

PROTOCOL FOR ACTIVE AMR SURVEILLANCE IN YOGURT

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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Foreword



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Foreword

The increasing prevalence of antimicrobial resistance (AMR) poses a critical threat to public health, food safety, and sustainable food systems. Effective surveillance mechanisms are essential to monitor AMR trends, inform risk mitigation strategies, and ensure the safety of food products. Recognizing this urgency, the Department of Food Technology and Quality Control (DFTQC) has been at the forefront of AMR surveillance in animal-based food products since December 2021.

In 2022, DFTQC successfully conducted a pilot study on active AMR surveillance in ready-to-eat meat products produced in the Kathmandu valley. This study provided valuable insights into the prevalence and patterns of AMR in foodborne pathogens, laying a strong foundation for future surveillance efforts. Building on these findings, DFTQC is now extending its surveillance initiatives to yoghurt, a widely consumed dairy product in Nepal.

To support the implementation of laboratory-based active AMR surveillance in yoghurt, the National Food and Feed Reference Laboratory (NFFRL) has developed a comprehensive protocol titled *Protocol for Active AMR Surveillance in Yoghurt*. This document defines the objectives, planning and preparation procedures, priority pathogens, laboratory analysis protocols, surveillance framework, sampling methodology, data management, and reporting requirements. It serves as a vital tool for ensuring systematic and effective AMR monitoring in yogurt.

I extend my sincere appreciation to the AMR Technical Working Group for the Food Sector (AMR TWG-Food), the experts involved in drafting this protocol, the microbiology laboratory team, and all contributors to this important initiative. I am also grateful to the Fleming Fund Country Grant for Nepal/FHI 360 for their support and technical assistance, as well as to the UK Department of Health and Social Care for their invaluable contributions to the development of this protocol.

I am confident that this protocol will play a crucial role in strengthening AMR surveillance in yoghurt, ultimately enhancing food safety and public health protection.

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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 Agriculture Organization
MDR	Multidrug-resistant
MRD	Maximum recovery diluent
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
TWG	Technical Working Group
WHO	World Health Organization

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1. Introduction

Antimicrobial resistance (AMR) presents a growing worldwide issue impacting human, animal, and plant well-being, as well as food safety and environmental health. The escalation of resistance to antimicrobial agents frequently employed in medical and veterinary practices is evident across both human and animal healthcare on a global scale. Active monitoring of AMR in zoonotic and commensal bacteria in processed food products from animal origin is part of AMR surveillance in food. Active surveillance of ready to eat dairy products is often used to generate representative estimates for monitoring the trends over time of resistances entering and moving through the food chain. Ready-to-eat dairy products like yogurt are a potential source of antimicrobial-resistant bacteria, posing a risk to consumers. The emergence and spread of AMR pathogens in yogurt have raised concerns regarding food safety. To track the presence of AMR in yogurt and control sources of contamination, active surveillance of AMR in yogurt is crucial.

The protocol for active AMR surveillance in yogurt in the Kathmandu valley serves as a comprehensive framework to assess the prevalence and patterns of AMR in yogurt. The surveillance will also include collecting swabs from yogurt packaging materials to trace the source of microbes. This will help understand how contamination occurs and identify points where interventions can be made.

The information gathered from this type of surveillance will be used to develop, revise, and implement strategies to reduce contamination and improve the safety of these popular dairy products. It will also help in creating guidelines and policies for better hygiene and production practices in the dairy industry. Additionally, it will provide a basis for educating producers and consumers about the risks of food-borne AMR and the importance of preventive measures. This comprehensive approach will ultimately contribute to the overall goal of reducing the impact of AMR on public health.

Effective surveillance strategies are crucial for identifying emerging trends, assessing risks, and implementing targeted interventions to prevent the dissemination of antimicrobial resistance through dairy products. This protocol aims to establish standardized procedures for systematic monitoring of AMR in ready-to-eat dairy products, encompassing sampling, laboratory testing, data analysis, and reporting mechanisms.

