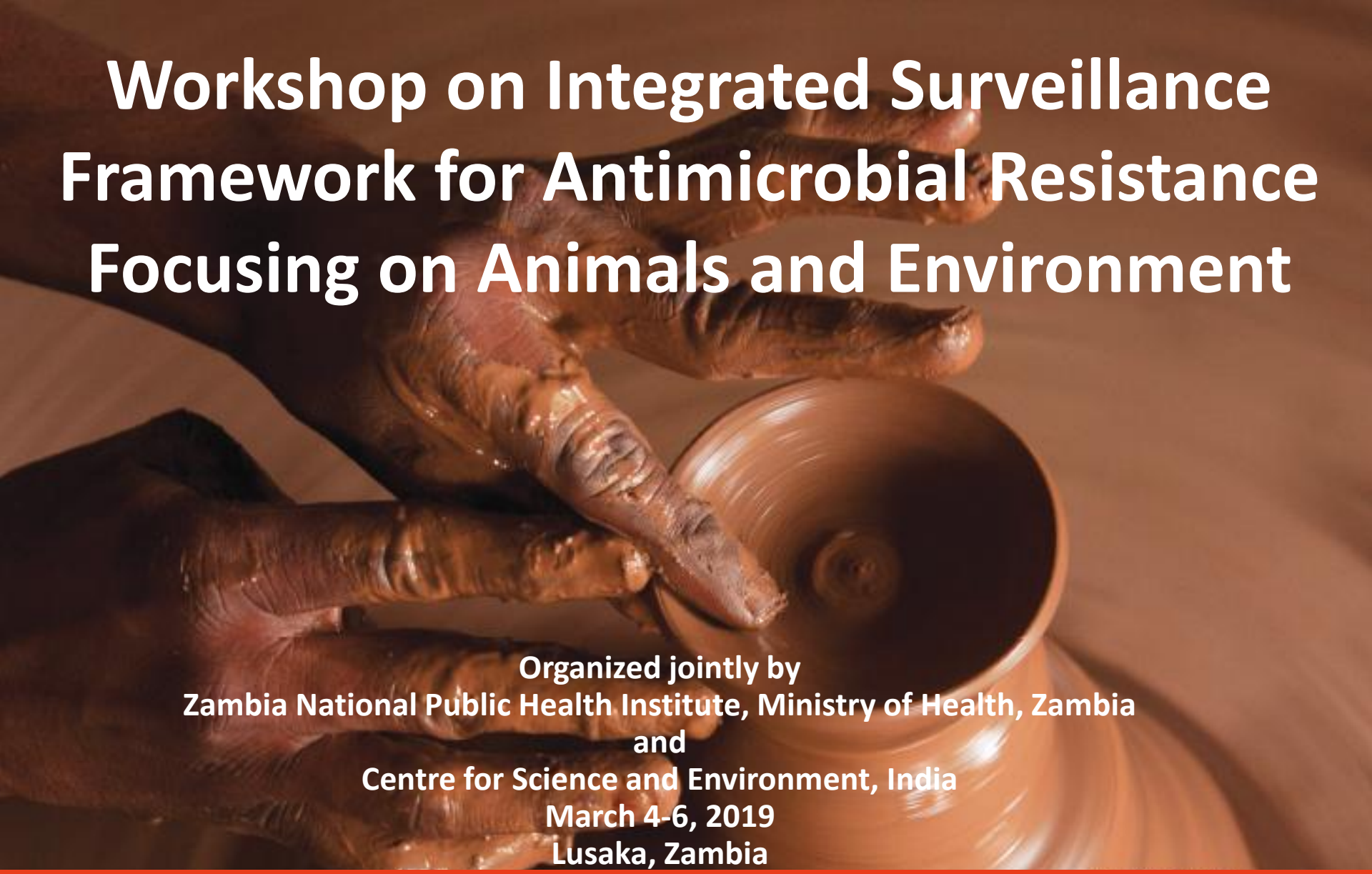
Workshop on Integrated Surveillance Framework for Antimicrobial Resistance Focusing on Animals and Environment

