PROTOCOL FOR SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN FOODBORNE BACTERIA IN NEPAL

Prepared by:

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Ministry of Agriculture and Livestock Development
Department of Food Technology and Quality Control
National Food and Feed Reference Laboratory
Babarmahal, Kathmandu, Nepal
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Disclaimer

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Protocol for Surveillance of Antimicrobial Resistance in Foodborne Bacteria in Nepal

Version 2.0 (2024)- Revised Edition

Key changes in this version include

1. Data management and reporting

The provincial food laboratories, which act as surveillance sites, are required to promptly submit their categorized test results to the DFTQC. These results should include both positive and negative findings. For foodborne AMR surveillance, the surveillance site laboratories must collect data from both passive and active surveillance. The data should either be recorded in an Excel sheet or uploaded into the Laboratory Information Management System (LIMS).

Once the data is collected and verified, the laboratories must submit it to DFTQC by the first week of each month. Even if no AMR cases are detected during a given period, the surveillance site laboratories are still obligated to submit a "zero data" report within the specified time frame. This ensures that DFTQC receives consistent and accurate updates from all surveillance sites.

2. Annex A: Bacterial isolates to be shared from provincial food laboratories to DFTQC for bio repository

Staphylococcus aureus= At least 25% of the all isolates instead of 50% of the isolates

Escherichia coli= At least 25% of the all isolates instead of 50%

Enterococcus spp.= At least 25% of the all isolates instead of all isolates.

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Foreword

Antimicrobial resistance (AMR) is a growing global concern affecting human, animal, and plant health, as well as food and the environment. To address this threat, the World Health Assembly approved a Global Action Plan (GAP) on AMR in 2015, which member nations are expected to adopt. One of GAP's five primary approaches to controlling AMR is the establishment of an effective surveillance system to generate the evidence and timely act on outbreaks. The food microbiology laboratory plays a crucial role in detecting and monitoring foodborne AMR and generating trustworthy evidence for policymaking.

In Nepal, the food microbiology laboratory at National Food and Feed Reference Laboratory (NFFRL) under Department of Food Technology and Quality Control (DFTQC) has initiated AMR surveillance in the food sector and has been designated as a reference laboratory for AMR surveillance activity in this sector. The NFFRL plans to expand this surveillance to provincial food laboratories, creating a network that can generate comparable, representative data on AMR in the food chain. This network may also establish an effective AMR surveillance system in the food sector, facilitating communication, reporting, and data sharing. Strong coordination and networking among the surveillance sites is essential, and all provincial food laboratories must follow the same standard protocol to ensure the generation of reliable and comparable data. To this end, the NFFRL has developed a national AMR surveillance protocol to facilitate the implementation of laboratory-based AMR surveillance in the food sector in provincial food laboratories. This protocol defines the objective of AMR surveillance, priority specimens and pathogens, data management and reporting requirements, quality assurance measures, monitoring and evaluation of surveillance activities, and documentation.

I would like to express gratitude to the AMR TWG-Food, experts involved in drafting the protocol, the microbiology laboratory team, and all those who contributed to this endeavor. I also thank the Fleming Fund Country Grant for Nepal/FHI 360 for their support and technical assistance, and the UK Department of Health and Social Care for their financial support in developing this protocol.

I strongly believe that this protocol will aid in the efficient implementation of laboratory-based AMR surveillance in the food sector in Nepal.

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Dr. Matina Joshi (Vaidya)
Director General

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Acronyms

AMR	Antimicrobial Resistance
NSC AMR	National Steering Committee for Antimicrobial Resistance
AST	Antibiotic susceptibility test
CLSI	Clinical and Laboratory Standards Institute
CVL	Central Veterinary Laboratory
DFTQC	Department of Food Technology and Quality Control
DQA	Data Quality Assurance
EQA	External Quality Assessment
IQC	Internal Quality Control
LWM	Laboratory Waste Management
MDR	Multidrug-resistant
MoALD	Ministry of Agriculture and Livestock Development
MoHP	Ministry of Health and Population
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCC	National Coordination Center
NEQAS	National External Quality Assessment Scheme
NFFRL	National Food and Feed Reference Laboratory
NPHL	National Public Health Laboratory
NTWC	National Technical Working Committee
OH	One Health
QA	Quality Assurance
QC	Quality Control
QSRD	Quality Standard and Drug Regulation Division
SOP	Standard Operating Procedure
TWG	Technical Working Group

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1. Scope

This protocol provides a concise overview of the national antimicrobial resistance (AMR) surveillance program, with a special focus on food. It outlines the sample types, monitored pathogens, and data management across food microbiology laboratories. The emphasis is on maintaining robust data quality and documentation in both the reference food microbiology laboratory and the surveillance site provincial food technology offices.