The active surveillance for foodborne AMR risks in yogurt guided by this protocol is designed to contribute to strengthening government food sector's ability to implement AMR surveillance. The implementation of this protocol and the AMR information generated will contribute to a One Health approach to AMR surveillance.

This protocol outlines the methodology for conducting active AMR surveillance of yogurt coupled with the collection of swabs from yogurt packaging materials and Knowledge, Attitude, and Practice (KAP) studies among dairy industry workers. By comprehensively assessing antimicrobial resistance levels in dairy products and gaining insights into the awareness and adherence to food safety standards among industry personnel, this protocol aims to inform targeted interventions and policy recommendations to enhance the safety and quality of dairy products.

2. Objectives

2.1 General objective

The general objective of active AMR surveillance in yogurt is to comprehensively detect antimicrobial-resistant bacteria within yogurt, and establish and/ or strengthen active AMR surveillance system within the manufactured food sector.

2.2 Specific objectives

- To isolate the target bacterial pathogens (*E. coli*, *Salmonella spp*, *Staphylococcus aureus* and *Enterococcus spp.*) from yogurt.
- Identify possible sources of contamination, specifically *S. aureus*, through swabs from packaging materials.
- To perform the antimicrobial susceptibility test on isolated pathogens and determine the antibiogram for the bacterial isolates.
- To generate evidence-based recommendations for enhancing food safety measures, promoting adherence to Good Manufacturing Practices (GMP), and preventing the spread of AMR in the dairy manufacturing process.

3. Justification for the need of active AMR surveillance in yogurt

- Yogurt is a commonly consumed dairy product that can potentially harbor antibiotic-resistant bacteria such as *E. coli*, *Salmonella species*, *Staphylococcus aureus*, and *Enterococcus species*.
- While passive AMR surveillance provides valuable data, active surveillance allows for targeted sampling and analysis to identify emerging AMR threats in yogurt and its packaging.
- Active AMR surveillance in yogurt is essential due to limited data on resistance, helping to identify emerging patterns, track resistant strains, and guide public health interventions through regular testing for pathogens like *E. coli*, *Salmonella*, *S. aureus*, and *Enterococcus*, along with their antimicrobial susceptibility profiles.
- *Staphylococcus aureus* (producing enterotoxin), can contaminate yogurt during production or handling and packaging, as it is commonly found on food contact surfaces. Collecting swabs from packaging materials, helps identify potential contamination sources. This data can be used to implement improved hygiene practices, sanitation protocols, worker training, and packaging guidelines to minimize enterotoxigenic and methicillin-resistant *S. aureus* transmission risks in finished yogurt products.
- Routine monitoring of these potential sources of contamination, alongside the testing of yogurt samples, provides a comprehensive approach to ensuring the microbiological safety and quality of yogurt products.
- Use the findings from AMR surveillance in yogurt to inform the design and priorities for future surveillance in other animal based food products. This also supports a One Health approach by integrating AMR data from the food sector with data from human, animal health, and environmental sectors, enhancing the overall understanding of consumer risks associated with AMR.

