

# PROTOCOL FOR ACTIVE AMR SURVEILLANCE OF READY TO EAT MEAT PRODUCTS PRODUCED IN KATHMANDU VALLEY

**Prepared by:**



**Government of Nepal**  
**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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# Contents

<b>1. Introduction</b>	<b>1</b>
<b>2. Objectives</b>	<b>1</b>
<b>3. Target food items</b>	<b>2</b>
<b>4. Target bacteria</b>	<b>2</b>
<b>5. Target antibiotics</b>	<b>2</b>
<b>6. Sample information</b>	<b>3</b>
<b>7. Laboratory analysis of sample</b>	<b>3</b>
<b>8. Characterization of isolates</b>	<b>3</b>
<b>9. Surveillance plan</b>	<b>3</b>
a. Sentinel area for active AMR surveillance in food sector	3
b. Sampling strategies	3
c. Sample calculation	3
<b>Annex I: Sample collection form for food industry</b>	<b>5</b>
<b>Annex II: Equipment</b>	<b>6</b>
<b>References</b>	<b>7</b>
<b>List of contributors</b>	<b>8</b>

# Acronyms

AMR	Antimicrobial resistance
AMU	Antimicrobial use
AST	Antimicrobial susceptibility testing
BPA	Baird Parker Agar
BPW	Buffered Peptone Water
CBS	Central Bureau of Statistics
CLSI	Clinical and Laboratory Standards Institute
CVL	Central Veterinary Laboratory
DFTQC	Department of Food Technology and Quality Control
<i>E. coli</i>	<i>Escherichia coli</i>
ESBL	Extended-spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agricultural Organization
GPS	Global Positioning System
MDR	Multidrug-resistant
MRD	Maximum recovery diluent
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
QGIS	Quantum Geographic Information System
SPSS	Statistical Package for the Social Sciences
SAS	Statistical Analysis System
TWG	Technical Working Group
WHO	World Health Organization

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## 1. Introduction

Bacterial infections resistant to antimicrobial drugs is a growing global issue that has effects on human, animal, and plant health, as well as food and the environment. In both human and veterinary health settings, resistance to antimicrobial compounds routinely employed in human and veterinary treatment has been rising. Active monitoring of antimicrobial resistance (AMR) in zoonotic and commensal bacteria in food products from animal origin is part of AMR surveillance in food. Active surveillance of ready-to-eat meat products is often used to generate representative estimates for monitoring the trends over time of resistances entering and moving through the food chain. The information gathered from this type of surveillance will be critical in determining the epidemiology of AMR in the food chain as well as monitoring the impact of antibiotic use in animals. This study will provide integrated One Health approach to monitor antimicrobial resistance in enteric bacteria from humans, food animals and foods. This study will also provide data on movement of bacterial populations among humans, food animals and food.

A comprehensive data on the AMR scenario in different sectors, for example, human, animal, food and environment in Nepal is lacking. The National Public Health Laboratory (NPHL) has been conducting the National AMR surveillance in human health for the last two decades. The Central Veterinary Laboratory (CVL) compiles data from passive AMR surveillance from four other veterinary laboratories in the country since 2018. Several independent research studies both from human and animal health have found increased resistance to the routinely used antibiotics in clinically important bacteria such as *E. coli*, *Salmonella*, *Neisseria*, *Staphylococcus*, *Streptococcus*, *Shigella*, and *Vibrio* (1,2,3).

## 2. Objectives

### a. General objective

To conduct active surveillance for antimicrobial resistance in ready-to-eat meat products produced in Kathmandu valley from manufacturers licenced with the Department of Food Technology and Quality Control (DFTQC) number.

### b. Specific objectives

- To isolate the target bacterial pathogens (*E. coli*, *Salmonella spp*, *Staphylococcus aureus*) from processed meat products.
- To determine the prevalence of pathogen of interest in the meat products
- To determine the antibiogram for the bacterial isolates
- To determine the multidrug-resistant (MDR), methicillin-resistant *Staphylococcus aureus* (MRSA), isolates.

### 3. Target food items

The sample for this pilot survey will only include ready-to-eat meat products that need minimal processing before consumption. This study will exclude unprocessed or raw meat products. Ready-to-eat meat products are defined as products consisting of or containing meat such as sausage, salami, and petties. These products do not require additional severe processing (bactericidal treatment) before consumption. A total of 105 samples will be collected.

### 4. Target bacteria

The target bacteria for the first round of AMR surveillance are.

- *E. coli*
- *Salmonella spp*
- *Staphylococcus aureus*

Active surveillance will focus on *Escherichia coli*, common intestinal bacteria that can serve as reservoirs of resistance genes and indicators of selection pressures in Gram-negative bacteria, respectively (4), *Salmonella spp.*, a major zoonotic bacterial cause of foodborne illness. Similarly, *Staphylococcus aureus* (*S. aureus*) is also one of the most important causes of food borne diseases. *S. aureus* is an opportunistic pathogen in humans and animals and is the third largest cause of food related illness throughout the world. *S. aureus* can cause a wide spectrum of infections, from superficial skin infections to severe, and probably fatal, invasive illness. *S. aureus* is a notorious bacterium, which quickly develops resistance to different antimicrobials.