2. Introduction

AMR is also a growing concern in the food chain, as it can result in the spread of drug-resistant bacteria and compromise food safety. AMR occurs when microorganisms become resistant to antimicrobials that previously killed/inhibited them and treated the infection. This phenomenon poses a severe risk to exposed workers, their families, and customers in the food production industry. Unsafe food can cause a variety of health issues, including acute or chronic food poisoning or foodborne diseases, reproductive and developmental issues, AMR, malignancies, and fatalities.

The emergence of AMR in the food chain requires comprehensive responses and a long-term commitment to combat the challenges posed by drug-resistant pathogens. The food chain and the wider environment provide an easy way for humans to become infected with drug resistant bacteria. Therefore, a system should be in place to recognize and report trends of AMR monitoring in zoonotic and commensal bacteria in food products of animal origin. AMR surveillance data can be used to identify and track the emergence of drug-resistant foodborne pathogens, as well as their patterns of spread over time. This will also recognize the importance of linking information on AMR from different sectors, such as human, animal, food, agriculture, environment, along with antibiotic use in human and animal populations and antibiotic residue in the environment.

It is vital to monitor the trends over time of resistances in bacteria entering and moving through the food chain to determine the epidemiology of AMR in the food chain, as well as monitoring the impact of antibiotic use in animals. A standardized surveillance system enables collection and analysis of valid and comparable data, which can be used for informed decision making, guiding national action, and providing the evidence base for action and advocacy.

3. Objectives

The purpose of this protocol is to guide the AMR reference and food laboratories in the provinces to conduct an efficient AMR surveillance in food sector. Specific objectives are as follows

- To define priority specimens and bacterial pathogens for AMR surveillance in regular food samples
- To define antibiotics to be tested against each priority bacteria (drug-bug combination for antimicrobial susceptibility testing (AST))
- To establish a comprehensive laboratory quality assurance program to ensure the accuracy, reliability, and timeliness of AMR data generation.
- To develop and implement a standardized documentation, data sharing, and reporting mechanism for AMR data that ensures the quality, accessibility, and usability of data.

4. National AMR surveillance program

The AMR surveillance in human health started in 1999 in Nepal, with the National Public Health Laboratory (NPHL) designated as the national reference laboratory for human health. In 2011, the Central Veterinary Laboratory (CVL), the national reference laboratory for animal health, was also included in AMR surveillance system led by NPHL encompassing the animal health in AMR surveillance system. In 2021, with the support of the Fleming Fund Country Grant for Nepal (FFCGN), the AMR surveillance program was introduced in the food sector. The Department of Food Technology and Quality Control (DFTQC) currently monitors the AMR in three foodborne bacterial pathogens.

Nepal has a multisectoral approach to AMR containment. The National Coordinating Center (NCC) for AMR surveillance is Quality Standard and Drug Regulation Division (QSRD). At the apex of the governance structure, is the National Steering Committee for Antimicrobial Resistance (NSC-AMR; formerly AMRMSC) chaired by the Secretary of the Ministry of Health and Population (MoHP) with the participation of senior government officials and experts from different sectors including food sector. The NSC-AMR provides strategic direction and oversight for AMR surveillance in Nepal. There is also a National Technical Working Committee (NTWC) formed in One Health (OH) approach to monitor the implementation of AMR activities in the country. In addition to these high-level committees, there are, currently, six Technical Working Groups:

- AMR Technical Working Group for Human Health (AMR TWG-HH)
- AMR Technical Working Group for Animal Health (AMR TWG-AH)
- AMR Technical Working Group for Food (AMR TWG-Food)
- Technical Working Group for Antimicrobial Consumption (AMC TWG-HH)
- Technical Working Group for Antimicrobial Use in Human Health (AMU TWG-AH)
- Technical Working Group for Antimicrobial Use in Animal Health (AMU TWG-AH)

The establishment of the AMR TWGs is a significant milestone in the development of AMR surveillance in Nepal. The TWGs plays a critical role in coordinating AMR surveillance activities across sectors and ensuring that the country has a comprehensive and effective AMR surveillance system in place. These groups are responsible for providing technical and operational guidance to the AMR/AMC/AMU surveillance program in One Health sectors, including the development of guidelines, protocols, and standard operating procedures (SOPs).