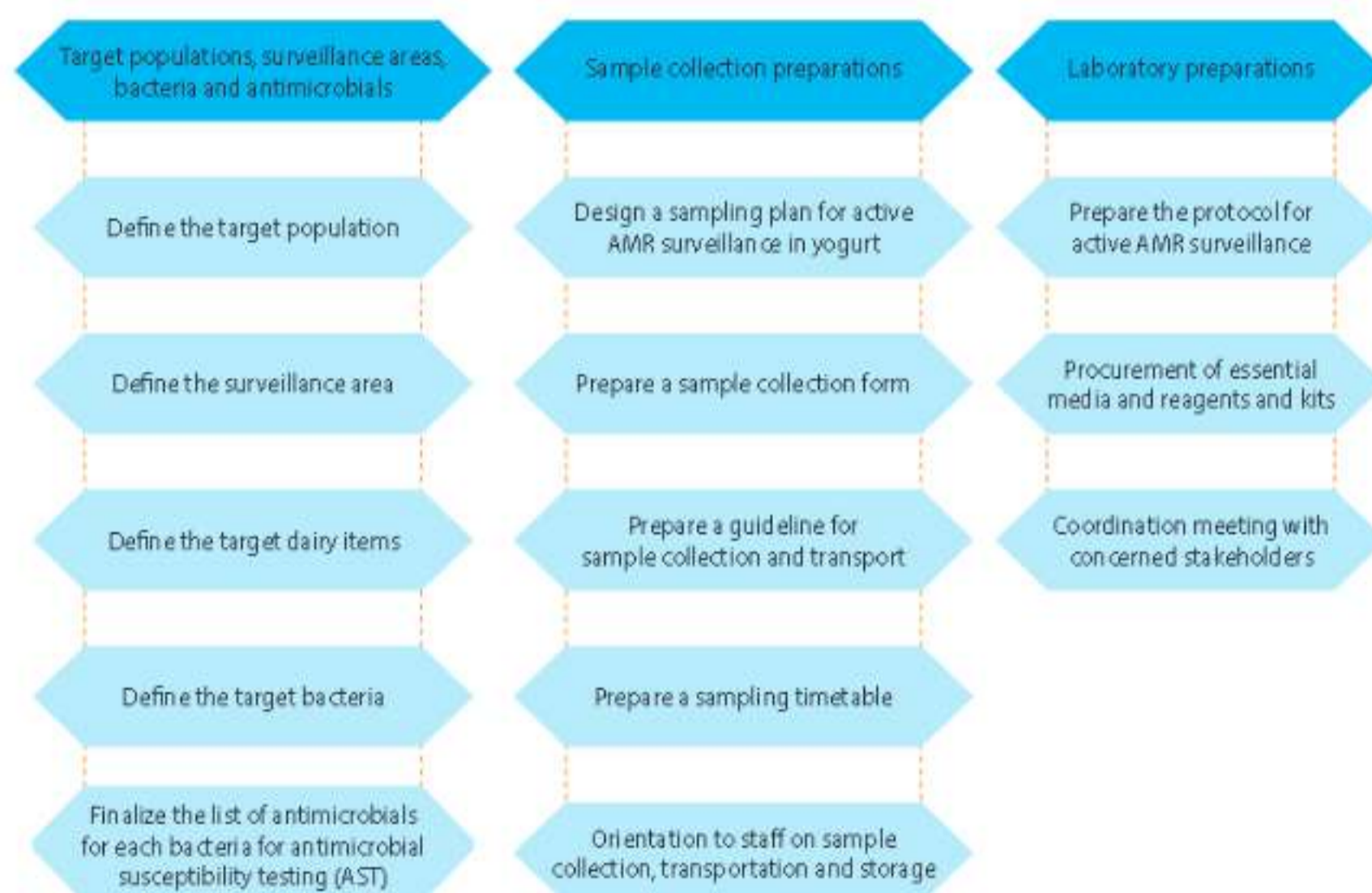
4. Collection of swabs from packaging material

AMR is a growing concern in food safety, particularly in ready-to-eat products like yogurt. In a recent study conducted by Asfaw *et al.* (2023) in Ethiopia, 29% of yogurt samples were found to be contaminated with AMR bacteria that were highly resistant to ampicillin and amoxicillin (> 93%). In addition, packaging materials, often overlooked, can harbor AMR bacteria, leading to contamination. Recent studies have shown that 15% of yogurt packaging tested positive for AMR bacteria, especially those resistant to tetracycline and ampicillin. This underscores the need for active surveillance, including swabbing packaging containers, to identify contamination sources and implement preventive measures. Such practices are essential for ensuring yogurt safety and supporting global AMR reduction efforts.

Research published in the *Journal of Food Protection* (2023) and a 2022 EFSA study highlight the importance of monitoring packaging materials in AMR surveillance. These studies reveal that packaging can harbor resistant microbes from various sources, posing a direct risk to consumers if not properly managed. Swabbing these materials helps detect and understand contamination points in the production chain, contributing to effective AMR control strategies.

