Integrated Antimicrobial Resistance Surveillance Framework for Kerala

focussing on food-animals, food-animal products and environment

Based on deliberations at the
Workshop on Integrated Surveillance Framework for Antimicrobial Resistance for Kerala
Kochi, Kerala

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Contents

List of abbreviations

AMR	Antimicrobial Resistance
AR	Antibiotic Residue
ARG	Antibiotic Resistant Gene
AST	Antimicrobial Susceptibility Testing
CIFT	Central Institute of Fisheries Technology
CLSI	Clinical and Laboratory Standards Institute
CSE	Centre for Science and Environment
ELISA	Enzyme-Linked Immunosorbent Assay
ESBL	Extended Spectrum Beta Lactamase
HPLC	High Performance Liquid Chromatography
KARSAP	Kerala Antimicrobial Resistance Strategic Action Plan
KSPCB	Kerala State Pollution Control Board
KUFOS	Kerala University of Fisheries and Ocean Studies
LCMS	Liquid Chromatography–Mass Spectrometry
MIC	Minimum Inhibitory Concentration
NAP	National Action Plan
STP	Sewage Treatment Plant

1. Introduction

Antimicrobial resistance (AMR), particularly antibiotic resistance, is recognized as a global public health threat today. It arises when microorganisms are able to survive exposure to a drug that could normally inhibit their growth or kill them. When AMR arises and spreads in infection-causing bacteria, treating common infections and managing surgical procedures becomes difficult, resulting in prolonged hospital stays, expensive treatments and high economic burden. AMR is estimated to claim more than 10 million lives per year by 2050[1] and result in lost outputs worth US $100 trillion, if not contained timely. It can also potentially impact food safety, nutrition security, livelihood and attainment of Sustainable Development Goals.

AMR has been recognized as a 'One Health' issue owing to its significant linkages with the health of humans, animals and environment. Antibiotic misuse and overuse in animals – particularly food-animals and in crops is an important driver of AMR. The environment also has a key role in the emergence and spread of AMR. Waste from drug manufacturing units, healthcare settings (human and veterinary), food production systems (such as farms, slaughter-houses, processing units), and community are considered as key contributors of AMR causing determinants such as resistant bacteria, antibiotic residues and resistance conferring genes in the environment. The environment is thus a large pool of these determinants. However, in terms of AMR containment, the human health side of AMR problem has received the most attention, followed by the animal side. The environmental aspects of AMR have received limited attention in comparison.

Responding to the global call on AMR containment, India released its multi-sectoral National Action Plan (NAP) on AMR[2] along with the Delhi Declaration on AMR[3]. The Indian NAP-AMR called for states in India to develop their own State Action Plans (SAP) on AMR to facilitate action on the ground, up to district and sub-district levels. Kerala became the first Indian state to develop its own SAP-AMR. The Kerala Antimicrobial Resistance Strategic Action Plan (KARSAP)[4] was formally released by the Hon'ble Chief Minister of Kerala, Shri Pinarayi Vijayan in October 2018.

Of the six key strategic priorities of the KARSAP, the second strategic priority outlines the need for surveillance of AMR in order to generate more knowledge and evidence. The KARSAP acknowledges that in addition to human health, surveillance in animals, fisheries and agriculture is equally critical. The KARSAP emphasizes on surveillance of AMR and antibiotic residues in food-animals, food-animal products, and environment.

The Centre for Science and Environment (CSE) has been engaging with the Department of Health, Kerala to contribute towards containment of AMR in the state. CSE is also an implementation partner to the KARSAP. As part of this engagement, CSE organized a workshop in Kerala to develop an integrated AMR surveillance plan for the state. The workshop was organized in Kochi in collaboration with the State Department of Health and Family Welfare and the Kerala State Pollution Control Board (KSPCB). An integrated surveillance encompassing all sectors would not only be useful for knowing present AMR trends in the state, but also help generate necessary evidence to guide future AMR containment policy and practice.

The workshop included representation from the various stakeholders from Kerala such as the state departments of Health, Animal Husbandry, Fisheries, Drug Control, along with the KSPCB, Kerala University of Fisheries and Ocean Studies, Kerala University, All Kerala Chemist and Druggist Association, Central Institute of Fisheries Technology, Centrient Pharmaceuticals, Meat Products of India, Government Medical College (Ernakulum and Trivandrum) and Aster Medicity. The framework for integrated surveillance of AMR in food-animals, food-animal products and environment has been developed based on the deliberations held in this workshop.