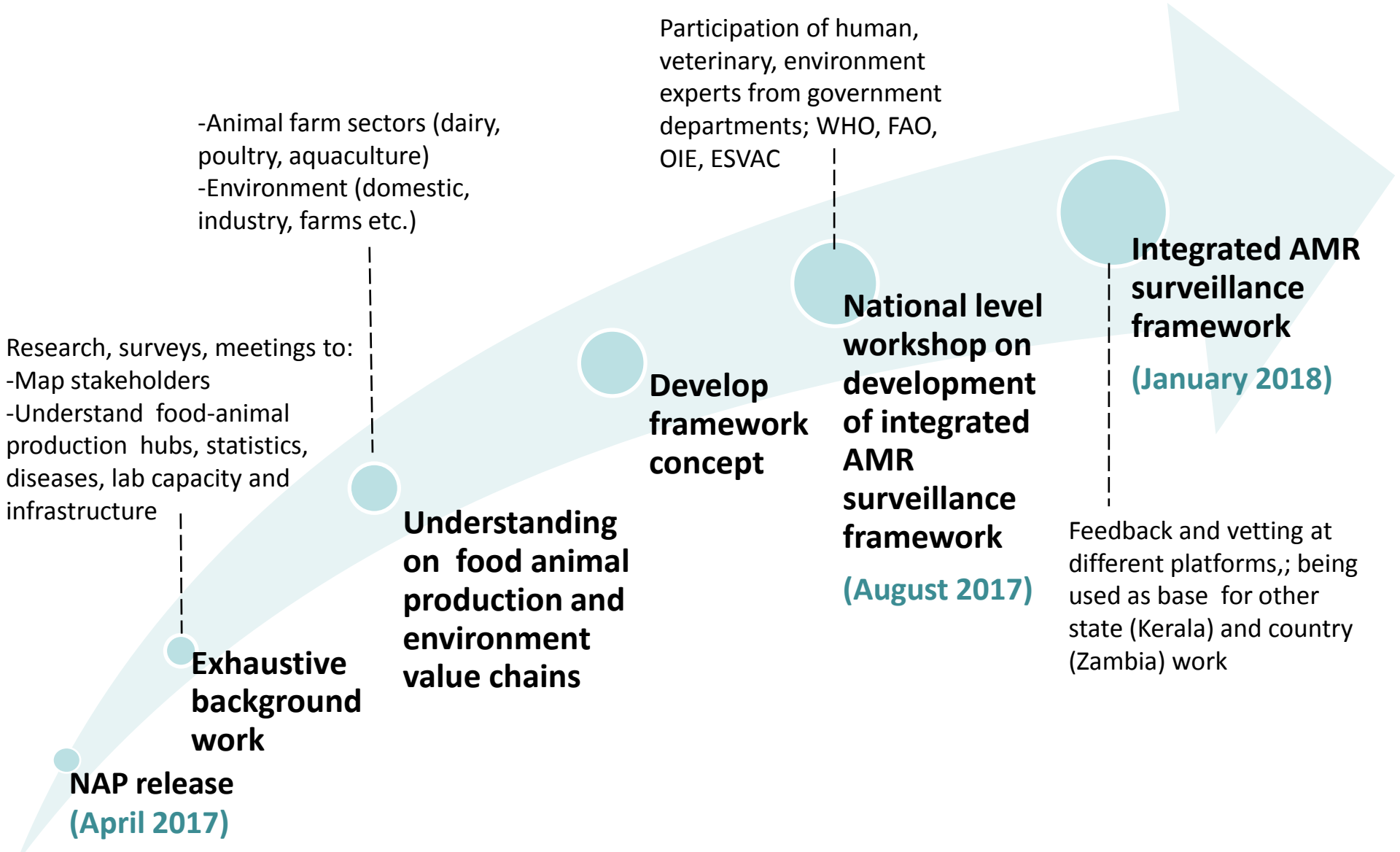


Organized jointly by
Zambia National Public Health Institute, Ministry of Health, Zambia
and
Centre for Science and Environment, India
March 4-6, 2019
Lusaka, Zambia