5. Planning and preparations before beginning AMR surveillance

Figure. Planning and preparation steps to prepare both the laboratories and the sample collection teams for the AMR surveillance. This diagram acts as a road map showing the relevant sections of the protocol to support each step.



6. Target food item for AMR surveillance

The target food item for this active AMR surveillance is yogurt produced in the Kathmandu valley. Yogurt is a widely consumed dairy product, appreciated for its nutritional benefits and taste. However, its popularity and widespread production make it a potential risk pathway for spread of antimicrobial resistant bacteria to consumers. Contamination can occur at various stages of yogurt production, including during the handling and packaging processes. By focusing on yogurt, this surveillance aims to detect and trace the presence of concern AMR bacteria (as described in section 8). Additionally, swabs from yogurt packaging materials will be collected to help identify possible sources of contamination. This targeted approach will provide valuable insights into the safety and quality of yogurt, ensuring consumer health and contributing to the broader efforts of AMR surveillance and control.

7. AMR surveillance area

Active AMR surveillance in yogurt will be concentrated in three districts of Bagmati province in the Kathmandu valley—Kathmandu, Lalitpur, and Bhaktapur districts—chosen for their high population density and substantial dairy food manufacturing presence. These areas are selected as sentinel sites due to their significant dairy production activities, which make them ideal for monitoring AMR in a widely consumed food product. Kathmandu Valley produces approximately 500,000 liters of yogurt daily, with Kathmandu alone accounting for about 300,000 liters. The high consumption rate in these areas underscores the importance of monitoring for AMR in this widely consumed product. The Department of Food Technology and Quality Control (DFTQC) will lead the active surveillance in Kathmandu, Lalitpur, and Bhaktapur. They will collect yogurt samples and swab sample from yogurt packaging containers from different yogurt manufacturing industries that have been assigned with DFTQC number to check for AMR. This will provide a clear picture of AMR in yogurt, helping to develop or revise the strategies to reduce AMR risks and improve consumer safety.

8. Target bacteria

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

1. *E. coli*
2. *Salmonella* spp.
3. *Staphylococcus aureus*
4. *Enterococcus* spp. (Mainly *E. faecalis* and *E. faecium*)

Escherichia coli (*E. coli*) and *Salmonella* spp. are recognized as priority organisms in the World Health Organization's Global Antimicrobial Resistance Surveillance System (GLASS) as of the 2020 report (WHO_GLASS_2020). These bacteria are not only significant due to their resistance patterns but also serve as key indicators of fecal contamination in various environments, including water and food sources. Their presence often signals potential health risks and the need for stringent hygiene practices. *Staphylococcus aureus* (*S. aureus*), on the other hand, is a major pathogen responsible for both nosocomial (hospital-acquired) and community-acquired infections globally. According to Rao et al. (2022), *S. aureus* is particularly concerning in food handling environments where it can contaminate food products and surfaces, leading to outbreaks of foodborne illnesses. Its ability to develop resistance to multiple antibiotics further complicates treatment and control measures. Furthermore, regional guidelines for AMR surveillance from the Food and Agriculture Organization (FAO) recommend the inclusion of *E. coli*, *Salmonella* spp., *Enterococcus faecium*, *E. faecalis*, in AMR surveillance programs to monitor AMR in the context of foodborne pathogens. As highlighted by Rivera-Gomis et al. (2021), *Enterococcus* can harbor and transfer resistance genes, making them important in the study of AMR and its spread within both clinical and environmental settings.