2. Surveillance framework for antimicrobial resistance in food-animals, food-animal products and environment for Kerala

With an aim to build a comprehensive surveillance framework for Kerala in the workshop, the expert group (*see List of expert contributors*) collectively finalized three sectors where surveillance would be carried out – livestock (poultry and cattle), fisheries and environment. The framework looks at surveillance of resistant bacteria, antimicrobial residues (AR) and antimicrobial resistance genes (ARGs). The framework is divided into two sections:

(a) Sampling framework: Across each sector, districts, sampling locations, sample sizes, sample types, frequency of sampling etc. has been identified.

(b) Key bacteria, antibiotics, genes, standard methods, data analysis and reporting: This section focuses on which bacteria, antibiotics or genes would be monitored, which standardized laboratory methods would be adopted, how data would be analyzed and reported.

The expert group also prioritized a phased approach for surveillance. The surveillance framework is intended to provide a five-year roadmap for carrying out AMR surveillance. Over a span of these five years, surveillance would be carried out in two phases – Phase 1 (1-3 years) and Phase 2 (4-5 years). The framework may be suitably adapted by other states with similar geographic and socioeconomic features.

2.1. Sampling framework

2.1.1. Poultry

A sampling framework for AMR surveillance in poultry sector of Kerala is provided in Table 1. The focus has been broiler poultry over layers, since poultry meat is the most consumed meat product in Kerala. Eggs from layer poultry are usually procured from other states. Two key stakeholders are involved in this surveillance –the Animal Husbandry Department and Food Safety Department. In Phase 1, sampling is to be done from only three districts. In Phase 2, sampling would be expanded to cover the remaining 11 districts so that all 14 districts are included in the surveillance exercise in the long run. Key sampling sites include broiler poultry farms and retail markets, wherein antimicrobial susceptibility testing (AST) would be done in cloacal swab samples (by the Animal Husbandry Department) and retail meat samples (by the Food Safety Department) respectively. For each sample type (e.g., cloacal swab or meat), the framework proposes testing of 30 samples per district per quarter in Phase 1 in three districts. These districts should continue with similar sampling strategy and surveillance in Phase 2 as well. Sampling for the remaining 11 districts in Phase 2 would include about 40 samples per district every year. A smaller sample size and lesser frequency for 11 districts in Phase 2 is proposed taking due consideration of the collective load on laboratories in the district. However, if the district has capacity to scale up in the long run, then sample size and frequency could be expanded for better understanding of district wise trends. For e.g., each district could consider expanding sample size from 40 to 120 samples in a year and increase sampling frequency from bi-annual to quarterly. Apart from AST, residue monitoring is to be carried out in all retail products by the Food Safety Department following a similar approach. This will help identify whether unapproved antibiotic has been misused or appropriate withdrawal periods have been followed or not.

Table 1: Sampling framework for poultry sector

	Districts	Sampling locations	Sample types	No. of sampling locations per district*	No. of samples per site	Samples per district	Sampling frequency	Samples per year per district	AMR, AR	Stakeholder
Phase 1 and 2	Ernakulam Kozhikkode Trivandrum	Broiler farms	Cloacal swab in healthy birds[#]	10	3	30	Quarterly	120	AMR	Animal Husbandry Department
		Retail outlets	Meat	10	3	30	Quarterly	120	AMR, AR	Food Safety Department
Phase 2	Alappuzha Idukki Kannur Kasaragod Kollam Kottayam Malappuram Palakkad Pathanamthitta Thrissur Wayanad	Broiler farms	Cloacal swab in healthy birds[#]	10	2	20	Biannual	40	AMR	Animal Husbandry Department
		Retail outlets	Meat	10	2	20	Biannual	40	AMR, AR	Food Safety Department

[#]Liver or heart blood samples may be taken from farms with diseased animals. This is not a part of routine monitoring.

*Rotation of sites sampled should be considered. For example, in each district, different sets of 10 sites should be sampled in different quarters. The first set of sites could be revisited again in year 2 and likewise.

