.2. Conduct cascade training of field officers on residue monitoring and control	6	FMAFS, FMLD, AMRCC								

4.0. SURVEILLANCE PROTOCOLS

4.1 Data Collection Methods and Standards

Laboratory Capacity: Laboratories should adhere to international standards, such as ISO/IEC 17025, and be equipped for accurate AMR detection. Data Management: Develop robust data management systems that facilitate the collection, storage, analysis, and sharing of AMR data.

4.1.1. Selection of target population

4.1.1.1 Poultry

There are three distinct categories of chickens produced and marketed in Nigeria: improved breeds grown and marketed for meat as "broilers," improved breeds grown for egg production and marketed and slaughtered as “spent layers,” and an indigenous breed of chickens (which may be mixed with improved breeds) that is raised under free-range conditions mainly in rural households and sold and slaughtered as “local chicken."
Chickens are raised in poultry farms across the four FAO operational classes as follows:
Sector 1: Industrial integrated system with high biosecurity systems.
Sector 2: Commercial poultry production system with moderate to high biosecurity.
Sector 3: Commercial poultry production system with low to minimal biosecurity.
Sector 4: Village or backyard production with minimal biosecurity.
The Animal Disease Control Act (1988) requires that only farms with 250 birds and above are required to register with the Veterinary Authority. In terms of population, the number of farms increases with increasing sector classification number; that is, fewer farms in sector 1 and farms in sector 4 being most numerous. A farm may contain one or more discrete houses. A number of combinations are available:

i. Farms with only one house with one type of bird (broiler or layer) of the same age. Farms with more than one house, with each house housing birds of the same type (broilers or layers) of different ages from the same or different hatcheries

ii. Farms with more than one house, with each house housing birds of different types of the same or different ages and from the same or different hatcheries

The majority of farms in Nigeria rear birds on a deep litter system, with occasional changes of litter material. Biosecurity practices are highly variable, including vaccination and drug use practices. Record keeping is at the discretion of the farmer in terms of type of record to keep, frequency, and consistency of recordings. It is not unusual to find farms without any records being kept. Farmers may also cull birds depending on availability of markets; "all-in all-out" practice is not strictly practiced. A large farm may cull birds gradually over days to take to distant or nearby live bird markets.

Birds in live bird markets may also be found in cages with or without separation of species. Birds may stay for days in the cages prior to sale and may mix with successive generations of birds awaiting marketing and/or slaughter. Feed and water may continue to be added to the same container without disinfection, and they may not be cleaned and/or disinfected between batches of birds. Such birds may be exposed to bacterial pathogens across generations from inhabiting the same cage over different times, though they may come from different sources. The birds are eventually slaughtered as individuals, when sold in singles, or as part of a batch of birds from the same source, when sold and slaughtered as a group.

There are very few poultry processing houses integrated with a commercial farm; such dedicated poultry processing plants are found in the south-west of Nigeria. The plants slaughter and process chickens from their own or other large-scale farms. There is a link between the slaughter records and farm records, so birds can easily be traced. Usually only one type of chicken from a common source is slaughtered at any given time. Local or rural chickens are not slaughtered in these plants (Table 4.1).

4.1.1.2 Ruminants and Pigs

Current data on the livestock population and their distribution in Nigeria is not available. However, recent estimates of the population of cattle, sheep, and pigs in Nigeria are put at 18 million, 42 million, 73.9 million, and 7.5 million, respectively (ASL 2050, 2019). There is also very limited information on the exact distribution in various production sectors. Generally, the population distribution for cattle is considered to be around 82% raised under pastoral systems, 17% under agro-pastoral systems, and 1% under intensive systems. These estimates correspond to the distribution of the other species along free-range (extensive), semi-intensive, and intensive production systems. Farms within the semi-intensive and intensive management systems that constitute less than 20% of each of the livestock species. A comprehensive, up-to-date, and reliable sampling frame must be generated prior to each stage of the surveys in consultation with governmental stakeholders (federal, state, and local governments) as well as non-governmental stakeholders, including producer associations, the Nigerian Veterinary Medical Association, and others.The following target populations of ruminants and pigs are selected for AMR surveillance and are arranged sequentially. The stages are not assigned a time frame; they represent the

prioritization sequence only, beginning with stages of the value chain closest to human consumption at existing animal health surveillance points (farms, abattoirs and slaughter slabs), as shown in Table 4.1

Table 4.1 Types of samples to be collected from target livestock species and surveillance points

Location		Livestock species and samples to be collected			
	Poultry	Cattle	Sheep	Goats	Pigs
Slaughter animals at abattoirs and slabs		Rectal	Rectal	Rectal	Rectal
Farms	Cloacal	Rectal Milk	Rectal	Rectal	Rectal
Retail markets		Meat	Meat	Meat	Meat
Live-birds market	Cloacal				

4.1.1.3 Aquaculture

In order to determine the contribution of aquaculture to spread of resistant pathogens of zoonotic importance, four sample types are usually collected for AMR surveillance in aquaculture, and these are the fishes, fish-feeds, pond water (within the pond and at point of discharge) and sediments. In Nigeria, the fish that are produced commercially under different scales of production for human consumption are catfish, tilapia and shrimps. All the stages of production in aquaculture (fry, fingerlings, juveniles, grow-out and adult) are very important in AMR dissemination, as such all the stages will be targeted in the current phase of aquaculture surveillance. Catfish is raised all over the country, while tilapia farming has substantial national spread and has attained economic significance, with the adoption of an intensive cage culture system of production. These two species are often reared in polyculture on many farms. Shrimp farming is very much limited with commercial shrimp farming recently established in Lagos state. It is expected over time that AMR surveillance will grow in aquaculture and include farmed shrimps, meanwhile catfish and tilapia represent the most important target species for surveillance due to their widespread production across the country and sampling will be exclusive on these two groups of food fishes.

4.1.2 Sampling Strategy

4.1.2.2 Poultry

The Avian Influenza Control Project (AICP) project created a list of registered poultry farms, but it is not routinely updated. Therefore, it will be important for the State Veterinary Services and the Poultry Association of Nigeria in each state to update the list of farms to be used as sampling frames.
For farms, Sectors 1 to 3 will be included in the surveys. The criteria for inclusion of the farms are:

I. Farms listed on the updated sampling frame are currently raising broilers near the point of sale (from the ages of 6 weeks and 50 weeks for layers). Farmers that disposed of their birds at point of lay (about 20 weeks) will be excluded. Samples will be collected from farms in Sectors 1, 2, and 3.
II. Samples will be collected from healthy birds only (excluding birds showing clinical illness).
III. One farm will be selected randomly out of every 4 farms on the sampling frame for each category of farms in Sectors 1 to 3.
IV. Where the same farm consists of more than one house housing the same bird type, the house with the oldest birds will be sampled. If the farm consists of broilers and layers, then one house for each category will be sampled.
V. Each farm will be visited once only.
VI. Geographical coordinates of selected farms should be captured to avoid repeat sampling of such farms within the study period.
VII. For LBMs, similarly, the AICP has documented LBMs, a list that can be updated with support from live bird marketer associations in each state to be used as a sampling frame. Inclusion criteria for the LBMs are:
VIII. Daily LBMs that have an adjoining slaughterhouse or slab and are located at an urban or periurban location.
IX. Maximum of two markets will be selected per state.

X. Live bird markets will be visited at two-week intervals until the required sample size is met.

4.1.2.3 Ruminants and Pigs

Animals at slaughter
The priority target population will be slaughtered healthy cattle, sheep, goats, and pigs at slaughter at abattoirs and slaughter slabs across the country. This means that all animals with obvious signs of disease at antemortem and postmortem will not be sampled.

A sampling frame will be generated by updating the list of all municipal abattoirs and slaughter slabs in each state of the Federation. Average slaughter figures will be generated for each slaughter facility. In each state, the slaughterhouse or abattoir with the highest number of average slaughter

figures will be enrolled. Abattoirs that slaughter more than one type of animal will be chosen for the surveillance. Where different slaughter facilities process different animal type, one each will be chosen for each animal type.

Prior to the surveillance, key stakeholders, including the butcher's association, will be engaged for their buy-in and support for the surveillance.

Animals on farms

For cattle, live cattle on farms, including dairy farms, will be targeted (Table 2). For sheep and goats, flocks kept on semi-intensive and intensive farms will be targeted for the surveillance. Similarly, pigs raised on semi-intensive and commercial farms will be sampled during the surveillance. The sampling frame will be provided by an updated list of farms in each study area. For the surveillance, only commercial and sedentary herds kept on farms or are part of cattle cooperatives, such as peri-urban dairy projects, will be included in the survey. For dairy herds, one bulk milk sample will be used to represent the herd.

Sheep and goat farms that house the animals separately will be included in the survey. Free-range flocks will not be sampled because they are generally more mobile, are not raised on farms, and are not easily organizable for surveys. Flocks that are raising animals for human consumption will be tested. Similar criteria will be used for pigs, with only animals on semi-intensive and intensive production systems being sampled. Free-range herds will be excluded. Individual herds or flocks will constitute the sampling unit, with one aggregate (pooled) sample representing the unit or farm.

For retailed meat surveys, the largest municipality in each selected state will be used. A sampling frame will be developed in conjunction with the State Butcher's Association to document all points where meat is retailed in markets and shops. The list of markets and meat shops by location will be used as a sampling frame. The number of samples to be taken will be allocated based on the number of sampling points in each state.

4.1.2.1 Aquaculture

The surveillance will be conducted across the country. The 36 states and the Federal Capital Territory will constitute the primary surveillance areas. The country is divided into six geopolitical zones namely North east, North central, North west, South east, South south and South west. Two states with the highest number of fish farms in each geopolitical zone will be enrolled. Making a total of 12 states out of the 36 states (see Appendix I).

For the sampling frame, a list of fish farms that produce catfish and or tilapia will be generated through multi-stakeholder consultation, involving Federal Department of Fisheries, State Veterinary Departments, Fish Farmers Associations, State Fisheries Departments, feed millers and other relevant stakeholders including fish-mongers and processors. The number of samples will then be proportionately distributed in accordance with the number of farms in the states.

Any pond with catfish and tilapia at all the stages of production (fry, fingerlings, juveniles, grow-out and adult) will be included in the survey. All fish farms or ponds currently experiencing disease episodes will be excluded.

4.1.3 Sample location

The entire country will be considered the surveillance area. Nigeria is divided into 6 geopolitical zones (GPZs); with each GPZ, there are 5 to 7 states (Appendix I). In each GPZ, the two (2) states with the highest estimated poultry population will be enrolled in the surveillance (Appendix II). A total of 12 states across the country will participate in the survey as follows (Table 4.2):

Table 4.2. Distribution of number of states to be selected in the 6 geopolitical zones

Geopolitical Zone	North East	North Central	North West	South East	South South	South West
Number of States	6	6	7	5	6	6
Number of studies areas	2	2	2	2	2	2
Laboratory	None	Ilorin NVRI 2	Zaria Sokoto	Nsukka	None	Ibadan

A study area is defined as two contiguous administrative areas (states) in a GPZ that have a higher poultry density. Each study area is matched to a designated laboratory, where samples from that area will be tested.

4.1.4 Sample size

4.1.4.1 Poultry

The approach for determining the sample size for cross-sectional studies will be adopted using a precision of 5% level of significance for the estimate. Since at the point of sampling the prevalence of resistance is unknown and likely to vary among different bacterial organisms, an assumed average prevalence of 50% will be used in calculating the sample size. Therefore, a calculated sample size of 384 will be used and adjusted to 400 samples for each bird type. Since two categories of birds (broilers and spent layers) will be sampled, the total number of samples will be 800, with 400 for each bird type. Here estimates of prevalence will be made for the entire country (see Appendix II for detailed distribution of samples). Due to the lack of complete and updated data on poultry farms, sampling will be based on the relative distribution of poultry farms across the sectors as described by Adene and Oguntade (2006). In each sampling area, a maximum of two farms from Sector 1 will be sampled. A ratio of 1:4:16 (number of farms in Sector 1: number of farms in Sector 2: number of farms in Sector 3) will be used to determine the number of farms to be sampled and to distribute samples across the sectors (Appendix VI). All farms will be sampled only once, and only one sample will represent each farm. If a farm has more than one house with different types of birds (that is, broilers in one house and layers within the eligible age groups as defined for the survey), each house will be treated as equivalent to a separate farm, with each differing house constituting a sample.

The remaining samples will be taken at live bird markets and will be equally divided between live birds and slaughtered birds. Birds from the same source will be sampled only once, either live or

slaughtered, but not both. Only apparently healthy birds will be sampled. In each selected market, a list of marketers will be used as the sampling frame; the sampling unit will be a marketer with a batch of birds, either broilers or layers. Information on the source of the birds will be obtained prior to sampling, including birds from farms that were previously sampled as well as birds whose origin is unknown. If a marketer has both broilers and layers, the birds will be treated as separate batches as long as they are not mixed in the cages, and each batch will be eligible for sampling. Marketers will be selected randomly, and up to a maximum of ten pooled samples will be taken per market (Appendix V1).

For slaughtered chickens, a batch of broilers or layers coming from the same source or farm will be considered a unit. Only one sample will be taken from a unit. Birds slaughtered later coming from the same source as those previously sampled will not be included in the survey. Up to a maximum of 10 batches of birds will be sampled per market. Where sampling in the market could not be concluded in one visit, a repeat visit will be undertaken after two weeks in order to ensure that the previous set of birds will not be encountered.

4.1.4.2 Ruminants and Pigs

Sample size will be calculated based on the available prevalence of target bacteria in each category of animals to be tested using the formula for a cross-sectional survey at 5% level of precision (95% confidence level). Equal prevalence (50%) will be assumed for all target bacterial species to allow testing of each sample for each target bacterial organism (FAO, 2019). For detailed breakdown of the sample sizes, see Appendix VI1. Table 4.3 provides a summary of the number of samples to be taken from each of the four livestock species from abattoirs, farms, and markets.

Table 4.3: Types and number of calculated samples to be taken from target species of livestock for AMR surveillance in Nigeria.

Species	Sample Types				
	Faecal (Farms)	Caecal (Abattoir)	Retailed Meat (Markets)	Bulk Milk (Farms)	Total
Cattle	2922	8064	8400	4858	24244
Goats	8960	2026	2414	-	13400
Sheep	9834	7074	3840	-	20748
Pigs	1214	3102	6451	-	10767
Total	22930	20266	21105	4858	69159

4.1.4.3 Aquaculture

The prevalence of AMR resistance among isolates from fish is presumed to be even more variable than poultry due to complexity introduced by varied production conditions. Aquaculture production systems varied and AMR resistance among both *Vibrio* and *Aeromonas* were reported, using different AMR test procedures. For the active AMR surveillance, an assumed prevalence of resistance of 50% will be used at

a precision of 5% and 95% confidence level to calculate the number of bacterial isolates for the survey in the different sampling units (fry, fingerlings, juveniles, grow-out and adult, fish-feeds, pond water and sediments). In order to determine the number of samples to take, the likelihood of isolation of target bacteria will be used as a factor to calculate the sample size as previously described (citation). For each of the sampling units a total of 3506 was calculated, however, it is rounded to 3528 for ease of distribution. The samples will be collected over a period of one year see Appendix (Va-Vg).

4.1.5 Target organisms

Pathogens of fish that are causal factors of common bacterial diseases of fish will be targeted for AMR surveillance. These organisms isolated from the routine clinical examinations in the sentinel laboratories will be subjected to antibiotic sensitivity test, and the AMR status determined, as these are pathogens that have probably been exposed to antibiotic mis-use over time. They may not be of importance in foodborne diseases, but their resistant genes may be shared with poultry, livestock and human bacterial pathogens in the environment, increasing the AMR burden. These fish pathogens include: *Aeromonas* spp., *Flavobacterium columnare*, *Pseudomonas* spp., *Edwarsiella tarda*, *Streptococcus* spp., *S. agalactiae* and *Vibrio* spp.

It should be noted that the incubation temperature for fish bacterial pathogens is 30^0C and **NOT** 37^0C. Also, some of these bacterial organisms are fastidious, and will require a special growth medium.