CSE perspective on AMR surveillance framework and strategy

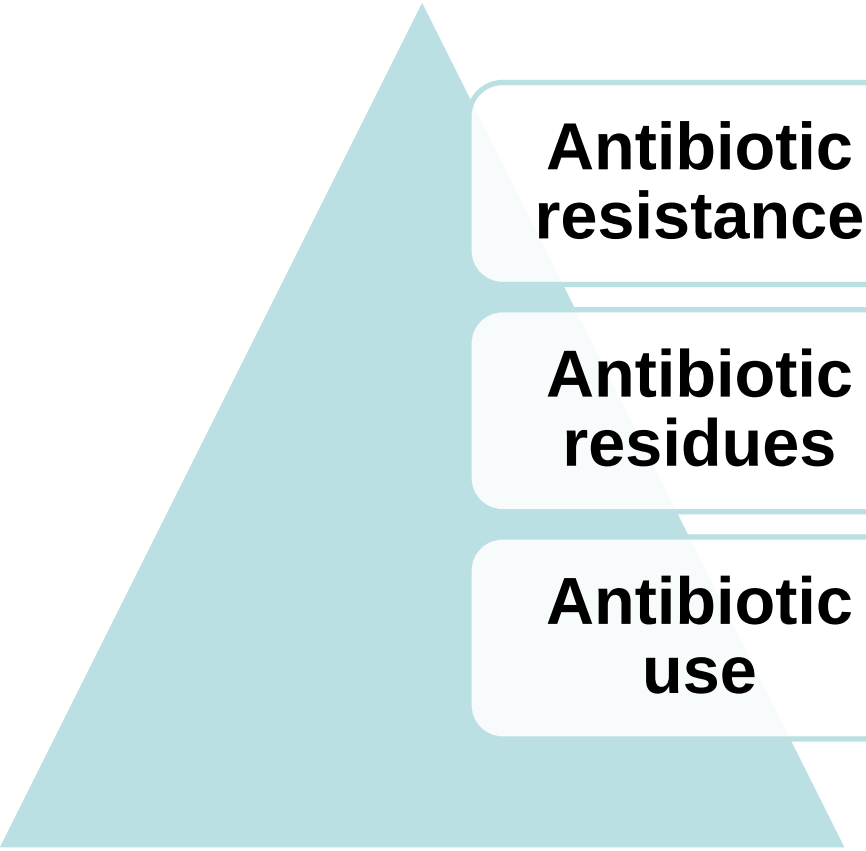
Dr Rajeshwari Sinha, Deputy Programme Manager, Centre for Science and Environment

Road to integrated AMR surveillance framework



AMR surveillance framework for India

Thematic areas



Antibiotic resistance

- Food animals, food-animal products, crops
- Environmental samples

Antibiotic residues

- Food-animal products
- Environmental samples

Antibiotic use

- Human and veterinary settings

Surveillance of antibiotic resistance in food animals, food-animal products and crops

Approach taken

1. Identify target bacteria and antibiotics
2. Identify prevalence rates of each bacteria in each sample type
3. Decide on number of isolates per year and sample size
4. Identify sampling location, sample type, collector
5. Testing method(s)
6. Laboratory network(s)
7. Data analysis and reporting

Target bacteria and antibiotics (consideration set!)