Note: Presently, the Animal Husbandry Department has a state disease diagnostic laboratory, four regional disease diagnostic laboratories and 14 clinical laboratories across districts[5]. In the long term, these should be strengthened and capacitated to execute the proposed framework across all districts.

2.1.2. Cattle for milk

Table 2 shows sampling framework for dairy sector (cattle for milk) in Kerala. This includes milk (raw and processed) as well as milk products such as cheese, curd, yoghurt etc. While the framework is largely similar to that of the poultry sector, an additional sampling location has been identified in this case which is cooperative societies. The Animal Husbandry Department will carry out surveillance of samples collected from the farms and cooperative societies, while those from retail markets will be carried out by the Food Safety Department. Other than processed milk, raw milk sold at market places or delivered at households for consumption could also be tested by the Food Safety Department.

Table 2: Sampling framework for cattle for milk

	Districts	Sampling locations	Sample types	No. of sampling locations per district *	No. of samples per site	Total samples per district	Sampling frequency	Samples per year per district	AMR, AR	Stakeholder
Phase 1 and Phase 2	Ernakulam Kozhikkode Trivandrum	Farms	Milk[#]	5	2	10	Quarterly	40	AMR	Animal Husbandry Department
		Cooperative society	Milk and milk products	2	5 (2 milk, and 3 milk products, if any)	10	Quarterly	40	AMR	Animal Husbandry Department
		Retail outlets	Milk and milk products	5	2 (1 milk, and 1 milk product, if any)	10	Quarterly	40	AMR in all samples, AR in milk	Food Safety Department
Phase 2	Alappuzha Idukki Kannur Kasaragod Kollam Kottayam Malappuram Palakkad Pathanamthitta Thrissur Wayanad	Farms	Milk[#]	5	2	10	Biannual	20	AMR	Animal Husbandry Department
		Cooperative society	Milk and milk products	2	5 (2 milk, and 3 milk products, if any)	10	Biannual	20	AMR	Animal Husbandry Department
		Retail outlets	Milk and milk products	5	2 (1 milk, and 1 milk products, if any)	10	Biannual	20	AMR in all samples, AR in milk	Food Safety Department

[#]Mastitis pus samples may be taken from farms with diseased animals. This is not a part of routine monitoring.

*Rotation of sites sampled should be considered. For example, in each district, different sets of 5 sites should be sampled in different quarters. The first set of sites could be revisited again in year 2 and likewise.

Note: Presently, the Animal Husbandry Department has a state disease diagnostic laboratory, four regional disease diagnostic laboratories and 14 clinical laboratories across districts[5]. In the long term, these should be strengthened and capacitated to execute the proposed framework across all districts.

2.1.3. Cattle for meat

Beef, after poultry, is the most consumed meat product in Kerala. This section presents the sampling framework for cattle (for meat) in Kerala (Table 3). The framework is designed in line with the dairy and poultry sector. In this case, slaughterhouses have been introduced as a new sampling site. The Animal Husbandry Department would conduct AST in rectal samples collected from farms and meat samples from slaughterhouses. The Food Safety Department will carry out AST and residue monitoring in meat samples from retail outlets.

Table 3: Sampling framework for cattle for meat

	Districts	Sampling locations	Sample types	No. of sampling locations per district*	No. of samples per site	Total samples per district	Sampling frequency	Samples per year per district	AMR, AR	Stakeholder
Phase 1 and Phase 2	Ernakulam Kozhikkode Trivandrum	Farms	Rectal samples	10	3	30	Quarterly	120	AMR	Animal Husbandry Department
		Slaughter houses	Meat	5	3	15	Quarterly	60	AMR	Animal Husbandry Department
		Retail outlets	Meat	5	3	15	Quarterly	60	AMR, AR	Food Safety Department
Phase 2	Alappuzha Idukki Kannur Kasaragod Kollam Kottayam Malappuram Palakkad Pathanamthitta Thrissur Wayanad	Farms	Rectal samples	10	2	20	Biannual	40	AMR	Animal Husbandry Department
		Slaughter houses	Meat	5	2	10	Biannual	20	AMR	Animal Husbandry Department
		Retail outlets	Meat	5	2	10	Biannual	20	AMR, AR	Food Safety Department

*Rotation of sites sampled should be considered. For example, in each district, different sets of 5 sites should be sampled in different quarters. The first set of sites could be revisited again in year 2 and likewise.