The following priority pathogens from livestock species intended for human consumption will be targeted. *Escherichia coli* and *Enterococcus* spp. are considered commensal organisms, while *Salmonella* spp. and *Campylobacter* species are important foodborne zoonotic pathogens that have a major impact on human health worldwide. These organisms will be isolated from four animal types from different samples obtained from abattoirs, farms, and markets. In addition, milk obtained from dairy farms will also be tested for two important milk-borne zoonotic pathogens, namely *Staphylococcus aureus* and *Listeria monocytogenes*. For aquaculture, *Vibrio, Streptococcus*, *Aeromonas* species, E. coli, Campylobacter and Salmonella spp. will be isolated (Table 4.4).

Table 4.4: Priority bacteria to be isolated from sampled target animals at abattoirs, farms and retail markets.

SN	Animal type	Sampling locations	Sample type	Target Bacteria
1	Aquaculture	Farm	Fish sample	*E. coli, Salmonella, Campylobacter, Enterococcus* spp., *Vibrio* spp., *Aeromonas* spp., *Streptococus* spp.

			Water	*E. coli, Salmonella, Campylobacter, Enterococcus,* spp. *Vibrio* spp., *Aeromonas* spp., *Streptococcus* spp.
			Sediments	*E. coli, Salmonella, Campylobacter, Enterococcus,* spp. *Vibrio* spp., *Aeromonas* spp., *Streptococcus* spp.
			Feed	*E. coli, Salmonella, Campylobacter, Enterococcus,* spp. *Vibrio* spp., *Aeromonas* spp., *Streptococcus* spp.
		Feed Mill	Feed	*E. coli, Salmonella, Campylobacter, Enterococcus,* spp. *Vibrio* spp., *Aeromonas* spp., *Streptococcus* spp.
2	Poultry	Farms	Faecal	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
		LBMs	Faecal	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
3	Cattle	Abattoir	Caecal	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
		Farms	Faecal Milk	*E. coli, Salmonella, Campylobacter, Enterococcus* spp., *Staphylococcus aureus, Listeria monocytogenes*
		Retail Market	Meat	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
4	Sheep and Goats	Abattoir	Caecal	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
		Farms	Faecal	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
		Retail Market	Meat	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
5	Pigs	Abattoir	Caecal	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.
		Farms	Faecal	*E. coli, Salmonella, Campylobacter, Enterococcus spp.*
		Retail Market	Meat	*E. coli, Salmonella, Campylobacter, Enterococcus* spp.

4.1.6 Sample collection

Sample collection from poultry farms

In poultry farms, birds will be captured randomly, and cloacal swabs will be taken. Up to ten cloacal swabs can be pooled into one sample representing the house if they come from the same type of bird. Each pooled sample constitutes only one sample. After proper restraint, the bird should be held with both wings folded by an assistant, ensuring that the bird is not in distress. The person taking the sample should then insert the tip of a sterile cotton swab stick into the vent of the bird while avoiding contact with the surrounding skin. The swab is then gently rotated inside the vent while rubbing on the mucosal surface of the vent. The swab tip should be covered with fecal material from the vent. Swabs for Campylobacter culture should be placed into 10 ml of transport medium, such as Cary Blair broth medium. The long handle of the swab stick may be cut with sterile scissors and discarded, while swab tips are retained for testing. All swabs should be transported on ice to the laboratory.

Sample collection from live bird markets and poultry abattoirs

In live bird markets, cloacal swabs will be taken from birds in the cages and will be used as samples. Up to 10 cloacal swabs can be pooled if belonging to the same type of bird and source in a cage. Caecal samples will be collected from live bird markets at slaughter. Caecal samples can be collected in groups of five in a pool or individually as previously described (McKenzie *et al.,* 2019). Background epidemiological information from the LBM will be collected using the form outlined in Appendix (X).

In processing plants, pooled caecal samples as described above will be used to represent a batch of birds from the same source. Systematic random sampling will be used to select five birds from the processing line. From each bird, intact caecum will be used and pooled in sets of five caeca. Each pooled sample represents only one sample.

Ruminants and Pigs

Rectal samples

Samples will be collected over time using periodic visits at a two-week interval. During each visit, the population of slaughter-stock for each category will be determined. Systematic random sampling will be used using an interval of 5 to randomly select animals for sampling. Where the number of slaughtered animals cannot be determined at the beginning of the day, the sequence of slaughter or entry into the slaughter hall will be used to count and select every 5^{th} animal to the end of the day. Repeat visits will be made after two weeks until the required sample size is met.

From each selected slaughtered animal, rectal samples will be collected after evisceration. About 5-10g of rectal or approximately 10 g of rectal contents will be collected into a sterile tube after incising the rectal just before the junction with the intestines. If the animals were obtained from the same farms, only one animal should be tested.

Faecal samples

Each farm selected from the sampling frame will be visited at roughly 3 monthly intervals, as much as possible, varying the season of sampling. During each visit, the number of houses on the farm will be determined, and each house will be randomly selected.

Due to the nature of housing in farms in Nigeria, where most farms do not use concrete flooring and animals are sometimes kept in open compounds, boot socks will not be used. Faecal samples will be obtained from freshly laid pen floor fecal deposits. From each deposit, a tongue depressor or sterile spoon will be used to take approximately 10 g of fecal material from the pile. For smaller

animals like sheep and goats, freshly laid fecal droppings on the pen floor that have not been contaminated should be picked. Up to five deposits can be picked and pooled into a sterile plastic bag to represent the herd or flock. Where fresh fecal deposits are not available. Five randomly selected animals can be restrained and fecal material obtained directly from the rectum without compromising the welfare of the animals. The five fecal samples can be pooled into one sample to represent the farm.

Meat samples

In each point of sale or sampling point, meat samples will be collected as offered to the public for sale. Approximately 10 g of muscle meat will be collected in a sterile container and transported on ice to the laboratory.

Milk samples

The first morning bulk milk sample will be collected from each herd. The bulk milk sample will represent the milking herd. From each herd, samples will be collected from the bulk tank after pooling and mixing the morning milk yield. Where there is more than bulk milk tank aliquots will be taken from each tank and pooled into one sample. From each bulk tank, approximately 100 ml of milk sample will be collected aseptically into a sterile tube. The sample will be immediately placed in a cold box for onward transportation to the laboratory.

Collection of attribute information from abattoirs and farms

Attribute data will be collected that will identify the animal by sex, breed, age group, and source (data collection template to be developed for each surveillance plan). Where the origin of the animal cannot be determined, the last market or location from where the animal came to the abattoir should be determined and recorded. (See Appendix for sample collection form).

Aquaculture

Data regarding the collection of fish, water, sediment and feed samples will be recorded in the form in Appendix VIII.

Fish Farms

On each farm, catfish and tilapia at different production stages (fingerlings, juveniles, grow-out and adult), fish-feeds, pond water and sediments would be sampled. Where there is more than one pond with such a type of fishes, then one pond should be randomly sampled to represent the farm for each fish group. The location from which the fish would be picked will depend on the size of the pond. For small and moderately sized ponds, fish should be captured from the centre of the pond. For large ponds, the pond will be divided into equi-distant grids and one grid is randomly selected for the capture of the fish. Two samples from each of the four aquaculture production stages should be collected in any case, placed in sterile plastic bags, covered with ice and transported to the laboratory for bacterial isolation. Each sample containers will carry the identity and characteristics of the sample collected.

Sampling Water

Water samples can be collected from the centre of the pond using sterile 500mls sampling bottles, during the collection the water surface should be devoid of floating organic matter such as leaves or sticks and as much as possible dirt from the sediment should not be disturbed while collecting the water sample. The sampling bottle will be quarter-filled with water sample collected about 50

cm below the surface and rinsed three times with the pond water before finally filling the bottle with the water sample leaving a small air space at the top. Water samples also should be kept on ice as soon as collected for transportation to the laboratory. Each sample containers will carry the identity and characteristics of the sample collected.

Sampling Sediments

Sediments from the bottom of the pond should be collected using a Van Veen grab or sterile scoop, water pipe scooping or using a conventional suction tube, 5-10 cm thickness of the sediments and about 100g will be transferred into sterile plastic bag and place also on ice for transportation to the laboratory. Each sample containers will carry the identity and characteristics of the sample collected.

Sampling Fish-Feeds from Farms and Feed-Mills

For fish-feeds, using sterile latex hand gloves feed sample should be collected from the portion of the feed meant for use on the day of sample collection. The feed should be collected at 10 cm thickness of the feed and about 50 g will be transferred into sterile plastic bag and place on ice for transportation to the laboratory. Each sample containers will carry the identity and characteristics of the sample collected.

Sampling Team

In order to ensure a smooth sampling exercise, a multi-stakeholder team pre-trained on the surveillance protocol will undertake sampling. Each team should consist of:

- A veterinary officer from state veterinary services
- A private veterinarian
- A microbiologist from the zonal or central laboratory
- Federal Epidemiology Officer in the State

4.1.6 Sample Preservation and Transport/Shipment of Samples to the Designated Sentinel Laboratories

Terrestrial Animals (Poultry, Ruminants and Pigs)

For samples caecal and fecal that will be subjected to *Campylobacter* isolation, a duplicate sample will be taken and inoculated into a 10 mL broth containing Cary Blair medium. All the various sample types in bags or tubes must be securely stored such that the mouths of the bags and tubes are tightly sealed to avoid leakages. After the initial packaging, the sample will be properly labeled with mandatory information, including sample identity, source, sampler, and date, in a manner that it could withstand temporary storage before the laboratory, particularly by use of water-proof labels and labeling. All samples will be kept immediately collected in a cold box packed with ice and transported to the laboratory within 3-5 hours after collection. If samples are going to stay for longer periods, such samples will be kept in a refrigerator at 5^0C. All samples will be transported by road on ice to the laboratory. Shipment of samples from farms or live bird markets should be in accordance with WHO recommended procedures for shipment of biological samples (WHO, 2019).

Aquaculture

All fish (fry, fingerlings, juveniles, grow-out and adult), feeds, pond water and sediment samples will be labeled and transported in clean and sterile plastic bags placed on ice or preserved in a refrigerator immediately after collection and transported to the laboratory at temperature of less than 5^0C for processing within 3-5 hours after collection. Post-mortem changes are rapid in fish and should be avoided.

For the active surveillance, whole fry will be macerated in sterile normal saline, in a clean, sterile, ceramic mortar and pestle to generate homogenate. In the fingerlings, juveniles, grow-out and adults, cotton swab moistened with sterile normal saline will be used to collect inoculum from the skin and the gills. However, in the passive surveillance, while the same protocol will be used for the fry, autopsy will be conducted on the fingerlings, juveniles and the adult fish samples, and inoculum will be aseptically collected from organs with lesion(s), including the dorsal kidney.

It must be noted that some of the common bacterial pathogens of fish are fastidious, and requires specialized or selective growth medium.

4.2 Laboratory Procedures and Quality Assurance

The isolation and antibiotic sensitivity testing of *E. coli, Salmonella, Enterococcus,* and *Campylobacter, Listeria, Staphylococcus aureus, Vibrio* spp. and *Aeromonas* spp. will be performed following the procedures described in the surveillance guidelines (Appendix III). However, procedures for isolation, identification of bacteria, and antimicrobial susceptibility testing (AST) shall be conducted in accordance with the routine approach to samples submitted for bacteriological examination in the OIE guidelines (Buba, 2018). All tests shall be performed in accordance with laboratory Standard Operating Procedures (SOPs) for surveillance in animal health laboratories.

4.2.1 Sample Processing

Poultry

Each sample will be cultured for *Escherichia coli*, *Salmonella* species, *Enterococcus* species, and Campylobacter species using standard microbiological protocols (Appendix III). Where appropriate, transport medium should be used to sustain samples to the laboratory. Bacterial identification may involve biochemical testing, serological tests, or molecular identification or confirmation of isolates or species according to standardized procedures (Appendix III).

Ruminants and Pigs

Preliminary Processing of Meat Samples: Meat samples will be processed prior to bacteriological isolation for specific target bacteria according to the methods specified by USDA-FSIS (USDA-FSIS, 2020). The target bacteria will be isolated using standard bacteriological procedures specified in Appendix III.

4.2.2 Isolation, identification, and handling of bacterial isolates

Inoculation of samples on suitable solid culture media:
The inoculation method on solid and liquid culture media is designed to ensure that isolated bacterial colonies are present to facilitate subculture and bacterial identification. The goals of primary inoculation are:

1. To cultivate bacterial priority pathogens while minimizing the growth of contaminants.
2. To obtain discrete colonies of organisms to allow selection of the pathogen(s) for subculture and identification. See Appendix IIIa.

To culture bacteria from a specimen, inoculate the edge of an agar plate with the sample material using a swab or sterile wire loop. After streaking out the inoculum, dilute the inoculum to a point when individual and separate colonies will develop. On well-prepared culture plates, individual bacteria will grow into discrete colonies, and the growth characteristics of these colonies can then be described. If streaking is not done properly, non-pathogenic contaminants may overgrow the more fastidious pathogenic organisms, making them difficult to detect.
Identification of bacteria
After primary culture and incubation, the plates are examined, and colonies can be described based on their characteristics. If, after 24 h, bacterial growth is not readily observed, the plates should be replaced in the incubator and read again after a further 24 h. The number of colonies of each type of bacteria may not directly relate to the number of bacteria present in the sample, nor do they indicate the relative significance of each type of bacteria isolated. Pure or predominant growth of one type of organism is usually a significant result. see Appendix IIIa

Bacterial colony characteristics
Colonial morphology typical of target bacteria based on shapes, size, consistency, color will be used for cultural identification.

Interpretation of culture results:
To interpret microbiological findings, it is important to know the characteristics of the normal commensal bacteria found in different species of animals and to have a good case history. The case history should be provided by the submitter on the submission form. Once a bacterial culture has grown, it is routine practice to subculture and stain bacteria from individual colonies using Gram stain (or other stains) to assist in the identification of bacterial isolates of interest.
Biochemical Testing
Preliminary biochemical tests will be carried out. Further biochemical tests and selective media are usually necessary to identify the organisms to family and genus level. Before this can take place, a subculture is made of each colony of interest to produce a ‘purity’ plate. These plates are incubated further until a good pure growth of bacteria is present. From the purity plate(s), a suspension of bacteria can be prepared for inoculation of secondary media and biochemical reagents. Detailed biochemical tests should not be performed on mixed cultures, as the results will be impossible to interpret. Broths made from purity plates can be used for a wide range of biochemical tests and can also be used to determine the antibiotic sensitivity of the suspect pathogen(s).

4.2.3 Antimicrobial susceptibility test

Four bacterial organisms *Escherichia coli*, *Salmonella*, *Campylobacter,* and *Enterococcus* species are selected as priority organisms for the One Health-integrated AMR surveillance because of their importance in zoonoses and antimicrobial resistance and inclusion in the WHO GLASS. In addition to these four organisms, *Listeria monocytogenes* and *Staphylococcus aureus* will be considered in ruminants and pigs. In aquaculture *Vibrio* spp and *Aeromonas* spp will be considered. *Escherichia coli* and *Salmonella* species are important zoonotic pathogens. *Campylobacter* species, though commensal bacteria in birds, are among the most important causes of human gastroenteritis. *Enterococcus* is a commensal organism highly associated with antimicrobial resistance, facilitating the sharing of ARGs between Gram-negative and Gram-positive bacteria.

These priority bacterial pathogens (isolated from sources in this survey) will be subjected to antimicrobial sensitivity testing (AST) in accordance with the recommendations of the Clinical Laboratory Standards Institute (CLSI). In general, the disk diffusion method will form the core of the resistance testing. In some instances, such as when evaluating resistance to colistin, vancomycin, or other peculiar situations, the minimum inhibitory concentration of the antibiotic as expressed by the isolate will be determined using E-test strips, microbroth dilution, or other prescribed methods at the National Reference Laboratory at NVRI.

The principle of the antibiotic sensitivity test is to determine whether bacterial growth occurs when cultured colonies are exposed to a selection of antibiotics. This does not precisely mimic the conditions found in the animal host, but the results can still be useful when choosing a specific antibiotic treatment. The minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) of selected antibiotics for an isolate are accurately measured. It is usually sufficient to use a simple qualitative assay, which distinguishes sensitive from resistant isolates to a particular antibiotic in line with the probable outcome of therapy:

Manual methods

1. **Disc diffusion antibiotic sensitivity testing using Kirby-Bauer disc diffusion assay**

The Kirby-Bauer disc diffusion assay uses a selection of small discs of a standard filter paper containing predetermined amounts of chosen antibiotics. These discs are placed on large (140 mm) Petri dishes of culture medium. The choice of culture medium used in the assay will determine the interpretation criteria used for determining sensitivity. If Muller-Hinton agar (MH) +/- 5% sheep blood is used, the interpretation criteria of the Clinical and Laboratory Standards Institute (CLSI) can be used. If Luria-Bertani (LB) or Brain-Heart Infusion Agar (BHI) are used, interpretation criteria will be determined by comparing inhibition zone measurements between the sample strain and control strains.