5. AMR surveillance program in food

a. Priority pathogens

The AMR surveillance in Nepal currently focuses on monitoring the following priority pathogens in the food sector:

- *Escherichia coli*
- *Salmonella spp,*
- *Staphylococcus aureus*

In addition to the current pathogens, the AMR surveillance program in food will also include *Enterococcus* spp., *Campylobacter* spp. and *Listeria monocytogenes*.

b. Food samples

The priority specimen

Food specimen	Pathogen for AMR surveillance
Milk and dairy products	*Escherichia coli* *Salmonella* spp. *Staphylococcus aureus* *Listeria monocytogenes* *Enterococcus* spp.
Poultry products	*Escherichia coli* *Salmonella* spp. *Staphylococcus aureus* *Listeria monocytogenes* *Campylobacter jejuni, Campylobacter coli, Campylobacter lari* *Enterococcus* spp.
Meat products	*Escherichia coli* *Salmonella* spp. *Staphylococcus aureus* *Enterococcus* spp.
Fish products	*Escherichia coli* *Salmonella* spp. *Staphylococcus aureus* *Listeria monocytogenes*

6. Data management and reporting

The designated provincial food laboratories acting as surveillance sites will be responsible for maintaining comprehensive records of their testing activities.

The provincial food laboratories, which act as surveillance sites, are required to promptly submit their categorized test results to the DFTQC. These results should include both positive and negative findings. For foodborne AMR surveillance, the surveillance site laboratories must collect data from both passive and active surveillance. The data should either be recorded in an Excel sheet or uploaded into the Laboratory Information Management System (LIMS).

Once the data is collected and verified, the laboratories must submit it to DFTQC by the first week of each month. Even if no AMR cases are detected during a given period, the surveillance site laboratories are still obligated to submit a "zero data" report within the specified time frame. This ensures that DFTQC receives consistent and accurate updates from all surveillance sites.

Throughout the process of data collection and reporting, a strong emphasis will be placed on upholding the authenticity, reliability, timeliness, accuracy, and integrity of the data generated by both the surveillance sites

and reference laboratories. To facilitate the continual enhancement of data quality, regular data verification and comprehensive Data Quality Assessments (DQA) will be carried out, with a frequency ranging from quarterly to yearly. These assessments will be informed by prior data verification and DQA results.

To ensure data integrity, surveillance sites must avoid duplicate entries and thoroughly assess data quality before submitting to DFTQC. This practice minimizes errors, discrepancies, and guarantees reliable data for informed decision-making. The food microbiology laboratory at the National Food and Feed Reference Laboratory (NFFRL) is responsible for collating, analysing and periodically sharing the data with sites and other stakeholders. In case of unusual antibiogram, NFFRL verifies and communicates these findings to the DFTQC, initiating necessary actions.

Additionally, the compiled AMR data is shared with the AMR Technical Working Group on Food (AMR TWG-Food), the National Coordination Committee (NCC), National Technical Working Committee (NTWC) and the NSC-AMR.

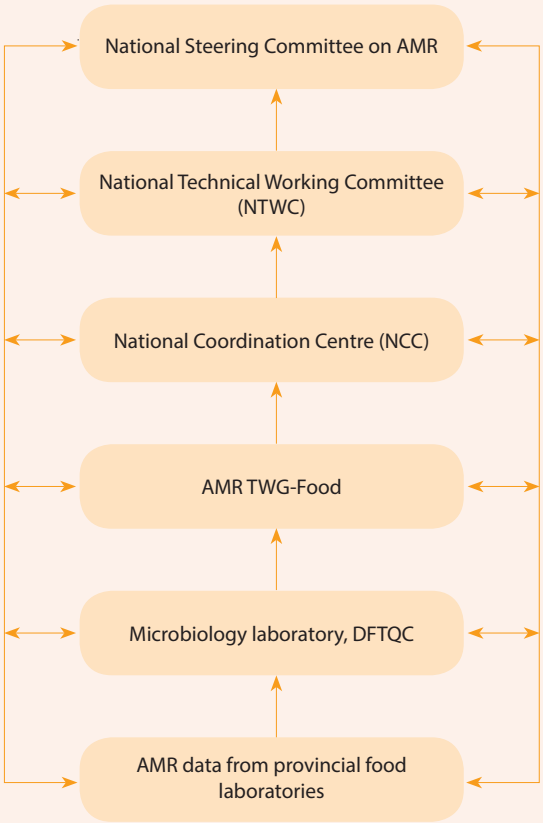
7. Quality assurance of surveillance data