Note: Presently, the Animal Husbandry Department has a state disease diagnostic laboratory, four regional disease diagnostic laboratories and 14 clinical laboratories across districts[5]. In the long term, these should be strengthened and capacitated to execute the proposed framework across all districts.

2.1.4. Sampling framework for surveillance in fisheries sector

This section discusses the sampling framework for fisheries sector in Kerala (Table 4). To begin with, AMR surveillance will be done in two key fish-producing districts of Kerala in Phase 1 across farms, landing centres and wholesale markets. Surveillance will subsequently expand to cover all five major fish-producing districts of the state in Phase 2, and include retail market as an additional sampling site. The framework suggests monitoring of not just fish meat, but also of environmental samples like soil, water and feed, particularly from fish farms. Frequency of sampling from farms is based on culture period, unlike other cases where samples are to be collected quarterly. The State Fisheries Department and the Food Safety Department would be the key stakeholders. The State Fisheries Department will initially be required to be supported by the resources and infrastructure from the Central Institute of Fisheries Technology and the Kerala University of Fisheries and Ocean Studies.

Table 4: Sampling framework for fisheries

	Districts	Sampling locations	Sample types	No. of sampling locations per district**	No. of samples per site	Total samples per district	Sampling frequency	Samples per year per district	AMR, AR	Stakeholder
Phase 1	Ernakulum Kollam	Farms*	Soil, water, feed, fish meat	2% of total farms	At least 2 per type	Based on no. of farms sampled	Twice during culture period	Based on no. of farms sampled	AMR in all samples, AR in fish meat	State Fisheries Department
		Landing centres#	Fish meat	2-3	At least 2	Based on variety of marine captures	Quarterly	Based on variety of marine captures	AMR, AR	
		Wholesale markets	Fish meat	10% of total markets	At least -2	Based on no. of markets sampled	Quarterly	Based on no. of markets sampled	AMR, AR	
Phase 2	Allepy Ernakulum Kannur Kollam Palakkad	Farms*	Soil, water, feed, fish meat	2% of total farms	At least 2 per type	Based on no. of farms sampled	Twice during culture period	Based on no. of farms sampled	AMR in all samples, AR in fish meat	State Fisheries Department
		Landing centres#	Fish meat	2-3	At least 2	Based on variety of marine captures	Quarterly	Based on variety of marine captures	AMR, AR	
		Wholesale markets	Fish meat	10% of total markets	At least 2	Based on no. of markets sampled	Quarterly	Based on no. of markets sampled	AMR, AR	
		Retail markets	Fish meat	10% of total markets	At least 2	Based on no. of markets sampled	Quarterly	Based on no. of markets sampled	AMR, AR	Food Safety Department

*Farms include freshwater and brackish water farms; #Landing centres for marine capture; **Rotation of sites sampled should be considered.

2.1.5. Sampling framework for surveillance in environment sector

The broad structure of the environment sector w.r.t. AMR has been categorized into point sources and non-point sources. Point sources refer to a single pollution source that could contribute AMR determinants through waste from these sources. These include factories, healthcare settings, farms and the community. AMR determinants may then directly, or after treatment, reach the larger external environment. Non-point sources are part of this larger environment, which include rivers, lakes, ponds, oceans, groundwater and soil. These act as sinks of AMR determinants contributed by point sources. In addition, they also act as sources since AMR determinants can find their way back into humans, animals and the food chain from the larger environment.

The sampling framework for surveillance across point and non-point sources in environment sector in Kerala is shown in Table 5 and 6 respectively. Sampling will expand from three districts in Phase 1 to all districts in Phase 2. For point sources, effluent samples will mostly be monitored, except in case of municipal solid waste dump sites, where soil and water will be monitored. For non-point sources, seasonal sampling (i.e., before, during and after monsoon) of surface or ground water samples will be done and monitored for resistant bacteria only. The key stakeholder involved would be the KSPCB. The KSPCB could initially seek technical and capacity building support from the other stakeholders in the state such as the state Animal Husbandry Department, Fisheries Department, Central Institute of Fisheries Technology etc. In the meantime, laboratories of the KSPCB could be strengthened and resources trained w.r.t. microbiology, analytical chemistry and molecular biology in order to conduct AMR surveillance independently.