Petri dishes are spread uniformly with an inoculum of the bacterial isolate to be tested, and after incubation at 37°C for 18–24 h, determination of sensitivity or resistance is made by measuring the visibly clear area around the discs, which is the zone of inhibition of bacterial growth. The 'zone of inhibition' depends on the diffusion of the antibiotic from the discs into the surrounding agar, and the size depends upon characteristics of the growth medium and of the antibiotic compound that influence diffusion of the antibiotic and does not directly relate to the degree of sensitivity or resistance of the bacteria to the antibiotic (that is, a large zone of inhibition does not by itself indicate resistance).

To perform the test, it is more convenient to use dry antibiotic discs prepared and supplied commercially. The discs are 6 mm in diameter and consist of absorbent (filter) paper impregnated with a known amount of antibiotic. Each disc is marked with a letter to show which antibiotic is present.
Kirby-Bauer disk diffusion is the most used method for performing AST. Results for disk diffusion AST should be reported as zone inhibition diameters only. Each antibiotic's zone inhibition diameter should be measurable. Overlapping zones prevent accurate measurement. Measure the diameter of the zones of complete inhibition, including the diameter of the disk. Hold the Petri plate a few inches above a black background illuminated with reflected light. AST zone diameters of vancomycin for *Enterococcus* spp and linezolid for *Staphylococcus aureus* are to be measured using transmitted light.

Note:

i). The disk diffusion method cannot be used to determine the susceptibility to colistin in Gram-negative bacteria

ii). *Staphylococcus aureus* resistant to linezolid by disk diffusion should be confirmed by determining the MIC by broth micro-dilution or using an automated AST system.

2. **Agar Pour screen method**:

AST for Vancomycin in *S. aureus* requires testing by vancomycin agar screen (VAS) on Brain Heart Infusion (BHI) agar containing 6 µg/ml vancomycin. Strains that show growth on 6 µg/ml BHI vancomycin plate should be confirmed by broth micro-dilution.

3. Broth micro-dilution (BMD): AST for colistin in all isolates of *E. coli*, *Klebsiella* species and *Pseudomonas aeruginosa* requires testing by broth micro-dilution. Results should be reported as MIC.

Colistin Agar dilution test: AST for colistin in all isolates of *E. coli*, *Klebsiella* species and *Pseudomonas aeruginosa* can also be done using the colistin agar dilution test. Results should be reported as MIC.

Automated AST systems

If a facility performs AST using an automated AST system, results should be reported as MIC for all pathogen-antibiotic (i.e., "bug-drug") combinations tested. If AST by BOTH manual disc diffusion and automated testing methods are performed, MIC results are preferred

Broth dilution method for colistin testing

This SOP describes the standard broth micro-dilution method used to determine the *in vitro* susceptibility to Colistin for aerobic Gram-negative bacterial isolates in Clinical Microbiology laboratory. It addresses the preparation of Cation-adjusted Mueller Hinton Broth (CAMHB) and colistin stock solution for dilution tests, testing conditions (including inoculum preparation, use of selective quality control (QC) strains, incubation time, and temperature), determining minimal inhibitory concentration (MIC), result analysis using approved breakpoints and QC procedures.
The broth micro-dilution method should be used to quantitatively measure colistin's *in vitro* activity against aerobic Gram-negative bacterial isolate. The method described herein is intended

primarily for testing aerobic Gram-negative isolates that grow well after overnight incubation in un-supplemented CAMHB.

Selected Antibiotics

The antibiotics listed in Table 4.5 below that are critically important in human medicine will be tested against the four priority organisms in poultry and additional two pathogens in ruminants and pigs using the CLSI testing and interpretation guidelines. In the event there is no cut off for CLSI use EUCAST.

Table 4.5: Priority bacteria and target antibiotics

SN	*Escherichia coli*	*Salmonella* spp.	*Campylobacter* spp.	*Enterococcus* sp.	*Vibrio* spp.	*Aeromonas* spp.	*Streptococcus* spp.
1	Gentamicin		Gentamicin Streptomycin	Gentamicin Streptomycin	Gentamicin	Gentamicin	Gentamicin
2	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol
3	Meropenem	Meropenem	Erythromycin	Erythromycin		Erythromycin	Erythromycin
4	Ertapenem	Ertapenem		Teicoplanin	Imipenem	Imipenem	Imipenem
5	Ceftriaxone	Ceftriaxone		Vancomycin	Cefotaxime	Cefotaxime	Cefotaxime
6	Cefepime				Ceftazidime	Ceftazidime	Ceftazidime
7	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Quinipristin/Dalfopristin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
8	Nalidixic acid	Nalidixic acid					
9	Ampicillin	Ampicillin	Ampicillin	Ampicillin	Ampicillin	Ampicillin	Ampicillin
10	Colistin	Colistin		Linezolid		Vancomycin	Vancomycin
11	Tetracycline	Tetracycline	Tetracycline		Tetracycline	Tetracycline	Tetracycline
12					Sulphamethoxazole - Trimethoprim	Clindamycin	Clindamycin
13						Rifampicin	Rifampicin

* A separate panel each will be used for *S. aureus* and *L. monocytogenes* in accordance with CLSI (19, 20) recommendations, detailed procedure provided in Appendix III include streptococcus on the table

4.2.4 *Molecular Identification and Whole Genome Sequencing of Priority Pathogens*

Utilize a standardized operating protocol (SOP) for nucleic acid extraction, PCR amplification for pathogen identification using specific primers, and electrophoresis conditions for nucleic acid separation. This includes the adoption of specific PCR conditions, mixtures, and reactions tailored for the identification of priority pathogens. The PCR conditions should be optimized for conventional PCR techniques. Confirmed isolates at the reference laboratory (NVRI, NCDC) will be subjected to whole genome sequencing.

4.2.5 Quality Assurance

Clear, Standard Operating Procedures (SOPs) should be developed for sample processing, culture, isolation in all sentinel laboratories, and confirmation of isolates in the reference laboratory. Internal quality control should be used to ensure that the laboratory procedures are functioning correctly. This includes positive and negative controls for every batch of samples tested. External Quality Assurance Proficiency Testing: Participate in external proficiency testing programs where unknown samples are tested and results are compared against a standard to evaluate laboratory performance. Inter-laboratory Comparisons should be conducted regularly between sentinel laboratories to ensure consistency in test results across different laboratories involved in the surveillance network. Relevant quality control strains (ATCC or NTCC) should be used at all stages of sample processing, cultural identification, and antimicrobial susceptibility testing.

Mandatory Clinical and Epidemiological Details of Isolates from terrestrial and aquatic animal Samples

The demographic and clinical data fields must be reported. All the variables are mandatory for data entry and attempts should be made to collect the details of the patients, including the location of the farm, hatcheries and retail outlets. All attempts should be made to retrieve and enter demographic data for each unique isolate. See relevant Appendices

4.2.6 Isolates Shipment

If a bacterial isolate is being shipped from one laboratory to another, as in the case of isolates for confirmation or biobanking from a sentinel laboratory to a national reference laboratory, it will be transported in accordance with the provisions of shipment of Category A & B (UN 3373) biological material (WHO, 2019), see Appendix (IIIb)

4.3 Data Management, Analysis, and Interpretation

4.3.1. General Considerations in Data Management

The data from expanded AMR surveillance will be in accordance with the data management strategy provided in the terrestrial and aquatic animals surveillance protocol.

The quality of data is key to the success of any surveillance system. Therefore, attention must be given to the accuracy of data capture and entry at all stages. Data capture across the surveillance areas must be uniform with the use of consistent authorized recording templates. Software to be

used needs to be compatible with other commonly used software and laboratory information management systems and WHONET. Relevant personnel must be trained on data management prior to the surveillance. The safety and confidentiality of the data must also be ensured at all stages of the surveillance, with data reporting only through officially designated channels. The obligations of the laboratories and FMARD and in data management will be specified under a Memorandum of Understanding developed under the Fleming Fund intervention in Nigeria.

4.3.2 Data Management

4.3.2.1 Data management during sampling

During field sample collection, information must be accurately captured. Sampling information consists of two linked components. The first component is the information on sample identity, while the second is the attribute information that provides the background epidemiological information about the sample captured using the forms in Appendices VIII-XIb. All the variables and responses recorded in the forms will be recorded into a spreadsheet by the veterinary epidemiologist. After each round of sampling, the information will be entered clearly, identifying the round of sampling dates, locations, the samples collected, and the person(s) responsible for sample handling. Locations and other detailed attribute information.

4.3.3 *Data management in the sentinel laboratories*

In the laboratory, information on submission of samples and sample identity, including source, date of submission, source, and persons responsible, will be recorded at the field or sentinel laboratories for each round of sampling. In the laboratory, such data can be entered into Microsoft Excel, WHONET, LIMS, or other related software (note a data management system is being developed for the project).

Sampling testing data constitutes the second component of the laboratory data. For each sample, the outcome of bacteriological isolation will be recorded alongside sample information in a template uniform across the laboratories. The results to be recorded include

1. Results of primary isolation
2. Results of biochemical testing for preliminary identification of the isolates. Isolation rates in percentage will be recorded for each of the target bacteria based on location (sampling area, geographical information), source (farm, pond water, fish-feed, sediments, market, processing plants), bird type (broiler, layer), and sample type (skin or gills, cloacal or faecal, milk and meat), type of fish (catfish and tilapia) cloacal, caecal).
3. Results of AST will be recorded according to the CLSI criteria for scoring. The zone diameters produced on disc diffusion testing for each disc will be recorded in millimeters. For each antibiotic in the panel tested and for each isolate type, the percentage sensitivity and/or resistance will be recorded.

4.3.4 Data management in the National Reference Laboratory

The National Reference Laboratory (NRL) at the NVRI will collate all isolates, results of bacterial isolation and identification, and that of AST from all seven sentinel laboratories and aggregate into

an AMR database based on WHONET, LIMS or related software. Outcomes of further testing will be recorded at the NRL, including:

a) Further serological characterization of isolates using serological tests, like in the case of *Salmonella* spp., or molecular identification methods, including detection of molecular mechanisms of resistance. Serological identification of *Salmonella* spp. should record the serotype identified in each sample submitted by the sentinel laboratories, as well as the species identified for other target bacteria from each sample. The rate of isolation of species, serotypes, or genotypes with the distribution of their sensitivity or resistance will be recorded.

b) Minimum inhibitory concentration (MIC) determination for specific antibiotics, particularly those required by the CLSI guidelines, such as in the case of colistin, vancomycin, and others, will be conducted and reported in the NRL. The MIC values as micrograms/mL will be recorded for each isolate for specific target antibiotics, and the outcomes and distribution of sensitivity and/or resistance will be recorded in percentages based on isolate types and other criteria.

Aggregate laboratory data from all sentinel laboratories and additional data generated at the NRL will be forwarded for further analysis and actions by NADIS and AMRIS.

4.3.5 Data management at the NADIS

All field data captured during sampling using the forms attached in Appendices VI and V will be collated from all sampling areas by NADIS. Also, all laboratory data generated from target bacterial isolation and identification and AST testing will be collected by NADIS from the NRL. The two compartments of the data will be merged for individual samples, sources, and other epidemiological categorizations and analyzed for distributions, trends, and further statistical analysis to work out the prevalence of individual resistance, multidrug resistance, and resistance mechanisms in the country based on various classifications done by the epidemiologists in NADIS. Finally, NADIS will report relevant findings from the Chief Veterinary Officer, who maintains the prerogative of reporting to the National AMR Steering Committee and other national, regional, and international stakeholders as may be required. Interpretation of the AST result should be interpreted using the CLSI-Vet standards for the national AMR surveillance network.

4.4 Data Analysis and Interpretation

AST data will be analyzed using descriptive measures by providing an overview of the resistance pattern through isolate listing and presentation of a chart on the resistance profile of isolates against tested antimicrobials in WHONET. A heat map can be used in Excel to show categorized isolates as wild type, non-wild type, sensitive, intermediate, or resistant. Resistance pattern analysis can be done to identify the most common pathogens and their associated resistance patterns across different sectors and regions. Analyze the distribution of resistance to specific antibiotics within and between sectors (aquaculture, poultry, ruminants, and pigs). Temporal and spatial analysis can be done to identify changes in resistance patterns over time, such as monthly, quarterly, or yearly trends. Spatial analysis using geographic information systems (GIS) to map the distribution of AMR and identify hotspots or clusters. Use multivariate statistical techniques (e.g., logistic regression, cluster analysis) to explore relationships between multiple variables, such as antibiotic usage, resistance patterns, and environmental factors. Identify factors associated with increased

resistance rates, such as specific animal husbandry practices, geographic regions, or seasons. Trend and predictive modeling can be done to predict future AMR patterns based on historical data. Machine learning algorithms and predictive models to forecast AMR emergence and spread under different scenarios. These analyses can provide information such as emerging resistance trends, high-risk areas, or populations at greater risk. Emphasize significant correlations and associations that could inform targeted interventions.

4.5 Reporting and Dissemination of Findings

4.5.1 Reporting AST Data

The data generated from AST testing of isolates from healthy animal populations and their edible food products and some environmental samples represent an outcome from epidemiological surveillance for resistance rather than clinical surveillance for resistance. For epidemiological surveillance purposes, resistance is classified as expected for the wild-type population (wild type) of the bacteria or as differing from the wild population, that is, isolates in the tested sample have mutated to be more resistant than the wild population (non-wild type) (FAO, 2019). The resistance test parameter that distinguished the two populations, wild type and non-wild type, is referred to as the Epidemiological Cut-off Value (ECV) by the CLSI. The ECV or ECOFF (EUCAST) may or may not correspond to the clinically relevant MIC.

When the disc diffusion method for antimicrobial susceptibility testing (AST) is used, zone diameter measurements without an interpretive category should not be reported. Recommended MIC and disk diffusion interpretive criteria are based on usual dosage regimens and routes of administration in the United States.

Susceptible, intermediate, or resistant interpretations are reported and defined as follows:

(a). Susceptible (S) The susceptible category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agents when the dosage recommended to treat the site of infection is used.

(b). Intermediate (I) The intermediate category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and E-lactams in urine) or when a higher than normal dosage of a drug can be used (e.g., beta-lactams). This category also includes a buffer zone, which should prevent small, uncontrolled technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

(c). Resistant (R) The resistant category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs or zone diameters that fall in the range where specific microbial resistance mechanisms (e.g., beta-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies.

(d). Non-susceptible (NS): A category used for isolates for which only a susceptible interpretive criterion has been designated because of the absence or rare occurrence of resistant strains.

Isolates that have MICs above or zone diameters below the value indicated for the susceptible breakpoint should be reported as non-susceptible.

Results and aggregated background epidemiological data will be collated on an appropriate database, such as WHONET and LIMS for submission. Data collected from Sentinel laboratories

will be reported along the official lines of reporting through NADIS. Similarly, data from the reference laboratory will also be transmitted through the same channel. A dedicated reporting template in accordance with WHO recommendations will be developed for reporting.
Data obtained from laboratory testing will be entered using suitable laboratory software and/or WHONET for submission via NADIS to AMRIS and INFARM.

4.5.2 Dissemination of Findings

Findings will be presented in national AMR surveillance reports, policy briefs, and technical documents that summarize key findings and recommendations. Reports will be periodically shared with DVSs and published on official government websites, health department portals, and other relevant stakeholders. Online databases, dashboards, and interactive maps will be used to share data with stakeholders. Social media channels will be employed to reach a broader audience and disseminate simplified messages and info-graphics. Regularly newsletters, bulletins, and fact sheets will be issued to provide updates on AMR surveillance findings and trends. Educational materials such as brochures, posters, and videos will be developed targeting different audiences, including the public, farmers, and healthcare professionals.