9. Target antibiotics

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

Table 1: The panel of target antibiotics for each of the bacteria

Antibiotic Class	<i>E. coli</i>	<i>Salmonella spp.</i>	<i>Enterococcus spp.</i>	<i>Staphylococcus aureus</i>
Aminoglycosides	Amikacin, Gentamicin,			Amikacin, Gentamicin
Amphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol	Chloramphenicol
Beta-lactamase Inhibitor	Amoxicillin-Clavulanate, Ampicillin-sulbactam, Piperacillin-Tazobactam		Amoxicillin/ clavulanic acid	
Carbapenems	Imipenem	Imipenem		
Cephalosporins II				Cefoxitin
Cephalosporins III	Cefotaxime, (Or Ceftriaxone),	Cefotaxime, (Or Ceftriaxone),	Ceftriaxone	Ceftriaxone
For ESBL detection	Cefotaxime-Clavulanate			
Glycopeptides			Vancomycin	
Macrolides		Azithromycin		Azithromycin
Penicillins	Ampicillin, Benzylpenicillin	Ampicillin, Benzylpenicillin	Ampicillin, Benzylpenicillin	Ampicillin, Benzylpenicillin
Phenicol		Chloramphenicol	Chloramphenicol	Chloramphenicol
Quinolones	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin, Levofloxacin	Ciprofloxacin
Sulfonamides	Trimethoprim Sulfamethoxazole	Trimethoprim Sulfamethoxazole		Trimethoprim Sulfamethoxazole
Tetracyclines	Tetracycline,	Tetracycline,	Tetracycline,	Tetracycline,
Others	Nitrofurantoin		Nitrofurantoin	

Note: The majority of the antibiotics panels listed above are currently in use in AMR surveillance in the food sector at microbiology laboratory, NFFRL, DFTQC. EUCAST zone sizes of the aforementioned antibiotics are given in Appendix III to Appendix VI

10. Sample information

It is critical to keep record of the basic details 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 AMR in food chain. The following basic information should be included with each specimen: date, processing plant identification (location), 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 2.

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

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 or EUCAST 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 (ESBL) positive Enterobacteriaceae by plating directly onto selective media (MacConkey Agar, ChromID ESBL Agar, Cefotaxime or Ceftazidime-containing media).

12. 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.

13. Surveillance plan

13.1. Sentinel area for AMR surveillance in yogurt sector

Three areas have been selected for AMR surveillance in dairy products having high population density and many food manufacturing industries. Those areas are Kathmandu, Lalitpur and Bhaktapur district. DFTQC will be responsible for leading this active surveillance in those selected areas.

13.2. Sampling strategies

The following criteria are to be considered:

- Ensure that samples are collected from various producers and locations within the Kathmandu valley to capture a representative picture of yogurt production.
- Samples will be collected from manufacturing industries that have been assigned a DFTQC number or are in the process of receiving one.
- Status of sample such as, packaged and airtight hermetically sealed, vacuum packaged.
- Collect swabs from yogurt packaging materials to trace potential sources of contamination and evaluate hygiene practices during packaging.

13.3. Excluding criteria

- Yogurt products not produced within the Kathmandu valley.
- Yogurt products imported from outside the Kathmandu valley region.
- Yogurt products lacking clear identification or labelling regarding their batch number, yogurt manufacturers that are not registered in DFTQC.

13.4. Sampling design

- Sampling frame: All yogurt manufacturing industries from the Kathmandu valley will be included if the total number of factories is below 30. If the total number of industries are above 20 in each district, then systematic random sampling will be conducted.

- Sample population: Batches of yogurt are the sampling population.
- Sampling unit: Yogurt packet representing each batch will be the sampling unit.
- Sampling strategy:

Out of all registered dairy industries in Kathmandu valley, the industries that produce yogurt will be selected. The ideal number of batches of yogurt available at each factory were around two batches, as per the preliminary enquiries with the factories. For ensuring consistency, equal number of batches from all 25 yogurt factories will be collected i.e., total batches from each factory will be total sample divided by total number of factories.