### 5. Target antibiotics

Clinically important antimicrobial agents or classes used in human and veterinary medicine should be included in active antimicrobial resistance surveillance programs. The panel of antibiotics selected for each of the three bacteria is given below in the Table 1

**TABLE 1: The panel of target antibiotics for each of the bacteria**

Antibiotic Class/antibiotic	<i>E. coli</i>	<i>Salmonella spp.</i>	<i>Staphylococcus aureus</i>
<b>Aminoglycosides</b>	Gentamicin/ Amikacin		Gentamicin
<b>Amphenicol</b>	Chloramphenicol	Chloramphenicol	Chloramphenicol
<b>Carbapenem</b>	Meropenem Imipenem	Meropenem Imipenem	
<b>Cephalosporins II</b>			Cefoxitin
<b>Cephalosporins III</b>	Cefotaxime (Or Ceftriaxone) Ceftazidime	Cefotaxime (Or Ceftriaxone) Ceftazidime	
<b>For ESBL detection</b>	Cefotaxime-Clavulanate		
<b>Quinolones</b>	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
<b>Macrolides</b>		Azithromycin	Azithromycin/ Erythromycin
<b>Penicillin's</b>	Ampicillin	Ampicillin	Penicillin
B-lactam	Amoxicillin-Clavulanate, Ampicillin-sulbactam, Piperacillin-Tazobactam		
<b>Tetracyclines</b>	Tetracycline	Tetracycline	Tetracycline
<b>Sulphonamides/ Trimethoprim</b>	Co-trimoxazole	Co-trimoxazole	Co-trimoxazole

Note: Most of the antibiotics panels listed above are currently in use in AMR surveillance in the food sector at the Food Microbiology Laboratory, NFFRL, DFTQC.

## 6. Sample information

It is critical to keep a record of the basic information for each sample. This will allow for a more thorough examination of laboratory data, as well as the clarification of potential biases for various sample types and the identification of critical control points (CCPs) for preventing the emergence and spread of antibiotic resistance in food chain. The following basic information should be included with each specimen: date, type of food (processed, or ready-to-eat), animal species, processing plant identification (location), freshness or frozenness, and whether the food was pre-packaged or subjected to in-store processing. The majority of information can be obtained by submitting a copy of the package label. The detail of sample collection form is given in Annex 1.

## 7. Laboratory analysis: isolation, identification, and antimicrobial susceptibility testing (AST) of samples

Detailed instructions for culture and identification of the target bacteria are provided in Annex 1. AST will be conducted for each bacterial isolate against the panel of antibiotics listed using modified Kirby Bauer's disk diffusion method. After the sensitivity tests, the diameter of the inhibition zone shall be measured, recorded and result will be interpreted as Susceptible (S), Intermediate (I) or Resistant (R) following CLSI guidelines.

In addition to testing resistance patterns in pure isolates of the target bacterial species/groups, each sample will be tested for the presence of extended-spectrum beta-lactamase positive Enterobacteriaceae by plating directly onto selective media.

## 8. Characterization of isolates

Characterization of foodborne bacterial isolates (genus, species, and additional microbial subtyping) is important. Serotype information is fundamental to understanding the epidemiology of Salmonella and its multidrug resistant isolates. The most common serotypes in a given area should be known in order to ensure an adequate supply of antisera.

## 9. Surveillance plan

### a. Sentinel area for AMR surveillance in food sector

Three areas have been selected for AMR surveillance in food sector having high population density and many food manufacturing industries. Those areas are Kathmandu, Lalitpur and Bhaktapur, and selected meat products (Sausage, Petties and Salami). DFTQC will be responsible for leading this active surveillance in those selected areas

### b. Sampling strategies

Sampling should be conducted on a purposive random sampling basis. The sampling strategy should ensure:

- I. The sample is representative of the population of interest and meets the objectives of the surveillance
- II. The robustness of the sampling method

The following criteria are to be considered:

- I. Samples from manufacturing companies with a DFTQC number or are in the process of receiving one
- II. Category of sample such as processed and ready-to-eat meat products
- III. Status of sample such as, packaged and airtight hermetically sealed, vacuum packaged

### c. Sample calculation

A study in China shows that, prevalence of antibiotics resistance for staphylococcus aureus was 7% in food products. The number of samples will be calculated by taking staphylococcus aureus as prevalence

factor for this survey. For the final calculation, confidence interval will be taken as 95% assuming marginal errors as 5% making the sample of  $\pm 105$ . So, 105 number of meat item will be taken from different manufacturing companies inside Kathmandu valley.