4.5.3 Integration of AMR data with other sectors (e.g., public health, environmental monitoring)

At the reference laboratory, AMR data should be harmonized with other AMR data sources by submitting AST and meta data to NADIS for onward submission to AMRIS. AMRIS is a centralized, secure database or data repository that allows for the storage, management, and sharing of AMR data across sectors (AST data from human samples, environmental samples, and plant samples). The national database supports interoperable data formats and has the capacity to handle data from multiple sources. The database allows for harmonization and data sharing.

5.0 CAPACITY BUILDING

Capacity building in antimicrobial resistance (AMR) surveillance is important to effectively track and fight AMR. The components are:

5.1 Training and Education Program for Veterinary and Surveillance Personnel

It is important to equip veterinary and surveillance personnel with the knowledge and skills necessary to effectively monitor and combat AMR.

5.2. Development of Modules

Developing training curricula and modules on AMR detection, surveillance, and data management for veterinary professionals and surveillance staff is important. Important components are: The fundamentals of AMR, such as epidemiology, including mechanisms of disease resistance and the impact of AMR on animal and human health, will be covered.

5.3 Surveillance Techniques

Provision of hands-on training workshops to personnel in order to build practical skills in areas like sampling methods, sample collection, laboratory testing, epidemiological analysis, and reporting protocols.
Data collection with electronic systems for accurate and efficient data entry and management. There should be a centralized data hub, with the development of a centralized, secure database where all AMR data is stored and managed; the platform integrated with existing health information systems and laboratory databases for seamless data sharing and aggregation; Real-Time Data Collection procedure whereby mobile and web-based tools are used by the veterinary and surveillance and laboratory personnel for real-time data entry. Reliable internet and communication infrastructure should be put in place to facilitate data sharing and real-time reporting. Standardized methods, formats, and terminologies should be used to facilitate data integration and comparison. Establishing or strengthening national and regional networking platforms to facilitate data sharing and harmonization.

5.4 Antimicrobial Stewardship

Adopting global best practices for antibiotic use in veterinary settings to minimize resistance development. This will include training of healthcare providers, veterinarians, and the public on the importance of responsible antimicrobial use and the risks of injudicious use of antimicrobial agents.

5.6 Formats for training

5.6.1. Workshops and seminars

Organising regular in-person training workshops and seminars, including e-learning modules (online courses that can be accessed remotely), for personnel and lab technicians on AMR detection, reporting, and data interpretation.

5.6.2 Certification and continuous education

Certification programs will be developed to standardize skills and knowledge in AMR surveillance. Personnel who complete the training programs will be awarded a certificate of attendance. Continuous education will provide opportunities for ongoing learning to keep up with the latest developments in AMR surveillance and management.

5.7 Infrastructure Development and Resource Needs

It is necessary to put in place infrastructure development and resources to support effective AMR surveillance and response.
The components are:

5.7.1 Laboratory infrastructure

Investment in upgrade or new laboratory facilities, modern equipment, and supplies to enable robust pathogen identification and antimicrobial susceptibility testing, such as advanced diagnostic tools (such as high-throughput sequencing and automated susceptibility testing systems). Also investing in innovative diagnostic technologies, such as rapid tests and whole-genome sequencing, to improve pathogen identification and characterization.

5.7.2 Animal, laboratory biosafety, biosecurity, and animal welfare in AMR surveillance

Adherence to biosafety protocols and guidelines when handling samples and isolates to protect personnel and prevent the spread of resistant pathogens. Laboratories should be equipped to the appropriate biosafety levels to safely handle pathogenic organisms. Laboratories should be designed according to the required biosafety levels (BSL-1 to BSL-4) based on the risk of pathogens being handled in IATA (CDC,NIH, 2020).

Biosecurity measures should be implemented to safeguard laboratories and prevent the accidental or intentional release of AMR pathogens.
Provision of appropriate personal protective equipment and training to personnel involved in AMR surveillance to minimize risks. Establishing emergency response plans to address accidental exposures or biosecurity incidents.
Animal welfare standards should be upheld during sample collection and other surveillance activities involving animals.

5.7.3 Resource allocation

Funding should be secured for infrastructure development and equipment. Ensuring sustainable funding sources and budget allocation for the ongoing operations and maintenance of AMR surveillance systems.

5.8 Strategies for Enhancing Laboratory Capacity and Data Analysis

This aims at improving laboratory capabilities and data analysis for more effective AMR surveillance.
The components are:

5.8.1. Laboratory capacity building

Provision of specialized training for laboratory personnel on advanced diagnostic techniques and quality control. Also, implementing standardized testing protocols and quality assurance measures to ensure reliable and comparable AMR data. There is also the need to offer technical support and troubleshooting for laboratory equipment and processes.

5.8.2. AMR data interpretation and management

Implementation of quality control and validation procedures to ensure data accuracy and reliability is important. The development of data management systems (e.g., databases, electronic medical records) to store and organize AMR surveillance data and analyzing AMR data to identify trends, patterns, and emerging threats using statistical and epidemiological methods. Interpretation of data in the context of local, national, and global AMR trends to inform decision-making. Data analysis approaches are to be regularly reviewed and updated to reflect evolving needs and best practices. Implementation of strict access controls to protect sensitive data from unauthorized access and encryption to secure data during storage and transmission.

5.8.3. Quality assurance

Development of Standard Operating Procedures (SOPs) and enforcement of their use. Conducting regular audits and proficiency testing to maintain high standards of laboratory performance.

5.9 Collaboration with international and local partners for capacity strengthening

This will leverage partnerships and collaborations to enhance AMR surveillance and capacity building.

5.9.1 International partnership

Engaging with global health organizations and networks (e.g., WHO, FAO) and initiatives like GLASS, CAESAR, and the Fleming Fund to align with international AMR surveillance standards and practices. Also participating in international conferences and workshops to exchange knowledge and best practices.

5.9.2 Local collaborations

Fostering regional and cross-border collaborations to share knowledge, harmonize data collection, and jointly address shared AMR challenges. Strengthening multi-sectoral collaboration between human health, animal health, and environmental sectors to enable a comprehensive "One Health" approach to AMR surveillance.
Partnering with academic institutions, research organizations, and professional associations to leverage their expertise and resources for capacity building. Establishing or strengthening national and regional networking platforms to facilitate data sharing and harmonization. Collaboration with private sector entities, including pharmaceutical companies and diagnostic labs, to support AMR initiatives.

5.9.3 AMR awareness and communication

Developing targeted awareness campaigns to educate healthcare providers, veterinarians, policymakers, farmers and the general public about the importance of AMR surveillance and containment. Diverse communication channels (e.g., workshops, social media, and mass media) are utilized to disseminate key messages about AMR trends, risks, and prevention strategies.

Engaging with professional associations, academic institutions, and community organizations to amplify AMR awareness. Coordinating AMR awareness efforts across human health, animal health, and environmental sectors and monitoring the impact of awareness and communication activities to refine and improve their effectiveness over time.

6.0 MONITORING AND EVALUATION OF AMR SURVEILLANCE IN TERRESTRIAL AND AQUATIC ANIMALS

Strategic Objective 1: Enhance the evidence base by strengthening food animal AMR surveillance and effective research for informed decision-making.					
Outcomes	Outputs	Activity	Indicator(s)	Time Frame	Responsible
Knowledge and skill gaps amongst field surveillance officers, laboratory and data management personnel filled based on needs assessment	Knowledge and skill gap analysis	To design a questionnaire for gap analysis	Pretested questionnaires	Q1, 2025	FDVPCS
		To administration questionnaires for capacity gap analysis	No. of survey and personnel tested	Q1, 2025	FDVPCS
	Trainings conducted to fill identified gaps	Meeting of stakeholders to approve list of trainings to be conducted following gap analysis	Meeting to approve training list	Q1, 2025	FDVPCS

		Conduct of approved trainings	No of training conducted No. of trainees	Q2, 2025	FDVPCS AMR Focal Person
Outcomes	Outputs	Activity	Indicator(s)	Time Frame	Responsible
Capacities and readiness of veterinary services and laboratories improved for testing of surveillance samples	Additional equipment installed in sentinel and reference laboratories	Audit of laboratories to identify additional equipment required for effective surveillance activity	Report indicating Number of equipment and laboratories needing additional equipment	Q1, 2025	FDVPCS AMR Focal PersonSentinel and Central lab heads
		Procurement and installation of additional equipment	No. of additional equipment installed	Q2, 2025	FDVPCS AMR Focal Person Sentinel and Central lab heads

	Existing equipment serviced and recertified in sentinel and reference laboratories	Audit of laboratories to identify existing equipment that require servicing and or recertification	Audit conducted with a report indicating No. of equipment needing servicing	Q1, 2025	FDVPCS AMR Focal Person Sentinel and Central lab heads
		Contract for servicing and recertification of identified equipment awarded and executed	Contract award No. of equipment serviced	Q2, 2025	FDVPCS AMR Focal Person Sentinel and Central lab heads
	Required sampling materials, consumables and reagents provided at surveillance points and laboratories	Develop list of surveillance items, consumables and reagents, computer software and hardware	Report of required materials and their quantities	Q1, 2025	FDVPCS AMR Focal Person
		Procure surveillance materials and other inputs	Contract awarded	Q1, 2025	FDVPCS AMR Focal Person

		Distribute surveillance items to field, reagenst to labs and others	No. of items, types and destination of items, consumables, reagents etc.	Q2, 2025	FDVPCS AMR Focal Person
Antimicrobial resistant priority bacterial pathogens isolated from terrestrial food animals across Nigeria	Nation-wide sampling of food animals conducted for detection of priority zoonotic bacteria	Sampling of broilers and layers on farms and live bird markets	No of samples from broilers and layers	Q3, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Person State DVS
		Sampling of cattle, sheep and goats on farms and slaughter locations	No of samples from cattle, sheep and goats	Q4, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Person State DVS
		Sampling of pigs on farms and slaughter locations	No of samples from pigs	Q4, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Person State DVS

	Antimicrobial susceptibility testing conducted to detect antimicrobial resistance amongst priority zoonotic bacteria	Isolation and identification of priority zoonotic bacteria using microbiological methods	No. of priority zoonotic bacteria isolated from each food animal type	Q4, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Person Heads of Sentinel laboratories
		Antimicrobial susceptibility testing of isolated priority bacteria at the sentinel labs	No. of Resistant Phenotypes from each priority bacterial pathogen	Q4, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Person Heads of Sentinel laboratories
Antimicrobial resistant bacteria from sentinel laboratories further characterized and resistant mechanisms determined in the central laboratory	Identity of *Salmonella* and other bacteria confirmed by serological and molecular methods	Serotyping of *Salmonella* isolates	No. of Salmonella serotypes identified	Q4, 2025, 2026, 2027, 2028	Central AMR lab Chief

		PCR-based identification of other bacterial isolates	No of various species confirmed	Q4, 2025, 2026, 2027, 2028	Central AMR lab Chief
	Resistance phenotypes from sentinel labs confirmed	Repeat of AST on isolates	No of isolates tested No of resistant phenotypes	Q4, 2025, 2026, 2027, 2028	Central AMR lab Chief
		MIC determination for some classes of antibiotics like colistin, vancomycin	No of isolates above breakpoint	Q4, 2025, 2026, 2027, 2028	NRL
		Identify resistant profiles including MDR and XDR	Number of isolates Number of resistance profiles	Q4, 2025, 2026, 2027, 2028	NRL
	Mechanisms of resistance determined	Phenotypically confirm known resistance mechanisms	Number of isolates Number of resistance mechanisms	Q4, 2025, 2026, 2027, 2028	NRL

		Identify newly emerged resistance genotypes	Number of isolates Number of new genotypes	Q4, 2025, 2026, 2027, 2028	NRL
Impact and socioeconomic and other drivers of AMR identified	AMR data analyse for reporting to FMAFS and OIE	Generate descriptive data on AMR in Nigeria	No of occurrences (%) Prevalence (%)	Q4, 2025, 2026, 2027, 2028	NRL
		Generate AMR risk maps	No. of maps generated	Q4, 2025, 2026, 2027, 2028	NRL
		Report to NADIS and OIE	No. of reports	Q4, 2025, 2026, 2027, 2028	NRL
	Risk factors for emergence and dissemination of AMR in Nigeria identified	Conduct Socio Economic analysis	No. of analysis reports	Q4, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Point
		Conduct spatial analysis	No. of maps, No. of reports	Q4, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Point

		Conduct risk analysis	No. of risk factors	Q4, 2025, 2026, 2027, 2028	FDVPCS AMR Focal Point
Biorepository of resistant bacterial isolates establish to enhance research and control of AMR	Facilities for storage upgraded to cope with expanding surveillance for AMR	Conduct need analysis to guide expansion of facilities	Need Analysis report	Q3, 2025	FDVPCS AMR Focal Person Central Lab Manager
		Procure additional biobanking facilities	No. of additional storage items procured	Q3, 2025	FDVPCS AMR Focal Person Central Lab Manager
		Install additional biobanking facilities	No of additional storage items installed	Q4, 2025	NRL

	SOPs for collection, identification and biobanking at central laboratory developed and operationalized	Produce Guidelines for submitting isolates	Guidelines developed	Q3, 2025	NRL
		Develop SOPs for storage and retrieval	SOPs developed	Q3, 2025	NRL
	Protocols for sharing of isolates to collaborators developed and operationalized	Publish, disseminate and post guidelines	No. of publications, posts on guidelines	Q3, 2025	NRL
		Collate isolates and revalidate identity	Produce protocol for obtaining or sharing to collaborators including MTA	Q3, 2025	NRL

7.0 BUDGET AND RESOURCES

The costs of AMR surveillance are high, involving considerable resources. Thus, efficient utilization of available resources is key to the success of the AMR surveillance programme.

7.1 Resource Mobilization

Domestic and international resources, including public and private sectors:

- **Federal and state government funding (dedicated** line item in the national and state budgets for AMR surveillance).
- Tertiary Education Trust Fund (TETFUND)
- **International Grants/Aids and Collaborations (from** international partners such as WHO, FAO, and World Bank, Fleming Fund, USAID).
- **Private sector engagement (**engage with pharmaceutical companies, livestock farmer groups, and agribusinesses to co-fund AMR surveillance activities as part of their corporate social responsibility (CSR) initiatives.
- **Public-Private Partnerships (PPP)**.
- **Long-term sustainability through revenue** generation from service charges (e.g., laboratory testing fees).

7.2 Cost-Effectiveness Analysis and Resource Optimization

- Annual national AMR active surveillance to be conducted over the next five years to evaluate the cost-effectiveness of surveillance activities, reductions in AMR prevalence, improved number of sentinel laboratories reporting.
- **For resource optimization** pooled procurement for laboratory supplies and equipment to reduce costs.

8.0 RISK MANAGEMENT

8.1 Potential Challenges and Risks Related to AMR Surveillance in Terrestrial and Aquatic Animals

Tracking of antimicrobial resistance (AMR) in terrestrial and aquatic animals is a critical public health initiative aimed at monitoring and controlling the emergence and spread of resistant pathogens within livestock and wildlife populations. With the continuous use of antibiotics in agriculture, the potential for the development, evolution, and transfer of AMR from animals to humans poses significant risks to food safety and public health. Effective surveillance systems enable the identification of resistance patterns, informing strategies for responsible antibiotic use, and guiding policies to mitigate the impact of AMR. By understanding the dynamics of AMR in animal populations, stakeholders can work collaboratively to enhance food security and protect human health from the threats posed by resistant infections.

Surveillance of antimicrobial resistance (AMR) in terrestrial and aquatic animals in Nigeria presents several challenges and risks that can hinder effective monitoring and control efforts. These challenges can be categorized into several key areas:

8.2 Data Collection and Quality

1. Lack of comprehensive data: There is a significant gap in reliable and comparable data on AMR across different animal species and regions in Nigeria. This is worsened by limited laboratory capacity and sporadic data collection methods, which often rely on clinical samples rather than systematic monitoring of AMR in indicator bacteria (Dawuf *et al.*,2020; Achi *et al.*,2021).
2. Measurement challenges: The measurement of AMU is complicated by numerous factors, including the lack of standardized definitions and methods for data collection. Many farmers are unaware of the antibiotic contents in animal feed, which complicates tracking AMU (Dawuf *et al.*,2020).