Laboratory quality assurance (QA) is an essential component of the process in ensuring the accuracy, reliability and reproducibility of data collected for AMR surveillance in food. The laboratory manual for AMR surveillance in food outlines the internal and external quality control measures that will be employed.

The food microbiology laboratories at the provincial level should maintain Internal Quality Control (IQC) processes. All surveillance sites are expected to enroll in External Quality Assessmnet (EQA) scheme. DFTQC is enrolled in the National External Quality Assessment Scheme (NEQAS) conducted by CVL.

The EQA process entails distributing proficiency testing panels to surveillance site food laboratories. These panels comprise isolates used to assess proficiency in identifying bacterial strains and conducting AST. Another important measure would be confirming the reports on the subsets of isolates referred from surveillance site laboratories. Furthermore, EQA encompasses conducting on-site assessments at provincial food laboratories within the surveillance network. These evaluations offer a direct appraisal of laboratory practices, equipment, and personnel expertise. This proactive approach ensures the maintenance of elevated testing standards. Participating laboratories within the EQA framework are obligated to abide by standardized testing procedures during bacterial strain identification and AST.This adherence to established protocols is paramount for generating consistent and comparable outcomes. Following testing, laboratories communicate their findings back to the NFFRL. Subsequently, these results undergo comprehensive feedback, serving both retrospective analysis and the implementation of corrective actions to address any disparities.The effectiveness of the EQA process is enhanced by the active involvement of the DFTQC and AMR TWG-Food.

Figure: Surveillance data reporting and feedback mechanism



The laboratory manual on AMR surveillance in food comprehensively outlines the quality assurance and quality control (QA/QC) measures required to validate the performance of laboratories engaged in AMR surveillance in food.

8. Reposition of isolates

The surveillance sites are required to periodically send a specific subset of resistant bacterial isolates to the food microbiology laboratory at NFFRL, DFTQC. These will be reposited in an inventoried biorepository.

DFTQC will validate the accuracy of the results linked to these resistant isolatesand then preserve them at a temperature of -80°C.

For precise guidance on the types of isolates suitable for inclusion in the biorepository, detailed information can be found in Annex A.

Further information about managing the biorepository, including procedures for transporting samples, ensuring their safekeeping, preserving them, and facilitating their retrieval for future investigations, is thoroughly explained in the SOP dedicated to biorepository management for AMR surveillance.

9. Document control

The provincial food laboratories in various surveillance sites generate a data on foodborne AMR on a regular basis. To ensure efficient and effective management of AMR data, a standard template will be used to electronically transmit the AMR data from these laboratories to the NFFRL. Once received, the data will be stored securely in the dedicated computers of the respective laboratories and will only be accessible to authorized personnel.

To ensure effective monitoring and control of AMR data, the collated data will be analyzed by the NFFRL. The surveillance sites will be encouraged to analyze the data themselves after receiving training. The NFFRL will then share the findings with the surveillance sites, policymakers, and other stakeholders. The data will provide valuable insights into the status and trends of AMR, which will be used to inform policies, guidelines, and interventions to control the spread of antibiotic-resistant infections.

In addition, the DFTQC will publish data on the status and trends of AMR on their website, in annual bulletins, and other relevant publications. This information will be widely disseminated to inform the public, healthcare providers, researchers, and other stakeholders. The aim is to raise awareness and promote understanding of AMR, and to encourage appropriate antibiotic use to prevent the emergence and spread of resistant bacteria in food production chain.

Overall, the systematic collection, collation, and analysis of AMR data is critical for effective management of antibiotic resistance. By sharing this data, stakeholders can work together to develop evidence-based policies and strategies to tackle this global health threat.