The selected meat items for the survey are sausage, salami and patties.

#### d. Sampling techniques and distribution

Altogether there are 17 manufacturing companies inside the Kathmandu valley producing 3 meat items. So, the sample will be collected as per weightage sample based on available companies in each meat items.

Food items	Available manufacturing companies (in number)	Weightage sample
Sausage	17	76%
Salami	10	18%
Patties	3	6%

Referencing to the table above, 76% of sausage, 18% of salami and 6% of patties will be collected for companies. In this regard, 4 sample will be collected from each company for each meat items which means 80 sausage samples, 21 salami samples and four patties samples need to collect for this survey. Nonfunctional manufacturing industries will be excluded from this survey. To meet the target sample size, samples will be collected from other companies based on their numbers. This will be applicable in all three meat items.

**Number of samples:** On each sampling period, 4 to 6 samples will be collected.

#### e. Review sampling plan

The experience with sample collection and results of antibiotic sensitivity testing will be reviewed after the first one to two months of sampling to identify if any changes need to be made to the sampling plan.

#### f. Sample transport

Store all samples in secure containers in polystyrene boxes with ice pads while all samples are being collected. Keep chilled ( $<10^{\circ}\text{C}$ ), but not freezing, to prevent overgrowth of samples.

#### g. Note: Never freeze samples as this may kill the bacteria.

Samples should ideally be transported to the laboratory on the same day of collection. If they cannot be transported on the same day, they must be stored in a refrigerator at no more than  $4^{\circ}\text{C}$  and transported to the laboratory the next day.

#### h. Data collection form

Data collection form for each sample will capture descriptive information that will help correctly interpret the AMR results. Forms will include the information shown below.

It is important to ensure that a unique sample identification numbering system is put in place so that every sample, regardless of the area from which it comes, has a unique sample ID.

*Note: Ensure that the ID number written on the form matches the ID number on the sample tube.*

#### i. Data management and analysis plan

Data recorded in the paper-based ledger will be entered and stored in the Microsoft Excel sheet. The data will be analysed at frequent interval to present the trends during the bi-monthly meeting of the TWG-Food and other higher-level AMR committees. A dashboard will be used for quick data visualization. For longer term data analysis using epidemiological information collected during sampling, appropriate statistical software such as Epi Info, SPSS, SAS or R shall be used.

# Annex

## Annex I: Sample collection form for food industry

**Sample collection form for food industry**

1. Sample collection: Date: ..... Time: .....

2. Sample identification number: (Any format?) .....

3. Information on industry:

Name of industry: .....

Address of industry: .....

Tole: ..... Ward no: .....

Local level: .....

District: ..... Province: .....

Renewal status of DFTQC no: .....

Responsible person of industry: ..... contact no. ....

4. Information about food sample:

a. Type of food (circle one):            Raw            Processed            Ready-to-eat

b. Name of food: .....

c. Animal species (circle one):            Chicken            Goat            Pigs            Buffalo            Others

d. Condition of food (circle one):            Fresh            Frozen

e. Storage temperature: .....

f. Other ingredients:            Additives            Preservative            Colour

g. Packaging status (circle one):            Packaged            Non-Packaged            Vacuum packaged

h. Label information: Date of mfg.            Exp. date            Batch no.

i. Industry classification (circle one):            Cottage            Small            Medium            Large

## Annex II: Equipment

### Essential equipment

1. Autoclave, with temperature recording device(s), capable of holding 121°C for 15 minutes within a waste load.
2. Incubator, 35°C, aerobic (capable of holding 35 +/- 1°C)
3. Incubator, 36 +/- 1°C, aerobic (capable of holding 37 +/- 1°C)
4. Incubator, 42°C, aerobic (capable of holding 42 +/- 1°C),
5. Refrigerator or cold room capable of holding 2-8°C
6. Freezer, -40°C.
7. McFarland 0.5
8. Disk dispensers with desiccant (NB they are disk-brand dependent) or desiccator for storing opened antimicrobial disk cartridges.
9. Forceps (metal) if disk dispensers are not used.
10. Coplin jars or similar for ethanol sterilizing scissors/clippers/forceps
11. Scissors/clippers, metal (for cutting swabs)
12. Bunsen burner(s) or Bacti-Cinerator (or similar) if gas is not available.
13. Callipers or ruler (for measuring zone sizes)
14. Loops, nichrome or plastic disposables
15. Micropipette capable of measuring 100 µl
16. Microscope light, with oil-immersion objective
17. Quality control organisms as specified by the relevant CLSI standards

### Equipment for sample collection

1. Cool box
2. Ice pads
3. Gloves

### Other equipment

1. Oven or microwave oven for drying desiccant (alternatively the 42°C incubator can be used)
2. Water bath capable of holding 42°C +/- 0.2°C

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