8.3 Laws, Regulations and Policy Issues

1. Weak regulatory framework: Although there are policies in place regarding AMR, they are new, and their implementation is in progress but weak. The Nigerian Centre for Disease Control (NCDC) and the Federal Ministry of Agriculture and Rural Development (FMARD) have made efforts, but the lack of a national policy on AMR hampers coordinated action (Achi *et al.*,2021; Federal Ministry of Agriculture, Environment, and Health, 2017).
2. Inconsistent Enforcement: Regulatory bodies like the National Agency for Food and Drug Administration and Control (NAFDAC) face challenges in enforcing existing regulations, leading to uncontrolled use of antimicrobials in aquatic and terrestrial animals (Federal Ministry of Agriculture, Environment, and Health, 2017).

8.4 Resource limitations

1) *Funding Constraints*: Limited financial resources hinder the establishment and maintenance of effective surveillance systems. Inadequate funding affects laboratory capabilities, training for personnel, and the overall infrastructure needed for AMR monitoring (Achi *et al.*,2021).
2) *Human Resource Shortages*: There is a shortage of trained personnel in the veterinary and public health sectors to carry out AMR surveillance. This limits the ability to conduct comprehensive studies and respond to emerging AMR threats (Dawuf *et al.*,2020; Achi *et al.*,2021).
3) *Infrastructural constraints:* inadequate infrastructural facilities.

4) *Logistical challenges:* inaccessible areas due to insecurity challenges and poor road networks (especially in rural areas), resulting in incomplete coverage.
5) *Data management:* Inconsistency in sample collection, handling, processing, and reporting practices (failure to follow standardized SOPs).

8.5 Public Health Implications

1) Zoonoses: The high levels of AMR in food animals pose significant public health risks, as resistant pathogens can be transmitted to humans through the food chain. This situation is compounded by inadequate monitoring of AMR in environmental settings, where waste from livestock and aquaculture can contribute to the spread of resistance (Oloso *et al.*,2023; Okon *et al.*,2022).
2) *Impact on Animal Health*: The rise in AMR not only threatens human health but also affects animal health, leading to increased mortality and reduced productivity in livestock. This creates a cycle of increased antimicrobial use, further exacerbating the resistance problem (Dawuf *et al.*,2020; Okon *et al.*,2022).

8.6 Mitigation Strategies and Contingency Plans

Mitigating the risks associated with antimicrobial resistance (AMR) in terrestrial animals in Nigeria requires a multifaceted approach that emphasizes surveillance, policy implementation, and collaborative efforts across various sectors. Here are some key strategies and contingency plans:

8.6.1 Surveillance systems

1. *Integrated Surveillance*: Establishing a comprehensive surveillance system that integrates data from human, animal, and environmental health is crucial. This "One Health" approach allows for the monitoring of AMR patterns and antimicrobial use (AMU) across different sectors, enabling timely interventions (Mudenda *et al.*,2023; Manyi-Loh *et al.*,2018).
2. *Strengthening Laboratory Capacity*: The Nigerian government, through the Federal Ministry of Agriculture and Rural Development (FMARD) and the Nigeria Centre for Disease Control (NCDC), has initiated efforts to enhance laboratory capabilities for AMR surveillance. This includes equipping laboratories and conducting point prevalence surveys to assess AMU in agricultural settings. Achi *et al.*,2021; NAP, 2017).
3. *Data Reporting and Analysis*: Regular reporting of AMU and AMR data to international bodies such as the World Organisation for Animal Health (OIE) is essential. This data helps in understanding the scope of AMR and informs policy decisions (Achi *et al.*,2021).
4. *Infrastructure investment*: upgrading of existing and establishment of more sentinel laboratories. Procurement of laboratory reagents and consumables from the same source and distribution to laboratories.

8.7 Policy Framework

1. *National Action Plan (NAP)*: Nigeria has developed a National Action Plan on AMR that outlines strategic objectives aimed at reducing AMR prevalence. This plan includes promoting rational use of antibiotics and enhancing public awareness about AMR (NAP, 2017; Federal Ministry of Agriculture, Environment, and Health, 2017).
2. *Regulatory measures:* Strengthening regulations surrounding the use of antimicrobials in agriculture is necessary. This includes enforcing guidelines on the prescription and sale of

antibiotics, as well as monitoring their use in livestock (Manyi-Loh *et al.*,2018; Federal Ministry of Agriculture, Environment, and Health, 2017).

3. *Public awareness campaigns:* Increasing awareness among farmers and healthcare providers about the risks of AMR and the importance of responsible antibiotic use is critical. Educational programs can help mitigate the irrational use of antimicrobials (Mudenda *et al.*,2023; Achi *et al.*,2021).

8.8 Advocacy for Government Commitment to Support AMR Surveillance Via Enactment or Enforcement of Policies and Regulations that Ascribe Penalties to Non-compliance

1. **Collaborative Efforts**
2. *Multisectoral Collaboration*: Engaging stakeholders from various sectors—human health, animal health, agriculture, and environmental health—ensures a coordinated response to AMR challenges. Collaborative initiatives can enhance resource mobilization and promote shared strategies for AMR control (Achi *et al.*,2021; NAP, 2017).
3. *International Partnerships*: Collaborating with international organizations and neighboring countries can provide technical support and resources to bolster Nigeria's AMR surveillance and response strategies (Manyi-Loh *et al.*, 2018).
4. **Engagement with local** communities and stakeholders (farmers and veterinarians) through awareness campaigns to build trust and cooperation.

8.9 Contingency Plans

1. *Emergency response protocols*: Develop protocols for rapid response to AMR outbreaks in animal populations is essential. This includes identifying containment measures and treatment options for affected animals (Achi *et al.*, 2021).
2. *Research and development*: Continuous research is necessary to understand the dynamics of AMR in Nigeria. This includes studying the impact of agricultural practices on AMR and developing innovative solutions for infection control and prevention (Mudenda *et al.*, 2023; Achi *et al.*,2021).
3. *Monitoring and evaluation*: Establish a framework for monitoring the effectiveness of implemented strategies and policies will help in adapting and improving AMR management efforts over time (NAP, 2017; Federal Ministry of Agriculture, Environment, and Health, 2017).
4. Develop contingency plans for unforeseen situations such as natural disasters, insecurity challenges.

By implementing these strategies, Nigeria can enhance its capacity to monitor and mitigate the risks associated with AMR in terrestrial animals, ultimately protecting public health and ensuring food safety.

8.10 Risk Assessment and Management Strategies

Antimicrobial resistance (AMR) in terrestrial animals in Nigeria poses significant public health challenges, necessitating comprehensive risk assessment and management strategies. The following outlines the current situation, strategies in place, and recommendations for improving AMR surveillance and management.

8.11 Risk Assessment Strategies

1. *Surveillance systems*: The establishment of robust surveillance systems is crucial. The NCDC, in collaboration with the Federal Ministry of Agriculture and Rural Development (FMARD), has been working to enhance laboratory capacities for monitoring AMR in both human, animal and environmental health sectors. This includes the training of personnel and the provision of necessary resources for effective data collection and analysis (Achi *et al.*,2021).
2. *Data Sharing*: Effective risk assessment relies on the availability of comprehensive data. There is a need for improved data sharing between sectors and institutions to facilitate better understanding and management of AMR trends and patterns (Federal Ministry of Agriculture, Environment, and Health, 2017).
3. *Public Awareness*: Increasing awareness about AMR among healthcare providers, veterinarians, and the public is essential. Educational campaigns should focus on the risks associated with inappropriate antimicrobial use and the importance of hygiene and sanitation in preventing the spread of resistant pathogens (Achi *et al.*,2021; Federal Ministry of Agriculture, Environment, and Health, 2017).
4. Conducting regular risk assessments to identify emerging threats to the surveillance program, such as funding shortages, insecurity challenges, adherence to the surveillance protocols, etc.
5. Development of contingency plans for potential disruptions (e.g., alternative funding sources, temporary reallocation of resources, change of sampling location, adjustment of sampling frame).

8.12 Management Strategies

1. *Antimicrobial Stewardship Programs*: Implementing antimicrobial stewardship programs in both human and animal health is critical. These programs should promote the rational use of antimicrobials and discourage unnecessary prescriptions, particularly in food-producing animals (Achi *et al.*,2021).
2. *Research and Development*: Enhancing research capabilities to study the socio-economic impacts of AMR and the effectiveness of intervention strategies is vital. This includes developing local capacity for vaccine production and exploring alternative treatments to reduce reliance on antimicrobials (Alhaji and Opeyemi, 2018; Federal Ministry of Agriculture, Environment, and Health, 2017).
3. *Collaboration across Sectors*: A multidisciplinary approach involving veterinarians, public health officials, environmentalists, and policymakers are necessary to create a unified front

against AMR. Collaborative efforts can lead to more effective interventions and policies that address the complexities of AMR (Alhaji and Opeyemi, 2018; Achi *et al.*,2021).

4. Implementation of a dynamic risk management approach that enables real-time adjustments to minimize the effect of the risk on the integrity of surveillance data.
5. Establishment of multi-sectoral and multi-level risk management teams for a quick and comprehensive response to emerging threats.
6. Regularly monitoring the effectiveness of risk management strategies and making relevant adjustments based on lessons learned.

9.0 IMPLEMENTATION PLAN

9.1 Detailed Timelines and Milestones:

- **Phase 1: Planning and Preparation (1st and 2nd quarters)**
    - Conduct stakeholder mapping analysis
    - Secure funding
    - Establish AMR surveillance core teams, using a One Health approach.
    - Conduct initial training sessions and workshops.
    - Develop the work plan/finalizing of the AMR surveillance strategy.
    - Procure and distribute laboratory reagents and consumables and sampling materials
- **Phase 2: Pilot Implementation (3rd and 4th quarters)**
    - Conduct AMR surveillance in selected states.
    - Conduct data collection and management processes.
    - Conduct the first round of cost-effectiveness analysis.
    - Production of the report of the pilot study.
- **Phase 3: Full-Scale National AMR Surveillance (1st quater 2025)**
    - Conduct comprehensive national AMR surveillance covering all regions of the federation.
    - Implement regular risk assessments and adjustment of strategies if necessary.
    - Put in place self-reliance and sustainability plans.
- **Phase 4: Evaluation and Scaling (2nd quarter 2025)**
    - Conduct comprehensive evaluation of the AMR surveillance program.
    - Scale successful strategies and interventions.
    - Publish findings and sharing of best practices with relevant national and international bodies.
    - Strengthen the national AMR surveillance infrastructure, capacity building, and production of the first comprehensive national AMR report.

9.2 Responsibilities and Roles of Stakeholders

Nigeria has a strong, multisectoral governance structure that demonstrates a One Health approach to addressing AMR activities. This structure shows the Federal Government's commitment to ensuring effective communication, collaboration, and coordination among sectors – provides a comprehensive model for operationalizing interventions that prevent and reduce AMR in Nigeria. The nation's One Health AMR Governance structure is comprised of the following entities: The National One Health Steering Committee (NOHSC), The Antimicrobial Resistance Coordinating Committee (AMRCC) and The National AMR Technical Working Group (NTWG).

The National One Health Steering Committee (NOHSC): This is the overall decision-making and policy-setting body for AMR in Nigeria, and is vested with the responsibilities of overseeing the activities of the National One Health Technical Committee (NOHTC) and the Antimicrobial Resistance Coordinating Committee (AMRCC).

The Antimicrobial Resistance Coordinating Committee (AMRCC): It is comprised of focal points from the Federal Ministry of Health, the Federal Ministry of Agriculture and Food Security, the Federal Ministry of Environment, and the chairs of the National AMR TWG. The AMRCC provides direct leadership and coordination of interventions from the multisectoral technical working groups. It oversees and coordinates all AMR related activities in all sectors, mobilises the needed resources for AMR activities and organises scientific meetings, seminars and serve as national scientific platform for AMR surveillance in the animal health and environment health sectors. AMRCC is also responsible for analysing and reporting of national AMR data to the AMRIS and other relevant international platforms.

The National AMR Technical Working Group (NTWG): The NTWG is comprised of five sub TWGs with a focus on awareness/education, surveillance, stewardship, infection prevention and control (including vaccination), and research. The NTWG on AMR surveillance provides technical support during the development and implementation of National Action Plan (NAP). The TWG also monitors the collection and reporting of data on antimicrobial use and AMR in the animal health sector.

International Partners:

These include WHO, FAO, WOAH, UNEP, Fleming Fund, USAID. They support building laboratory capacity to identify and report AMR organisms, provide technical (e.g. trainings, mentorship, assessments) and material (e.g. reagents, equipment) support towards implementation of AMR surveillance.

The National AMR Reference Laboratory (NRL):

The NRL is domiciled at the National Veterinary Research Institute (NVRI), Vom, Plateau State. The responsibilities of NRL include:

- Provision of technical support and guidance in AST testing and quality management to surveillance network laboratories
- Provision of bio-repository service for isolates with unexpected or unusual resistance profiles;
- Receives AMR data from sentinel laboratories and sharing same to the AMRCC/FMAFS;
- Confirmation/characterization of unusual or new resistance patterns before they are reported to the AMRCC/FMAFS;
- Participation in external quality assurance through appropriate international schemes;
- Liaises with the AMR-TWG in standardizing and verifying data from the sentinel laboratories;
- Provision external quality assurance across sentinel site laboratories and providing feedback;

• Assists sentinel laboratories to procure equipment, reagents and supplies;
Sentinel Laboratories: These are public (attached to the Veterinary Teaching Hospitals) and private (privately owned laboratories with capacity to perform AMR diagnostics) laboratories. The responsibilities of these laboratories include the following:
• receives/collects samples/specimen and process the for isolation, identification and AST according to protocol;
• Transportation of multi-drug resistant isolates (and samples) to the NRL for confirmation and further characterization;
• Performs initial /descriptive AMR data analysis;
• Shares AMR data with National Reference Laboratory for confirmation and analysis;
• Ensures availability of appropriate sample collection materials;
• makes quarterly (passive surveillance) and real-time (active surveillance) AMR reports to NRL and FMAFS (through the CVON).
State Directors of Veterinary Services (DVS), Practicing Veterinarians and Livestock/Poultry Farmers:
• Facilitate availability of MR surveillance data;
• Provide personnel for community engagement and AMR surveillance activities. The Non-Governmental Organizations (NGOs):
• Facilitate community engagement, awareness campaigns, and advocacy efforts.

9.3 Coordination and Communication Strategies

- AMRCC (a multi-sectoral committee) in conjunction with NCDC and NRVI coordinates AMR surveillance activities.
- Regular communication channels between surveillance teams, sentinel and reference laboratories, and FMAFS to ensure timely information sharing
- Prompt and transparent reporting of surveillance findings to relevant authorities and the public.
- Regular meetings and production of newsletters/bullets to ensure information flow among stakeholders.
- Public awareness campaigns through media, community outreach, and educational programs to inform the public about AMR risks and the importance of surveillance.

10. Glossary of Terms

Active AMR surveillance: The act of actively reaching out to sources to obtain AMR information (ie active collection of data on AMR from the population) .

Antimicrobial Resistance (AMR): The ability of microorganisms (bacteria, viruses, fungi, parasites) to resist the effects of an antimicrobial medication that they were once susceptible to.

Antimicrobial Use (AMU): The application of antimicrobial agents in humans, animals, and plants to treat infections. In livestock production, AMU includes the use of antimicrobials for growth promotion, disease prevention, and treatment.

Antimicrobials: Natural, semi-synthetic, and synthetic drug compounds used to treat infections

caused by bacteria, viruses, fungi, and other unicellular parasites. In livestock, antimicrobials are used for growth promotion, therapeutic, and prophylactic purposes.

Codex Alimentarius: A collection of internationally recognized standards, guidelines, and codes of practice to ensure food safety and quality. It includes guidance on AMR risk assessment and management in the food supply chain.

Commensal Bacteria: Non-harmful bacteria that normally reside in the body but can carry genes that confer resistance to antibiotics.

Control: Is the administration of an antimicrobial agent to a group of animals containing sick animals and healthy animals (presumed to be infected), to minimise or resolve clinical signs and to prevent further spread of the disease;

Critically Important Antimicrobial Agents: Antimicrobials that are essential in human and veterinary medicine, often targeted in AMR surveillance**.**

Multidrug-Resistant (MDR) Bacteria: Bacteria that are resistant to at least one drug in three or more classes of antimicrobial agents, making infections difficult to treat. MDR bacteria are a growing global health concern, particularly in the context of livestock production.