Table: Number of dairy industries in Kathmandu valley that produces Yogurt

District	Yogurt
Kathmandu	7
Bhaktapur	12
Lalitpur	7
Total	26

13.5 The sample size calculation of Yogurt from the factories to observe the AMR prevalence in yogurt

To calculate the sample size for AMR surveillance in yogurt from manufacturing industries in the Kathmandu valley, 95% confidence interval (CI), 5% margin of error (precision), and an estimated prevalence of 50% will be used. As the prevalence of AMR in yogurt in Nepal is unknown and may differ from industries to industries, thus 50% estimated prevalence was used. Following formula to calculate the sample size is used,

The formula to calculate the sample size n for estimating a proportion is:

$$n = Z^2 \cdot p \cdot (1-p) / E^2$$

Where:

- ▶ n = required sample size
- ▶ Z = Z-value (standard score corresponding to the desired confidence level)
- ▶ p = estimated prevalence
- ▶ E = margin of error (precision)

We know that,

- ▶ **Confidence interval:** For AMR surveillance in yogurt from factories, a 95% confidence level is chosen, corresponding to a Z-value of 1.96.
- ▶ **Margin of error:** The precision required for the surveillance is set at 5%, or 0.05.
- ▶ **Estimate the prevalence:** The expected prevalence of AMR in the yogurt samples is 50%, or 0.50.

Now, substituting the values into the formula:

$$n = 384.16 \sim 385 \text{ yogurt box}$$

Notes:

- ▶ The calculated sample size n is 384.16. Since the sample size cannot be a fraction, it is usually rounded up to the next whole number. Therefore, the required sample size is 385.

13.6. Sampling frequency:

As discussed in sampling strategy, as total industries that produces yogurt are 25 and total number for sample size is 385, yogurt of 15 batches from each industry will be collected. Each industry has two batches in their stores. The total sample duration is six months from December 2024 to May 2025.

13.7 Collection of swabs from yogurt packages

Pooled swabs will be collected from empty yogurt packages intended for packaging, which serves as a critical step in tracking contamination sources in the production chain. The handling and packaging stages are particularly vulnerable to contamination, as studies have shown that improper hygiene practices can facilitate the transfer of pathogens from workers to food products (Kikuchi et al., 2022). The risk of contamination is compounded by the fact that Methicillin-resistant *Staphylococcus aureus* (MRSA) can survive in various environments, making it a resilient pathogen in dairy settings (Taban et al., 2021). Furthermore, the emergence of MRSA in dairy cattle and its potential spill-over into human populations underscores the importance of comprehensive AMR surveillance in yogurt production (Hansen et al., 2019). By implementing a systematic approach to sampling and monitoring, the protocol aims to enhance food safety and mitigate the risks associated with resistant strains in yogurt.

14. Sampling techniques and sample distribution and data

14.1. Sample size

In the context of active AMR surveillance, the selection of yogurt packages weighing 250 grams is a strategic choice that facilitates effective sampling and analysis. The use of a random sampling technique to collect yogurt packets of this specific weight is essential for ensuring that the samples are representative of the yogurt population. This approach allows for a systematic and unbiased collection of data, which is crucial for accurately assessing the prevalence of antimicrobial-resistant bacteria in yogurt products (Walia et al., 2019).

14.2. Sample transport

Store all samples in secure containers in polystyrene boxes with ice pads while all samples are being collected. Keep chilled (<8°C), but not freezing, to prevent overgrowth of samples.

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°C and transported to the laboratory the next day.

14.3. 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 in Annex-II

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 package.

15. Data management and analysis plan

The data recorded entered 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.

16. Future vision

16.1. Knowledge Attitude and Practice (KAP) study

In the future, active AMR surveillance in dairy products will be strengthened by conducting a KAP study within the dairy industry. This study will help understand how dairy workers and stakeholders view their roles in the containment of antimicrobial resistance. It will identify gaps in their knowledge and practices, providing valuable insights into areas that need improvement. With this information, better training and awareness programs can be developed to promote safe practices and improve the overall quality of dairy production. This will ultimately support efforts to curb the spread of resistant bacteria through dairy products.