Table 5: Sampling framework for point sources in environment

	Districts	Sampling locations	No. of sampling locations per district	Sample types	No. of samples per site	Total samples per district	Sampling frequency	Samples per year per district	AMR, AR	Stakeholder
Phase 1	Kozhikkode Ernakulum Trivandrum	Sewage Treatment Plant (STP)	1 modern plant at Muttathara, Trivandrum	Effluent	3 (inlet, midpoint, outlet)	3	Quarterly	12	AMR, AR	KSPCB
		Residential STPs in high rise buildings	5 (initial pilot in Trivandrum)	Effluent	1	5	Quarterly	20	AMR, AR	
		Hospital	1 each of general hospital, medical college, corporate hospital, veterinary hospital, polyclinic (5 sites)	Effluent	1	5	Quarterly	20	AMR, AR	
		Slaughter houses	2	Effluent	1	2	Quarterly	8	AMR, AR	
		Biomedical waste treatment plant	Only 1 present in Palakkad	Effluent	1	1	Quarterly	4	AMR, AR	
		Farms (poultry, dairy, aquaculture)	5 (consider 5-10% of farms selected for surveillance in livestock/fisheries)	Effluent	1	5	Quarterly	20	AMR, AR	
		Municipal solid waste dump sites	2	Soil, water	2 per sample type	8	Quarterly	32	AMR, AR	
Phase 2	Alappuzha Ernakulam Idukki Kannur Kasaragod Kollam Kottayam Kozhikkode Malappuram Palakkad Pathanamthitta Thrissur Trivandrum Wayanad	STP	1 modern plant at Muttathara, Trivandrum	Effluent	3 (inlet, midpoint, outlet)	3	Quarterly	12	AMR, AR	KSPCB
		Residential STPs in high rise buildings	5 (initial pilot in Trivandrum)	Effluent	1	5	Quarterly	20	AMR, AR	
		Hospital	1 each of general hospital, medical college, corporate hospital, veterinary hospital, polyclinic (5 sites)	Effluent	1	5	Quarterly	20	AMR, AR	
		Slaughter houses	2	Effluent	1	2	Quarterly	8	AMR, AR	
		Biomedical waste treatment plant	Only 1 present in Palakkad	Effluent	1	1	Quarterly	4	AMR, AR	
		Aquaculture farms, poultry farms, dairy farms	5 (consider 5-10% of farms selected for surveillance in livestock/fisheries)	Effluent	1	5	Quarterly	20	AMR, AR	
		Municipal solid waste dump sites	2	Soil, water	2 per sample type	8	Quarterly	32	AMR, AR	

Note: Based on learning from Phase 1, number of sampling locations will be expanded for other point sources. Antibiotic residue testing could be done on 1/3rd of samples collected for AMR surveillance and involve qualitative testing before quantitative estimation.

Table 6: Sampling framework for non-point sources in environment

	Districts	Sampling locations	No. of sampling locations per district	Sample types	No. of samples persite	Total samples per district	Sampling frequency*	Samples per year per district	AMR, AR	Stakeholder
Phase 1	Ernakulum Kozhikkode Trivandrum	Rivers and Lakes[#]	At least 5	Water	3 (at different points along a river)	15	Seasonal	45	AMR	KSPCB
		Open wells	10	Ground water	1	10	Seasonal	30	AMR	
		Estuaries	5	Water	1	5	Seasonal	15	AMR	
		Coast line	5	Sea water	1	5	Seasonal	15	AMR	
Phase 2	Alappuzha Ernakulam Idukki Kannur Kasaragod Kollam Kottayam Kozhikkode Malappuram Palakkad Pathanamthitta Thrissur Trivandrum Wayanad	Rivers and Lakes[#]	At least 5	Water	3 (at different points along a river)	15	Seasonal	45	AMR	KSPCB
		Open wells	10	Ground water	1	10	Seasonal	30	AMR	
		Estuaries	5	Water	1	5	Seasonal	15	AMR	
		Coast line	5	Sea water	1	5	Seasonal	15	AMR	

[#]Examples: Pamba, Periyar rivers, Ramsar sites e.g. Vembanad lake, Ashthamudi lake (brackishwater lake), Sashthamkotta Lake (freshwater lake); *Seasonal sampling could be carried out pre-monsoon, monsoon, post-monsoon.