One Health: An integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems.

Passive AMR Surveillance: The routine collection of data on AMR from clinical samples submitted to the laboratory from the clinics/hospitals during the normal course of healthcare or veterinary services.

Prevent: It is the administration of an antimicrobial agent to an individual or a group of animals at risk of acquiring a specific infection or in a specific situation where infectious disease is likely to occur if the drug is not administered

Risk Assessment: A comprehensive process that evaluates the potential hazards, exposure, and consequences of a particular risk, such as AMR, to inform risk management strategies. Risk assessment in AMR involves identifying hazards, assessing exposure, and characterizing risks associated with antimicrobial use.

Sentinel Laboratories: Laboratories designated to perform microbial analysis and early detection of diseases, including those related to AMR.

Standard Operating Procedures (SOPs): In the context of AMR, SOPs are established procedures to ensure consistent and accurate surveillance and laboratory testing of AMR.

Surveillance of antimicrobial resistance: Collection, validation, analyses and reporting of relevant clinical, microbiological and epidemiological data on antimicrobial resistance in targeted pathogens from different sources (e.g. humans, animals, food, environment), and on relevant antimicrobial use in humans and animals.

Terrestrial Animals: Animals that primarily live on land, including mammals, birds, reptiles, and

bees. This category encompasses domesticated livestock (e.g., cattle, sheep, goats, pigs, poultry), pets (e.g., dogs, cats), and wild animals that inhabit terrestrial environments.

Treat: Is the administration of an antimicrobial agent to an individual or a group of animals showing clinical signs of an infectious disease

Zoonotic Health Threat: A health risk that occurs when diseases are transmitted from animals to humans.

10.2 Contact Information of Key Stakeholders and Partners

Federal Ministry of Agriculture and Food Security (FMAFS)

Address: Federal Secretariat Complex, Phase II, Abuja, Nigeria

Email: info@fmard.gov.ng

Nigeria Centre for Disease Control (NCDC)

Address: Plot 801, Ebitu Ukiwe Street, Jabi, Abuja, Nigeria

Email: info@ncdc.gov.ng

National Veterinary Research Institute (NVRI)

Address: PMB 01, Vom, Plateau State, Nigeria

Email: info@nvri.gov.ng

Federal Ministry of Livestock Development

Address: Office of the Secretary to the Government of Federation (SGF), 3 Arms zone, Maitama Abuja

Email: info@fmld.gov.ng

Website: https://fmld.gov.ng

Federal Ministry of Health (FMOH)

Address: Federal Secretariat Complex, Phase III, Abuja, Nigeria

Email: info@health.gov.ng

World Health Organization (WHO) Nigeria Country Office

Plot 617/618, Diplomatic Drive, Central Business District, Abuja, Nigeria

Email: afwconigeria@who.int

Food and Agriculture Organization (FAO) Nigeria

UN House, Plot 617/618, Diplomatic Zone, Central Business District, Abuja,

Nigeria

Email: FAO-NG@fao.org

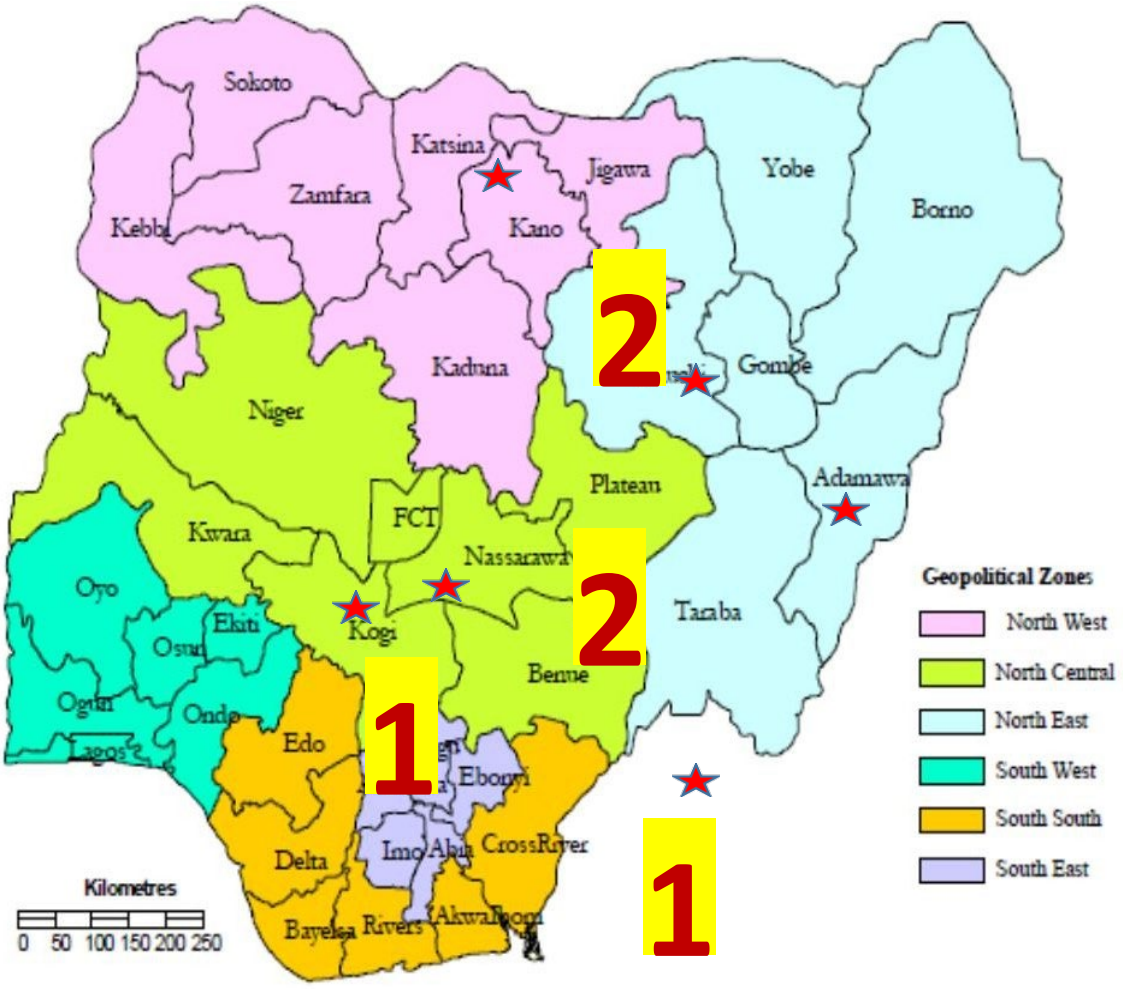
World Organisation for Animal Health (WOAH) - formerly OIE

Regional Office for Africa: BP 2954, Bamako, Mali

Email: africa@oie.int

10: APPENDICES

APPENDIX I: MAP OF NIGERIA SHOWING THE THIRTY-SIX STATES AND SIX GEOPOLITICAL ZONES



APPENDIX II: PROJECTED POULTRY POPULATION IN NIGERIA

States	*Total poultry population	**Total Area (km sq)	Poultry Density (poultry/km sq)
Abia	1631544	4900	333
Adamawa	4777851	38700	123
A/ibom	3530559	6900	512
Anambra	3162793	4865	650
Bauchi	13519620	49119	275
Bayelsa	1147432	90059	13
Benue	7796652	30800	253
Borno	6766906	72609	93
C/river	1471066	21787	68
Delta	3000976	17108	175
Edo	1426934	19187	74
Ebonyi	6818234	6400	1065
Ekiti	3383453	5435	623
Enugu	4310216	7535	572
Gombe	588427	17100	34
Imo	7428886	5288	1405
Jigawa	5590052	23287	240
Kaduna	3265767	42481	77
Kano	4459328	20280	220
Katsina	6031372	23561	256
Kebbi	8826399	36985	239
Kogi	4266093	27747	154
Kwara	3868905	35705	108
Lagos	3633534	3671	990
Nasarawa	676691	28735	24
Niger	3530559	68925	51
Ogun	4118986	16400	251
Ondo	3824773	15520	246
Osun	4118986	9028	456
Oyo	3604113	26500	136
Plateau	4398489	27147	162
Rivers	4413199	10575	417
Sokoto	1706437	27825	61
Taraba	3133372	56282	56
Yobe	3971879	46609	85
Zamfara	6766906	37931	178

APPENDIX IIIa

LABORATORY PROCEDURES FOR ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL SENSITIVITY TESTING OF TARGET BACTERIA
(Adapted from Standard Procedures References 15 and 20)

1. *Sample preparation and enrichment*

In poultry, the pooled caeca from one bag is pulverized with a rubber mallet and mixed well.
Or
The pooled fecal deposits in one bag are mixed well.
1 gram of caecal or fecal sample is added to 9 ml of buffered peptone water (BPW) in a sterile tube (50 ml tubes recommended) with a lid.
Samples should be gently mixed but not shaken to avoid spillage.
For *E. coli, Salmonella,* and *Klebsiella*: Incubate for 16–24 hours at 37°C aerobically.
For *Enterococcus*: a sub-sample of the BPW must be mixed with Azide Dextrose Broth or Brain Heart Infusion BEFORE the BPW mixture is incubated (see details below under Enterococcus).
For Campylobacter: The swab used to collect fecal material, which was placed in charcoal-containing transport medium (or a swab mixed through the pooled caecal sample), is added to 9 ml of an enrichment broth (e.g., Bolton broth) in a sterile tube (50 ml tubes recommended) with a lid. Incubate at 42°C for 48 hrs. in a microaerobic atmosphere using one of the options below:

1. A microaerobic gas pack (such as CampyGen™ or CampyPak™) in an anaerobic jar. Or
2. An anaerobic jar was gassed with pre-mixed microaerobic (5% O2, 10% CO2, 85% N2) gas mix. Or
3. A variable-atmosphere incubator if one is available in the laboratory.

1. *ESBL+ENTEROBACTERIACEAE*

Subculture
Following incubation, inoculate the enriched Buffered Peptone Water onto one of the three types of agar listed below using a sterile swab or 10 ul inoculum and streak for single colonies:

CHROMagarTM ESBL or BrillianceTM ESBL agar

OR

MacConkey agar mixed with 1 mg/L of ceftriaxone

OR

1. MacConkey agar mixed with 1 mg/L ceftazidime PLUS 1 mg/L cefotaxime. Bacterial growth on any of these plates is indicative of possible ESBL resistance.

Isolate purification

Subculture at least **two** typical *E. coli* and *Klebsiella pneumoniae* colonies from the plate to non-selective media such as blood agar or nutrient agar.
Incubate at 37 °C for 18–24 hours, aerobically.
Tentative identification

1. *E. coli* can be identified by the following options:
2. Roughly identified by bacteriology (oxidase, lactose fermentation, able to grow on MacConkey agar), and then
3. Further differentiated by further biochemical testing (such as triple sugar-iron agar, lysine decarboxylase, urease, and motility).
4. *Pneumonia* can be identified by the following options:
5. Roughly identified by bacteriology (oxidase, lactose fermentation, mucoid colony morphology, able to grow on MacConkey agar), and then
6. Further differentiated by further biochemical testing (such as triple sugar-iron agar, lysine decarboxylase, indole, urease, and motility). *C. Escherichia coli*

All purified isolates should be stored and sent to the AMR reference laboratory for confirmatory testing of the specific type of resistance.

2. Salmonella

Subculture 1
Either of the following two options:

Transfer 100 µl of enriched Buffered Peptone Water to 10 ml of warmed Rappaport Vassiliadis soy peptone.

Incubate aerobically at 42 °C, preferably in a water bath, for 20–24 hours.
OR

Transfer 1 ml of enriched buffered peptone water to 10 ml of tetrathionate broth + iodine.

Incubate aerobically at 35 °C for 20–24 hours.
Subculture 2
Subculture selective broths to XLD (with or without novobiocin).
Incubate aerobically for 20–24 hours at 35 °C.
Isolate purification
Subculture **three** typical colonies from the plates to non-selective media such as blood agar or nutrient agar and incubate at 35 °C for 18-24 hours, aerobically.
Identification
Salmonella can be identified by the following options:

1. Bacteriology (triple-sugar iron agar, urea, lysine decarboxylase)
2. Serology (Salmonella poly-O and poly-H antisera agglutination) e. Enterococcus

Antimicrobial susceptibility testing
Test isolates for antimicrobial susceptibilities by disk diffusion against the panel of antibiotics prescribed by EUCAST or CLSI.
All purified isolates, regardless of AST results, should be stored and sent to the AMR reference laboratory for confirmatory testing of the specific type of resistance.

Before BPW is incubated, add 1 ml of buffered peptone water mixture to approximately 10 ml of Azide Dextrose Broth1 (OR Brain Heart Broth + 3 mg/L vancomycin). Incubate broth for 18–24 hours at 35 °C.

3. Enterococcus

Before BPW is incubated, add 1 ml of buffered peptone water mixture to approximately 10 ml of Azide Dextrose Broth1 (OR Brain Heart Broth + 3 mg/L vancomycin).
Incubate broth for 18–24 hours at 35 °C.
Subculture

> Subculture broth to selective agar such as Slanetz and Bartley (Pleydell, 2010). Incubate for 18-24 hours at 42°C aerobically.

OR

> Subculture broth to CHROMagarTM VRE (Peltroche-Llacsahuanga *et al.*,2009) or BrillianceTM VRE (Gouliouris *et al.*,2016) and incubate 18–24 hours at 37°C aerobically.
> *Isolate purification*

Subculture **two** colonies (if present, choose colonies that appear different) from each plate (if present) to blood agar or nutrient agar.
Incubate at 35 °C for 18–24 hours, aerobically.
Identification
Enterococci are catalase-negative cocci with PYRase.
There is no need to identify Enterococci to species level at this stage.

4. Staphylococcus aureus (*Protocol in accordance with ISO 6888-1 (1999)* (25)).

Isolation
Staphylococcus aureus can be isolated from swabs taken from carcasses, nasal swabs, or bulk milk samples in accordance with the recommendations of ISO 6888-1 (1999) (25). Enrich swabs in 5 ml Tryptone soy broth with 6.5% NaCl and 0.3% yeast extract. Incubate at 37 oC for 18 hours. Bulk milk samples can be plated directly. Plate 10 ul of enriched or fresh milk samples onto Baird Parker Agar and egg yolk tellurite medium. Incubate at 37 oC for 24-48 hours. Typical colonies are black or grey, shining and convex (1 mm to 1,5 mm in diameter after incubation for 24 h and 1.5 mm to 2.5 mm in diameter after incubation for 48 h) and surrounded by a clear zone.
Identification
Preliminary identification of *Staphylococcus* species can be done using Gram staining, catalase, hemolysis tests on Colombian blood agar with sheep defibrinated blood, and DNAse tests. If

available, further confirmatory testing may be carried out using the API 32 Staph kit (Biomerieux, France) or the Microbact Staph 12S kit (Oxoid, UK).

Identification of Coagulase-positive S. aureus

Confirm coagulase positive Staphylococcus using tube coagulate test as follows: Pick a suspected colony with a sterile wire and transfer it to a tube or bottle containing 10 ml of brain-heart infusion broth. Incubate at 37°C for 24 hrs. Aseptically add 0.1 ml of each culture to 0.3 ml of the rabbit plasma and incubate at 37°C. By tilting the tube, examine for clotting of the plasma after 4 h to 6 h of incubation, and, if the test is negative, re-examine at 24 h of incubation. Consider the coagulase test to be positive if the volume of clot occupies more than half of the original volume of the liquid. As a negative control, for each batch of plasma, add 0.1 ml of sterile brain-heart infusion broth (5.4) to the recommended quantity of rabbit plasma (5.5) and incubate without inoculation. For the test to be valid, the control plasma shall show no signs of clotting.

Identification of MRSA

MRSA detection amongst isolates can be done using the cefoxitin disc diffusion method. Susceptibility of *S. aureus* isolates can be tested against cefoxitin (30µg) by the CLSI agar disc diffusion method. Adjust suspensions of overnight *S. aureus* cultures to turbidity of 0.5 McFarland standard. Dip sterile swabs into suspensions and streaked evenly onto Mueller-Hinton agar (MHA) and leave for a few minutes to dry. Then apply cefoxitin (30µg) discs aseptically on the plates and incubate at 35°C. The results read after 24 hours of incubation; isolates are considered MRSA when the inhibition zone diameter is ≤ 21 mm for cefoxitin. Use *S. aureus* ATCC 25923 as the control strain. Latex agglutination to detect penicillin-binding protein 2a (PBP2a) (Oxoid, UK) may also be used as a confirmatory method for MRSA.