Annex

Annex I: Sample collection form for yogurt industry

Sample collection form for dairy 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:
Food licence (circle one): Yes No Other
Responsible person of industry: Contact no.
4. Information about food sample:
a. Product name: b. Batch number:
c. Production date: d. Expiry date:
e. Storage temperature: :
f. Packaging type (circle one): Plastic Glass Pouch Others (Specify)
5. Sample weight (g):
6. Sample location (Put tick): Production line Cold storage Finished product storage Others (Specify)
7. Industry classification (circle one): Cottage Small Medium Large
8. Number of batches produced daily:
9. Total volume of yogurt produced per day (kg):
10. Hygiene practices in place (tick):
Handwashing Sanitizing Equipment
Regular Cleaning Use of Gloves/Masks Others (Specify)
11. Any past issues with contamination/recall (tick one): Yes No
12. Condition during sample transport: Refrigerated Room temperature

Annex II: Equipment

Essential equipment in

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 capable of holding 2-8°C
6. Freezer, -80/-40°C.
7. McFarland Densitometer 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. Scissors/dippers, metal (for cutting swabs)
11. Callipers or ruler (for measuring zone sizes)
12. Loops, nichrome or plastic disposables
13. Micropipette capable of measuring 100 µl
14. Microscope light, with oil-immersion objective
15. 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

Annex III: List of antibiotics against *E. coli* with EUCAST zone diameter breakpoints

Antibiotic	Disk content (μg)	Zone diameter breakpoints (mm)	
		Susceptible (\geq mm)	Resistant (< mm)
Amikacin	30	18	15
Amoxicillin-Clavulanate	20/10	19	15
Ampicillin	10	14	11
Ampicillin-Sulbactam	10/10	15	12
Chloramphenicol	30	17	13
Cefepime	30	26	23
Cefotaxime	5	26	23
Ceftriaxone	30	26	23
Ceftazidime	10	21	18
Ciprofloxacin	5	25	22
Doxycycline	30	19	15
Ertapenem	10	25	22
Imipenem	10	23	20
Meropenem	10	27	24
Piperacillin-Tazobactam	30/6	21	18
Trimethoprim-Sulfamethoxazole	1.25/23.75	16	13
Gentamicin	10	18	16
Tetracycline	30	19	15

Annex IV: List of antibiotics against *Salmonella* spp. with EUCAST zone diameter breakpoints

Antibiotic	Disk content (µg)	Zone diameter breakpoints (mm)	
		Susceptible (≥ mm)	Resistant (< mm)
Ampicillin	10	17	14
Azithromycin	15	13	13
Chloramphenicol	30	17	13
Cefotaxime	5	26	23
Ceftazidime	10	21	18
Ceftriaxone	30	26	23
Ciprofloxacin	5	31	28
Doxycycline	30	19	15
Imipenem	10	23	20
Tetracycline	30	19	15
Trimethoprim-Sulfamethoxazole	1.25/23.75	16	13

Annex V: List of antibiotics against *Enterococcus spp.* with EUCAST zone diameter breakpoints

Antibiotic	Disk content (μg)	Zone diameter breakpoints (mm)	
		Susceptible (\geq mm)	Resistant (< mm)
Amikacin	30	17	17
Amoxicillin/clavulanic acid	20/10	18	18
Ampicillin/Benzylpenicillin	10	18	18
Chloramphenicol	30	18	18
Ciprofloxacin	5	19	19
Clindamycin	2	19	19
Doxycycline	30	19	19
Gentamicin	10	17	17
Tetracycline	30	19	19
Trimethoprim/ Sulfamethoxazole	1.25/23.75	19	19
Vancomycin	30	12	12

Annex VI: List of antibiotics against *Staphylococcus aureus* with EUCAST zone diameter breakpoints

Antibiotic	Disk content (μg)	Zone diameter breakpoints (mm)	
		Susceptible (\geq mm)	Resistant ($<$ mm)
Amikacin	30	18	13
Ampicillin	10	19	15
Benzylpenicillin	1	29	22
Cefepime	30	22	15
Cefoxitin	30	22	17
Ceftriaxone	30	23	18
Chloramphenicol	30	18	13
Ciprofloxacin	5	21	15
Clindamycin	2	23	14
Doxycycline	30	19	13
Gentamicin	10	16	12
Sulfamethoxazole	23.75	19	15
Tetracycline	30	19	13
Trimethoprim	1.25	19	15

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