Annex

Annex A: Bacterial isolates to be shared from provincial food laboratories to DFTQC for bio repository

Organism	Antibiotic resistant phenotypes
Salmonella spp.	All isolates
Staphylococcus aureus	At least 25% of all isolates. All MRSA (where Vancomycin MIC not conducted)
Escherichia coli	At least 25% of all isolates All MDR isolates All Carbapenem resistant isolates
Enterococcus spp.	At least 25% of all isolates
Campylobacter spp.	All isolates
Listeria monocytogenes	All isolates

Annex B: Checklist for technical monitoring of food laboratories in the food AMR surveillance network

Name of the sentinel site: _____

Address: _____

Date: _____

Assessment team members: _____

Activities	Method	Yes	No	Remarks
1. Biosafety management and documentation				
Staff received orientation on laboratory safety; biosafety and biosecurity (BSS); laboratory waste management (LWM)	MI/SI/R			
Laboratory staff are aware on Biosafety labels, hazard signs, safety precautions	MI/SI/R			
Site has SOP on BSS and LWM	R			
Site follows SOP on BSS including good food microbiological techniques	SI/O			
2. Laboratory access				
Biohazard warning symbol and sign are displayed on the doors of the rooms and areas where applicable	O			
Only authorized persons are allowed to enter the laboratory working areas	R/O			
Laboratory doors are kept closed	O			
3. Personal protection				
Laboratory gowns or uniforms (aprons) are worn during laboratory work in the laboratory	SI/O			
Appropriate gloves are worn for all procedures that may involve direct or accidental contact with potentially infectious materials	SI/O			
Protective laboratory clothing that has been used in the laboratory are not stored in the same lockers or cupboards as normal use clothing	SI/O			
Personnel wash their hands after handling infectious materials, and before they leave the laboratory working areas	SI/O			
Closed-toed footwear and/or shoe covers over outdoor shoes are worn in laboratories	O			
Storing foods or drinks, eating, drinking, smoking, applying cosmetics, handling contact lenses and using mobile phone is not done in laboratory working area	SI/O			
4. Procedures				
Mouth pipetting, keeping materials in mouth or licking labels is not practiced in the laboratory	SI/O			
Spillage tool kit with written procedure for the clean-up of all spills is available and followed	SI/O			
All spills, accidents and overt or potential exposures to infectious materials are reported to the laboratory supervisor and a written record of such accidents and incidents are maintained	SI/O			

Method Notes: O = Observation SI = Staff interview R = Records review MI = Management interview

Activities	Method	Yes	No	Remarks
Contaminated waste including liquids are decontaminated within the laboratory complex (chemically or physically) before final disposal (discharge to the sanitary sewer for liquid waste)	SI/O			
Written documents that are expected to be removed from the laboratory are protected from contamination while in the laboratory	SI/O			
5. Laboratory working areas				
The laboratory area is kept neat, clean and free of materials that are not pertinent to the work	O			
Work surfaces are decontaminated after any spill of potentially dangerous material and at the end of the working day	SI/O			
All contaminated materials, specimens and cultures are decontaminated within the laboratory complex before disposal or cleaning for reuse	SI/O			
Packing and transportation of food samples is conducted following applicable national and/or international regulations	SI/O/R			
Functional biosafety cabinet is available and in use	SI/O			
6. Laboratory waste management				
A focal person for waste management has been identified at the site.	MI/SI/R			
Laboratory waste management system	MI/SI			
SOP on safe waste management is available at the site	MI/SI/R			
Staff aware on LWM SOP and BSS and following these practices according to SOP	MI/SI/O			
Staff follow the standard precautions while handling infectious materials	MI/SI/O			
Separate color-coded bins for collecting different types of waste as per LWMSOP are placed at lab	O			
7. Logbook maintained				
Logbook for biosafety cabinet use and calibration maintained	MI/SI/R			
Logbook for autoclave use maintained	MI/SI/R			
Temperature log for incubator maintained	MI/SI/R			
Temperature log sheet maintained for refrigerators and incubators	MI/SI/R			
Logbook of N/EQAS participation and score maintained	MI/SI/R			
Logbook for subculture of QC/ATCC strains maintained	MI/SI/R			
Staff training and evaluation record sheet maintained	MI/SI/R			
Logbook of equipment maintenance maintained	MI/SI/R			
Logbook for equipment file	R			
Logbook for IQC measures for laboratory reagent/ media maintained	MI/SI/R			
Logbook for IQC measures for Antibiotic disc maintained	MI/SI/R			

Method Notes: O = Observation SI = Staff interview R = Records review MI = Management interview

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