Antimicrobial susceptibility testing

Disk diffusion testing is performed using Mueller-Hinton agar plates containing 0.2% NaCl incubated at 37°C for 24 hours. Vancomycin resistance is confirmed using an E-test performed as recommended by the manufacturers using Mueller-Hinton agar containing 0.2% NaCl, with inoculums adjusted to 0.5 MaCFarland standard and incubated at 37°C in air for 18-24 hours. Isolates with vancomycin MIC of ≥ 16ug/mL are considered resistant.

5. Listeria monocytogenes

Procedure Isolation of *Listeria monocytogenes* according to the revised EN ISO 11290-2:2017 standard (26)

Isolation

Use 25 ml of test milk to 225 ml of buffered peptone water and incubate at 37 oC for 24 hours. Surface plate 100 ul onto Agar Listeria acc. Ottaviani and Agosti (ALOA) plates using a sterile spreader, incubate at 24-48 hours at 37 oC. Presumptive Listeria monocytogenes form regular round colonies with a blue-green color and an opaque halo.

Identification of *Listeria monocytogenes*

Pick presumptive *Listeria monocytogenes* colonies and further purify on Tryptone Soya Yeast Extract agar (TSYEA). Pinpoint colonies on TSYEA can be subjected to further identification procedures, including Gram's staining, catalase test, and oxidase test, methyl red, Voges- Proskauer, nitrate, and sugar fermentation tests with xylose, rhamnose, mannitol, and α methyl D-mannopyranoside.

Microbiological Techniques for Isolation of Fish Bacterial Pathogens

Preparation of media:

a- Liquid broth media

- Weigh the prescribed quantity of powder (as mentioned on the label of the pack) and dissolved in the required amount of water in a conical flask;
- Dissolve the ingredients by warming, poured into test tubes and sterilized in autoclave.

b- Solid plate/ slant/ deep tube media

- Weigh the prescribed quantity of powder and dissolved in required amount of water in a conical flask;
- Sterilize the prepared medium in autoclave and then allowed to cool for some time;
- Poured in to sterilized petridishes or test tubes while still warm and before it solidifies;
- Allow to solidify upon cooling to room temperature. (Note: Media in very hot condition should never be poured into containers, as it leads to condensation of water on the wall of the containers, which may lead to its contamination).

Sterilisation:

i- Glasswares are sterilized in hot air oven at 160℃ for 1 hour;

ii- Media in autoclave at 121℃ (15 psi pressure) for 15 minutes.

Note: Glass wares can also be sterilized in autoclave, but media **should never** be sterilized in the oven, as water escapes from the media and they dehydrate).

Inoculation:

i- Surface samples (gills or skin) is rubbed with a sterilized swab and the swab is touched to the surface of a sterilized nutrient agar/ TSA plate and from the point of touch, streaks are made by sterilized loop aseptically.

Incubation:

i- Inoculated broth tubes or agar plates are incubated at 30 -35℃ for 24 to 48 hrs.

Observation:

i- Turbidity in liquid broth and colony growth on agar plates indicate growth of bacteria.

ii- On observation, if colony growth has taken place on the agar plate, then there is bacterial pathogen in the sample

iii- Isolation.

iv- Select representative colonies (colonies having dissimilar characteristics) of bacteria from the mixed population.

v- Isolate the individual species of bacteria aseptically by inoculating onto separate agar plates to get the pure cultures as per the standard protocol.

vi- Observe and analyse the inoculated plates for colony characteristics:

Size : Pinpoint, small, moderate or large

- Pigmentation : Colour of colony
- Form : The shape of the colony is described as follows:
- (a) Circular : Unbroken peripheral edge
- (b) Irregular : Indented peripheral edge
- (c) Rhizoid : Root like spreading growth

Margin : The appearance of the outer edge of the colony is described as follows:

- (a) Entire : Sharply defined, even
- (b) Lobate: Marked identifications
- (c) Undulate : Wavy indentations
- (d) Serrate : Tooth like appearance

- (e) Filamentous : Thread like spreading edge

Elevation : The degree, to which colony is raised as described as follows

- (a) Flat : Elevation not discernible
- (b) Raised : Slightly elevated
- (c) Convex: Dome shaped elevation
- (d) Umbonate: Raised with elevated convex central region

6.Aeromonas and *Vibrio* species

Under aseptic conditions, swab samples should be taken from skin and gills.
The samples should be inoculated into Tryptic soy broth and incubated at 27- 30°C for 24 hours,
Then, a loop full of broth should be streaked onto Tryptic soy agar (TSA) plates and TSA plates with 2% NaCl (for *Vibrio* species), and incubated at 27- 30°C for 48 hours.
For purification purposes, pure colonies were further sub-cultured into Tryptic soy agar.
The suspected purified colonies should be picked up and streaked over Thiosulphate citrate bile salt agar (TCBS) as a selective diagnostic media.
Aeromonas base agar media were supplemented with ampicillin (5 mg/L); for *Aeromonas* spp.
A loop full of pure culture should be inoculated on nutrient agar slant and could be kept for further identification.
Take loop full for the second time and inoculate on semisolid nutrient agar and preserved for further biochemical testing.
Identification of the isolates should be carried out according to Quinn et al. (2002) and Austin & Austin (2012) using the routine study of the morphological character and biochemical as shown in the table below:

Table: The morphological and biochemical characteristics of *Aeromonas* spp., and *Vibrio* spp.

	Pathogens							
Tests	*A. hydrophila*	*A. veronii*	*A. sobria*	*A. cavae*	*A. jandae*	*V. alginolyticus*	*V. harvey*	*V. vulnificus*
Motility	+	+	+	+	+	+	+	+
Cytochrom oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Indole	+	+	+	+	+	+	+	+
6% NaCl	-	-	-	-	-	+	+	+
H_2S (TSI)	+	-	-	-	-	+	-	-
Citrate	+	+	+	+	+	-	+	+
Vogaus proskauer	+	+	-	-	+	+	-	-
Ornithen decarboxylase	-	+	-	-	+	+	-	+
Acid production from Arabinose	+	-	-	+	-	-		-

Sucrose	+	+	+	+	-	+	+	-
Maltose	+	+	+	-	+			
Mannitol						+	+	-

7. *Streptococcus* spp

To isolate *Streptococcus* species from fish:

I. Collect the fish samples, ensuring aseptic techniques to avoid contamination.

II. Homogenize the tissue in a suitable sterile buffer to release the bacteria.

III. Inoculate the homogenate onto selective media

IV. Subsequently to blood agar, which supports the growth of *Streptococcus* while inhibiting other microorganisms.

V. Incubate the plates at 35°C for 24 to 48 hours under aerobic conditions.

VI. After incubation, examine the colonies for typical Streptococcus morphology, such as smooth, circular colonies.

VII. Perform Gram staining to confirm the presence of Gram-positive cocci.

VIII. Further characterize isolates using panel of biochemical tests and identify by molecular methods using PCR

Antimicrobial Sensitivity Testing

Grow three to five colonies of *Listeria monocytogenes* in 5-ml Mueller-Hinton Broth (Oxoid, UK) and adjust turbidity to a density of 0.5 McFarland standards. Streak the suspension uniformly onto the Mueller-Hinton agar plate containing 5% horse blood (Oxoid, UK) with a cotton swab. Antibiotic discs were placed on the surface of each plate. Incubate at 37 oC for 24 h. The diameter of the growth inhibition zone surrounding each disc will be measured and interpreted using the interpretative criteria provided by CLSI for *Escherichia coli* ATCC 29922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 as control strains.

Broth Micro-Dilution Antimicrobial Susceptibility Testing

This SOP is prepared for *in vitro* broth micro-dilution AST of colistin sulphate for clinical reporting of MIC values.

Medium: Cation-adjusted Mueller- Hinton Broth (CAMHB)

• Use CAMHB for routine broth dilution susceptibility testing of rapidly growing aerobic Gram negative bacteria (*E. coli, Klebsiella* spp and *Pseudomonas aeruginosa*)

• Check each batch of MHB and ensure that the final pH is between 7.2 and 7.4.

• Perform MIC with each batch of MHB using a standard set of QC organisms

• If a new lot of MHB does not yield the expected MIC values, investigate the cation content along with other variables and components of the test

Preparation of Drug Stock Solution of Colistin

• The formulation of reference standard powder used for AST is Colistin sulphate (30,000 units/ mg or 30 UN/µg) as per CLSI Standard.

• The colistin powder available in the market has different potencies

• Before preparing the stock solution, please check the lot number and Certificate of Analysis

(CAS/ CoA) the supplier provided separately for each lot.

• Please ensure the lot number as mentioned in the colistin sulphate vial and the CAS are the same, as the potency might differ for each lot number

• ≥ 15000 UN/mg mentioned in colistin vial does not mean the potency is 15000 UN/mg

Each lot number of colistin vial supplied by manufacturer have different potency of UN/ mg

• If CAS is not provided with the colistin sulphate vial, request the vendor for the certificate of analysis for the Lot number of colistin purchased move to SOPs

***Potency calculation*:**

The pure agent (reference) of colistin sulphate has a potency of 30,000 UN/mg, which is 30 UN/ µg. The first step is to determine the potency of the colistin sulphate powder to be used for BMD. For this, divide the given potency results in UN/mg (as given in the CoA of the colistin powder used for BMD) by 30 UN/µg. This will give the potency of the colistin powder in µg/mg (microgram/mg)

The second step is to prepare the primary stock solution so that the final concentration of active colistin sulphate (calculated against referenced pure salt) is 1mg/ml. Label this as Primary stock Colistin

Use the above prepared primary stock solution (1000 µg/ml) to make the desired concentration of working stock solution move to SOPs

Example 1

If the potency of Colistin powder available in colistin sulphate salt mentioned in the CAS/ CoA Batch number SLBZ 2145 is 23299 UN/mg, then potency with reference to pure agent is =23299 UN/mg = 776 µg/mg 30 UN/ µg

To prepare stock solution of 1000 µg/ml (1mg/ml), weigh 10 mg of this colistin sulphate powder with potency of 776 µg/mg and add 7.76 ml of autoclaved distilled water. 7760 µg /7.76 ml = 1mg/ml

Example 2

If the potency of Colistin powder available in colistin sulphate salt mentioned in the CAS is 22974 UN/mg), then potency with reference to pure agent is =22974 UN/mg = 765 µg/mg 30 UN/ µg

To prepare stock solution of 1000 µg/ml (1mg/ml), weigh 10 mg of this colistin sulphate powder with potency of 765 µg/mg and add to 7.65 ml of autoclaved distilled water. 7650 µg /7.65 ml = 1mg/ml

Storage of Primary Stock Solution of Colistin

Dispense small volumes of the primary stock solutions (approx. 100 µl – 200 µl as per requirement) into sterile 1.5 – 2 ml cryovials/cryotubes; carefully label and seal them, and store them at −20°C. Never store them in a self-defrosting freezer.

Note:

Do not refreeze the stocks; repeated freeze-thaw cycles accelerate the degradation of antimicrobial agents, particularly polymyxins.

Stock solutions of antibiotics can be stored at -20℃ or less for one to two months without significant loss of activity. In all cases, directions provided by the drug manufacturer must be considered in addition to these general recommendations

Preparation of Working Stock Solution of Colistin

Take out from the freezer the required number of primary stock solution tubes and prepare working stock solution of 4 x final drug concentration. To achieve a final concentration of 16 µg/ml concentration, prepare a working stock solution of 64 µg/ml in a sterile microcentrifuge tube (MCT). To achieve this, add 64 µl from the primary stock solution to 936 µl of autoclaved MHB in another MCT. As an alternative, to avoid wastage, 64 µl of primary stock solution can also be used.

Appendix IIIb

Standard Operating Procedure (SOP) for the Shipment of Categories A (UN2814, UN2900) and B (UN3373) Biological Materials

a. Purpose

This SOP provides steps and requirements for the safe and compliant shipment of Category A and B biological materials as stipulated by the International Air Transport Association (IATA) and the United Nations (UN) regulations.

b. Scope

This SOP applies to all personnel involved in the preparation, packaging, labeling, and shipping of Category B biological materials.

3. Definitions

i. Category A Materials

Infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals

Infectious substance, affecting humans (UN2814)

Infectious substance, affecting animals only (UN2900)

ii. Category B Materials (UN 3373)

Biological substances that are not classified as Category A (UN3373). They pose a minimal risk to public health and safety.

4. Responsibilities

Shipping Personnel: Ensure proper packaging, labeling, and documentation.

Laboratory Personnel: Identify materials for shipment and ensure compliance with this SOP.

Safety Officer: Monitor compliance and provide training on shipping regulations.

5. Materials Required

Primary containment (e.g., leak-proof containers)

Secondary packaging (e.g., sturdy boxes)

Absorbent material (e.g., absorbent pads)

Labels (UN2814, UN2900, UN3373 label, danger and biohazard symbols)

Documentation (e.g., waybill, shipper papers, declaration forms)

6. Procedure

6.1 Preparation

Identify Biological Material Type: Confirm that the biological material is classified as Category A (UN2814, UN2900) or Category B (UN3373).

Risk Assessment: Conduct a risk assessment to determine necessary precautions.

6.2 Packaging

Category A Packaging Requirements:

i. Use triple packaging: primary container (leak-proof), secondary container (leak-proof), and outer packaging.

ii. Ensure all containers are clearly labeled with the appropriate UN number and biohazard symbol.

iii. Include absorbent material between the primary and secondary containers.

Category B Packaging Requirements:

i. Use triple packaging: primary container (leak-proof), secondary container (leak-proof), and outer packaging.

ii. Clearly label with the appropriate UN number and biohazard symbol.

iii. Include absorbent material to absorb any potential leakage

Note: In both categories, place the primary container in a secondary container that is rigid and durable. Place the secondary package in a sturdy outer box and ensure the box is appropriately labeled and sealed.

6.3 Labeling

Markings:

Clearly label the outer package with the UN number UN2814, UN2900 or UN3373 as appropriate. Include the biohazard symbol if applicable.

6.4 Documentation:

Complete required shipping documents:

i. Include a declaration of contents.

ii. Provide emergency contact information.

iii. Indicate the appropriate UN number and classification.

iv. Keep copies of all documentation for records.

6.5 Shipping

i. Courier Selection: Choose a courier service that is trained in handling biological materials.

ii. Transport: Ensure that the shipment is transported in accordance with applicable regulations.

7. Training

All personnel involved in the handling and shipment of biological materials must receive training on:

i. Classification and packaging requirements

ii. Relevant regulatory guidelines

Emergency procedures in case of spills or incidents

8. Emergency Procedures

In the event of a spill or exposure during shipment, follow the facility's emergency response plan.

9. Record Keeping

Maintain records of all shipments, including documentation and training records, for a minimum of three years.

This SOP should be reviewed annually and revised as necessary to comply with current regulations and best practices (IATA, 2007; https://gcbs.sandia.gov/tools/gbrmc/).