2.2. Key bacteria, antibiotics, genes, standard methods, data analysis and reporting

	Poultry (Broiler)	Cattle for milk and meat	Fisheries	Environment
Key bacteria (for AST)	*Escherichia coli* *Salmonella* spp. *Klebsiella* spp.	*Escherichia coli* *Staphylococcus aureus*	*Escherichia coli* *Vibrio parahaemolyticus* *Aeromonas* spp.	Phase 1: *Escherichia coli* (focus on ESBL producing *E. coli*) Phase 2: *Escherichia coli* *Enterococcus* spp. *Klebseilla* spp.
Key antibiotics (for AST)	*Escherichia coli and Klebsiella* spp. • Tetracyclines • Sulfonamides (e.g. suphaquinoxaline) • Fluoroquinolones (e.g. enrofloxacin) • Third generation cephalosporins (e.g. ceftriaxone) *Salmonella* spp. • Beta lactams (e.g. ampicillin) • Chloramphenicol • Sulfonamides (e,.g. cotrimoxazole)	*Escherichia coli* • Tetracycline • Beta lactams • Third generation cephalosporins (e.g. ceftriaxone) • Fluoroquinolones (e.g. ciprofloxacin) • Carbapenems (e.g. imipenem) • Aminoglycosides (e.g. gentamicin) *Staphylococcus aureus* • Tetracyclines • Beta lactams (e.g. oxacillin) • Macrolides	• Tetracyclines • Cephalosporins • Quinolones	• Carbapenems (e.g. imipenem) • Fluoroquinolones (e.g. ciprofloxacin) • Beta Lactams and Beta Lactamase Inhibitor
Method for bacterial isolation, identification, characterization	Biochemical identification system (VITEK)	Biochemical identification system (VITEK)	Biochemical identification system (VITEK)	Selective media and biochemical analysis
Method for AST*	Kirby Baeur Disc Diffusion, MIC and interpretation using CLSI			
Key antibiotics (for residue monitoring)	• Tetracyclines • Fluoroquinolones (e.g. enrofloxacin) • Third generation cephalosporins (e.g. Ceftriaxone) • Sulfonamides (e.g. suphaquinoxaline)	• Tetracyclines • Beta lactams (e.g. penicillin, ampicillin, amoxicillin, cloxacillin) • 3rd generation cephalosporins • Fluoroquinolones (e.g. enrofloxacin, ciprofloxacin) • Aminoglycosides (e.g. gentamicin)	• Tetracyclines • Nitrofurans	Antibiotics to be tested will be site-specific and also depend on its concentration and rate of breakdown (only for point sources)
Method for residue testing	ELISA; 10% of ELISA positive samples to be further validated using HPLC/LCMS			
Genetic markers**	• ESBL genes (blaCTX-M, blaSHV, blaTEM etc.) • Tetracycline resistance genes (tet-A, tet-B, tet-M, tet-O, tet-S etc.) • Sulfonamide resistance genes (sul1-3 etc.) • Quinolone resistance genes (*qnrA, qnrB, qnrS* etc.)	• ESBL genes (blaCTX-M, blaSHV, blaTEM etc.) • Tetracycline resistance genes (tet-A, tet-B, tet-M, tet-O, tet-S etc.) • Macrolide resistance genes (err, mef, msr, ere etc.)	• Tetracycline resistance genes (tet-A, tet-B, tet-M, tet-O, tet-S etc.)	• ESBL genes (blaCTX-M, blaSHV, blaTEM etc.)
Data harmonization and reporting	WHONET; Annual reporting			

*Minimum Inhibitory Concentration (MIC) method is ideal and recommended for large antibiotic molecules. Laboratories with necessary infrastructure may prefer MIC (if required in Phase 2); **Along with resistant bacteria and residues, presence of genetic markers responsible for AMR in bacteria should also be tested. This could however be initiated in Phase 2 of surveillance.

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