APPENDIX IV: DISTRIBUTION OF THE SAMPLES

Type of Samples	**Total no. of samples = 24,612**												
	Distribution of the samples in the six geopolitical zones												
	North-east (4,102 samples)		North-central (4,102 samples)		North-west (4,102 samples)		South-east (4,102 samples)		South-south (4,102 samples)		South-west (4,102 samples)		**Total**
	State A (2051)	State B (2051)	State A (2051)	State B (2051)	State A (2051)	State B (2051)	State A (2051)	State B (2051)	State A (2051)	State B (2051)	State A (2051)	State B (2051)	
Fingerlings	293	293	293	293	293	293	293	293	293	293	293	293	**3,516**
Juveniles	293	293	293	293	293	293	293	293	293	293	293	293	**3,516**
Grow-out	293	293	293	293	293	293	293	293	293	293	293	293	**3,516**
Adult	293	293	293	293	293	293	293	293	293	293	293	293	**3,516**
Feeds	293	293	293	293	293	293	293	293	293	293	293	293	**3,516**
Pond water	293	293	293	293	293	293	293	293	293	293	293	293	**3,516**
Sediments	293	293	293	293	293	293	293	293	293	293	293	293	**3,516**
Total	2,051	2,051	2,051	2,051	2,051	2,051	2,051	2,051	2,051	2,051	2,051	2,051	**24,612**

APPENDIX Va: SAMPLE SIZE CALCULATION FOR CATFISH FRY AND FINGERLINGS

Bacterial Organism	Prevalence in Chickens (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	42	Amande and Nwaka et al., 2013	384	914.3	45.7	960	
Enterococcus	77	Amande and Nwaka et al., 2013	384	498.7	24.9	524	
Salmonella	11.5	Ibrahim et al., 2014	384	3339.1	167.0	3506	**3516**
*Campylobacter**	42	Wilson and Moore, 1996	384	914.3	45.7	960	
Aeromonas	30.5	Omeje and Chukwu, 2014	384	1259.0	63.0	1322	
Vibrio	33	Amande and Nwaka et al., 2013	384	1163.6	58.2	1222	

* Prevalence figure for *Campylobacter* in shellfish, no data on catfish in Nigeria

APPENDIX Vb: SAMPLE SIZE CALCULATION FOR CATFISH JUVENILES

Bacterial Organism	Prevalence in Chickens (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	42	Amande and Nwaka et al., 2013	384	914.3	45.7	960	
Enterococcus	77	Amande and Nwaka et al., 2013	384	498.7	24.9	524	
Salmonella	11.5	Ibrahim et al., 2014	384	3339.1	167.0	3506	**3516**
*Campylobacter**	42	Wilson and Moore, 1996	384	914.3	45.7	960	
Aeromonas	30.5	Omeje and Chukwu, 2014	384	1259.0	63.0	1322	
Vibrio	33	Amande and Nwaka et al., 2013	384	1163.6	58.2	1222	

* Prevalence figure for *Campylobacter* in shellfish, no data on catfish in Nigeria

APPENDIX Vc: SAMPLE SIZE CALCULATION FOR CATFISH GROW-OUT

Bacterial Organism	Prevalence in Chickens (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	42	Amande and Nwaka et al., 2013	384	914.3	45.7	960	
Enterococcus	77	Amande and Nwaka et al., 2013	384	498.7	24.9	524	
Salmonella	11.5	Ibrahim et al., 2014	384	3339.1	167.0	3506	**3516**
*Campylobacter**	42	Wilson and Moore, 1996	384	914.3	45.7	960	
Aeromonas	30.5	Omeje and Chukwu, 2014	384	1259.0	63.0	1322	
Vibrio	33	Amande and Nwaka et al., 2013	384	1163.6	58.2	1222	

* Prevalence figure for *Campylobacter* in shellfish, no data on catfish in Nigeria

APPENDIX Vd: SAMPLE SIZE CALCULATION FOR ADULT CATFISH

Bacterial Organism	Prevalence in Chickens (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	42	Amande and Nwaka et al., 2013	384	914.3	45.7	960	
Enterococcus	77	Amande and Nwaka et al., 2013	384	498.7	24.9	524	
Salmonella	11.5	Ibrahim et al., 2014	384	3339.1	167.0	3506	**3516**
*Campylobacter**	42	Wilson and Moore, 1996	384	914.3	45.7	960	
Aeromonas	30.5	Omeje and Chukwu, 2014	384	1259.0	63.0	1322	
Vibrio	33	Amande and Nwaka et al., 2013	384	1163.6	58.2	1222	

* Prevalence figure for *Campylobacter* in shellfish, no data on catfish in Nigeria

APPENDIX Ve: SAMPLE SIZE CALCULATION FOR FISH-FEEDS

Bacterial Organism	Prevalence in Chickens (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	42	Amande and Nwaka et al., 2013	384	914.3	45.7	960	
Enterococcus	77	Amande and Nwaka et al., 2013	384	498.7	24.9	524	
Salmonella	11.5	Ibrahim et al., 2014	384	3339.1	167.0	3506	**3516**
*Campylobacter**	42	Wilson and Moore, 1996	384	914.3	45.7	960	
Aeromonas	30.5	Omeje and Chukwu, 2014	384	1259.0	63.0	1322	
Vibrio	33	Amande and Nwaka et al., 2013	384	1163.6	58.2	1222	

* Prevalence figure for *Campylobacter* in shellfish, no data on catfish in Nigeria

APPENDIX Vf: SAMPLE SIZE CALCULATION FOR CATFISH POND WATER

Bacterial Organism	Prevalence in Chickens (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	42	Amande and Nwaka et al., 2013	384	914.3	45.7	960	
Enterococcus	77	Amande and Nwaka et al., 2013	384	498.7	24.9	524	
Salmonella	11.5	Ibrahim et al., 2014	384	3339.1	167.0	3506	**3516**
*Campylobacter**	42	Wilson and Moore, 1996	384	914.3	45.7	960	
Aeromonas	30.5	Omeje and Chukwu, 2014	384	1259.0	63.0	1322	
Vibrio	33	Amande and Nwaka et al., 2013	384	1163.6	58.2	1222	

* Prevalence figure for *Campylobacter* in shellfish, no data on catfish in Nigeria

APPENDIX Vg: SAMPLE SIZE CALCULATION FOR SEDIMENTS FROM CATFISH POND

Bacterial Organism	Prevalence in Chickens (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	42	Amande and Nwaka et al., 2013	384	914.3	45.7	960	
Enterococcus	77	Amande and Nwaka et al., 2013	384	498.7	24.9	524	
Salmonella	11.5	Ibrahim et al., 2014	384	3339.1	167.0	3506	**3516**
*Campylobacter**	42	Wilson and Moore, 1996	384	914.3	45.7	960	
Aeromonas	30.5	Omeje and Chukwu, 2014	384	1259.0	63.0	1322	
Vibrio	33	Amande and Nwaka et al., 2013	384	1163.6	58.2	1222	

* Prevalence figure for *Campylobacter* in shellfish, no data on catfish in Nigeria

APPENDIX VI: PROPOSED DISTRIBUTION OF SAMPLES BY BIRD TYPE, LABORATORY AND SAMPLING LOCATIONS

SOURCE GPZ AND CHICKEN/SAMPLE TYPE	SECTOR*			TOTAL NUMBER OF SAMPLES FROM FARMS	LIVE BIRDS IN CAGES**		SLAUGHTERED		TOTAL NUMBER OF SAMPLES FROM LBM	PROCESSING PLANT***	NO. OF SAMPLES/ LAB
	1	2	3		Broilers	Layers	Broilers	Layers			
	Cloacal Swabs	Cloacal swabs	Cloacal swabs		Cloacal swabs	Cloacal swabs	Caecal	Caecal		Caecal	
NC1	2	8	32	42	20	20	20	20	80	0	122
NC2	2	8	32	42	20	20	20	20	80	0	122
NW1	2	8	32	42	20	20	20	20	80	0	122
NW2	2	8	32	42	20	20	20	20	80	0	122
SE	2	8	32	42	20	20	20	20	80	0	122
SW	2	8	32	42	20	20	20	20	80	68	190
BROILERS	6	24	96	126	120	0	120	0	366	34	400
LAYERS	6	24	96	126	0	120	0	120	366	34	400
TOTAL	12	48	192	252		240		240	732	68	800

* Sector 4 is excluded due to the difficulty of sampling in this sector and the lack of a standardized approach in studying birds in the sector.

** In Nigeria there is evidence (21) of use of antimicrobial drugs in live bird markets while the birds are awaiting sale. The birds might have come from farms that used drugs or not. This constitutes a different source of selection pressure. Birds in cages can be mixed types. Mixed bird types in cages do not constitute a homogenous epidemiological group so will be excluded from sampling. Only birds in cages that separate bird types will be

included.

***There are very few dedicated poultry processing plants associated with some large Sector 1 farms (e.g. Zartech farms in Ibadan, Oyo State, Southwest Nigeria)

APPENDIX VII: SAMPLE SIZE DETERMINATION FOR AMR SURVEY IN CATTLE, GOAT, SHEEP AND PIGS

CATTLE FAECAL/RECTAL SAMPLES – FARMS

Bacterial Organism	Prevalence in Cattle (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	13.8	Fashae *et al.* 2018	384	2782.6	139.1	2922	**2922**
Campylobacter	14.8	Thomas et al, 2020	384	2594.6	129.7	2724	

CATTLE INTESTINAL SAMPLES – SLAUGHTERED

Bacterial Organism	Prevalence in Cattle (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	26.8	Fashae *et al.* 2018	384	1432.8	71.6	1504	**8064**
Campylobacter	5	Thomas et al, 2020	384	7680.0	384.0	8064	

CATTLE MEAT SAMPLES

Bacterial Organism	Prevalence in Cattle (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	22.2	Smith *et al.*,2016	384	1729.7	86.5	1816	**8400**
Campylobacter	4.8	Thomas et al, 2020	384	8000.0	400.0	8400	

CATTLE – MILK DAIRYFARMS

Bacterial Organism	Prevalence in Cattle (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Staphylococcus aureus	15.3	Umaru *et al.*,2019	384	2509.8	125.5	2635	
Listeria monocytogenes	8.3	Usman *et al.*,2016	384	4626.5	231.3	4858	**4858**

GOAT FAECAL/RECTAL SAMPLES - FARMS

Bacterial Organism	Prevalence in Goat(%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	4.5	Umeh et al, 2014	384	8533.3	426.7	8960	**8960**
Campylobacter	19.9	Thomas *et al.*,2020	384	1929.6	96.5	2026	

GOAT INTESTINAL SAMPLES - SLAUGHTERED

Bacterial Organism	Prevalence in Goat(%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	26.7	Saleh *et al.*,2015	384	1438.2	71.9	1510	**2026**
*Campylobacter**	19.9	Thomas *et al.*,2020	384	1929.6	96.5	2026	

** Estimates used for faecal samples*

GOAT MEAT SAMPLES

Bacterial Organism	Prevalence in Goat (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	16.7	Smith *et al.*,2016	384	2299.4	115.0	2414	**2414**
Campylobacter	29.7	Thomas *et al.*,2020	384	1292.9	64.6	1358	

SHEEP FAECAL/RECTAL SAMPLES - FARMS

Bacterial Organism	Prevalence in Sheep (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
E. coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	4.1	Thomas *et al.*,2020	384	9365.9	468.3	9834	**9834**
Campylobacter	17.3	Thomas *et al.*,2020	384	2219.7	111.0	2331	

SHEEP INTESTINAL SAMPLES - SLAUGHTERED

Bacterial Organism	Prevalence in Sheep (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia Coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	40	Saleh *et al.*,2015	384	960.0	48.0	1008	**7074**
Campylobacter	5.7	Thomas *et al.*,2020	384	6736.8	336.8	7074	

SHEEP MEAT SAMPLES

Bacterial Organism	Prevalence in Sheep (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	36.6	Musa *et al.*,2019	384	1049.2	52.5	1102	**3840**
Campylobacter	10.5	Thomas *et al.*,2020	384	3657.1	182.9	3840	

PIGS FAECAL/RECTAL SAMPLES - FARMS

Bacterial Organism	Prevalence in Pigs (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
E. coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	40	Umeh *et al.*,2014	384	960.0	48.0	1008	**1214**
Campylobacter	33.2	Thomas *et al.*,2020	384	1156.6	57.8	1214	

PIGS INTESTINAL SAMPLES - SLAUGHTERED

Bacterial Organism	Prevalence in Pigs (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
E. coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	22.5	Amaechi and Ezeronye, 2006	384	1706.7	85.3	1792	**3102**
Campylobacter	13	Thomas *et al.*,2020	384	2953.8	147.7	3102	

PIG MEAT SAMPLES

Bacterial Organism	Prevalence in Sheep (%)	Reference	Number of resistant isolates (50% prevalence)	Number of samples to take for isolation of the bacterium	Adjusted for missingness (+5%)	No. of samples	Selected Sample Size (Largest)
Escherichia Coli	100	Commensal	384	384.0	19.2	403	
Enterococcus	100	Commensal	384	384.0	19.2	403	
Salmonella	6.25	Smith *et al.*,2016	384	6144.0	307.2	6451	**6451**
Campylobacter	8.5	Thomas *et al.*,2020	384	4517.6	225.9	4744	

APPENDIX VIII: SAMPLE COLLECTION FORM FOR FISH FARMS

IDENTIFICATION			
Round of Sampling		Date of Collection	
Name of Sampler			
Mobile Phone No.			

LOCATION OF FARM			
Name of Farm		Village/Town/City	
Name of Owner/ Phone contact			
Address			
Coordinates	Longitude:		Latitude:

INFORMATION ON FISH AND FARM SAMPLED			
Species/Type of Fish		Pond Type	
Water Source		Approximate Age	
Total No. of Fish/ Capacity of Farm		No. of Ponds	
Approximate No. of Fish from Pond Sampled			
Other species of birds or livestock on farm			
Antibiotic Use History on the Farm in the past six months			
Disposal of waste pond water			
Use of poultry litter to fertilize ponds			

SAMPLE INFORMATION			
Sample ID No.		Sample Type	
Single or Pooled Sample (if pooled indicate number pooled)			
Transport media type (if used)			
Total number of samples taken from farm			

APPENDIX IX: SAMPLE COLLECTION FORM FROM POULTRY FARMS

IDENTIFICATION			
Round of Sampling		Date of Collection	
Name of Sampler			
Mobile Phone Number			

LOCATION			
Name of Farm		Village/Town/City	
Name of Owner/Phone Contact			
Address			
Coordinates	Longitude:	Latitude:	

INFORMATION ON BIRDS			
Species		Production Type (Broiler, Layer, Others)	
Breed		Approximate Age	
Total No. of Birds on Farm		No. of housing units	
Biosecurity Sector Classification (Sector 1 -4)			
No. of Birds from House Sampled			
Type of Birds from House Sampled			
Other species of birds or livestock on farm			
Antibiotic Use History on the Farm in the last year			

SAMPLE INFORMATION			
Sample ID No.		Sample Type	
Single or Pooled Sample (if pooled indicate number pooled)			
Transport media type (if used)			
Total number of samples taken from farm			

APPENDIX X: SAMPLE COLLECTION FORM FROM LIVE BIRD MARKETS OR SLAUGHTER HOUSES

IDENTIFICATION			
Round of Sampling		Date of Collection	
Name of Sampler			
Mobile Phone Number			

FACILITY INFORMATION			
Name of facility		Village/Town/City	
No. of Marketers/Processors		Average Number of chickens slaughtered/day	
Name and contact of Representative			
Address			
Coordinates	Longitude:	Latitude:	

MARKETER/VENDOR INFORMATION				
Name of Marketer		Phone contact		
Average number of birds sold/slaughtered day		**Type of chickens**	**Number**	**Source**
		Broilers		
		Layers		
		Local chickens		

SAMPLE INFORMATION			
Sample ID No.		Sample Type	
Single or Pooled Sample (if pooled indicate number pooled)			
Transport media type (if used)			
Total number of samples taken from farm			

APPENDIX XIa: SAMPLE COLLECTION FORM FOR LIVESTOCK FARMS

IDENTIFICATION			
Round of Sampling		Date of Collection	
Name of Sampler			
Mobile Phone Number			

LOCATION			
Name of Farm		Village/Town/City	
Name of Owner/Phone contact			
Address			
Coordinates	Longitude:	Latitude:	

INFORMATION ON ANIMALS SAMPLED			
Species		Production Type	
Breed		Approximate Age	
Total No. of Animals on Farm		No. of housing units	
No. of Animals from House Sampled			
Other species of birds or livestock on farm			
Antibiotic Use History on the Farm in the last year			

SAMPLE INFORMATION			
Sample ID No.		Sample Type	
Single or Pooled Sample (if pooled indicate number pooled)			
Transport media type (if used)			

Total number of samples taken from farm	

APPENDIX XIb: SAMPLE COLLECTION FORM FOR ABATTOIRS

IDENTIFICATION			
Round of Sampling		Date of Collection	
Name of Sampler			
Mobile Phone Number			

FACILITY INFORMATION				
Name of Abattoir		Village/Town/City		
Average Number of slaughtered/day	Cattle	Goats	Sheep	Others
Name and contact of Representative				
Address				
Coordinates	Longitude:		Latitude:	

SAMPLE INFORMATION			
Sample ID No.		Sample Type	
Single or Pooled Sample (if pooled indicate number pooled)			
Transport media type (if used)			
Total number of samples taken from farm			

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