	Salmonella spp.	*E. coli*	*Enterococcus* spp.*	*Aeromonas hydrophila*	*Vibrio harveyi*	*Campylobacter* spp.*
Aminoglycosides	Gentamicin	Gentamicin	Gentamicin	Gentamicin	Gentamicin	Gentamicin
Amphenicols	Chloramphenicol	Chloramphenicol		Florphenicol	Florphenicol	
Carbapenems		Imipenem				
Cephalosporins I & II	Cefoxitin	Cefoxitin		Cephalexin	Cephalexin	
Cephalosporins III	Cefatoxime	Cefatoxime				Phase 2
Glycopeptides			Vancomycin			
Macrolides		Erythromycin	Erythromycin	Erythromycin	Erythromycin	Erythromycin
Penicillins	Ampicillin	Ampicillin	Ampicillin		Ampicillin	Ampicillin
Polymyxins		Colistin				
Quinolones	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin	Ciprofloxacin
Sulfonamides	Cotrimoxazole	Cotrimoxazole			Cotrimoxazole	
Tetracyclines	Tetracycline	Tetracycline	Tetracycline	Tetracycline	Tetracycline	Tetracycline

Largely drawn from **WHO AGISAR Integrated Surveillance of Antimicrobial Resistance in Foodborne Bacteria**

Prevalence rates of priority bacteria

Species	Sample Types	Prevalence (%)					
		E. coli	*Enterococcus* spp.	*Salmonella* spp.	*Campylobacter* spp.	*Aeromonas* spp.	*Vibrio* spp.
Fish	Skin meat at farm	80	80	10	n.a.	40-50	40
	Skin meat at retail	80	80	10	n.a	40-50	40
Broilers (chicken)	Ceca	90	90	20	40	n.a	n.a
	Meat (drumstick)	70	70	10	20	n.a	n.a
Layers (chicken)	Ceca	90	90	50	50	n.a	n.a
	Eggs	60	60	8	n.a	n.a	n.a
	Meat (spent)	70	70	15	15	n.a	n.a

n.a*:* not applicable

- **Higher the prevalence rate for bacteria , lesser samples, cost effective**
- **Bacteria tested for fisheries relevant for both public health and fish health**

No. of isolates and sample-size

- **Consensus upon 120 isolates per year**

- **Let us assume, *E. coli* prevalence in ceca of broiler chicken is 90%**
  - No. of samples required for 120 isolates /**year** /**state**=133
  - No. of samples collected /**quarter** / **state**=34
  - If surveillance is being done in **2 districts**, no of samples collected/**quarter**/**district** =17
  - **These 17 samples can be collected from 4 farms , 4-5 samples each**
  - Number of farms vary with the varying prevalence

- **Districts to be selected based on production statistics**
- **Random selection of farms and sample to ensure representativeness**
  - Same set of districts/farms can be followed for annual trends
  - Districts can be rotated in each quarter for broader scope

Surveillance framework: Fisheries

Example

Sector/ specie	Location type	Geographic location	Sample types	Sample Size (per quarter per state)						Sample collector
				E. coli	*Entero-coccus* spp.	*Salmon-ella* spp.	*Campy-lobacter* spp.	*Aeromo nas* spp.	*Vibrio* spp.	
Fish	Farm	Top 10 producer states; 2 districts in each state	Skin meat	38	38	300	n.a	60-75	75	State Fisheries Department
	Retail	State capitals from top 10 producer states+ 5 metros	Skin meat	38	38	300	n.a	60-75	75	Food Safety and Standards Authority of India (FSSAI)

n.a: not applicable

Surveillance framework: Poultry

Example

Sector/ specie	Location type	Geographic location	Sample types	Sample Size (per quarter per state)						Sample collector
				E. coli	*Entero-coccus* spp.	*Salmon-ella* spp.	*Campy-lobacter* spp.	*Aeromon as* spp.	*Vibrio* spp.	
Broiler (chicken)	Farm	Top 10 producer states; 2 districts in each state	Ceca	34	34	150	75	n.a	n.a	State Animal Husbandry Department
	Slaughter -house	Top 10 producer state	Ceca	34	34	150	75	n.a	n.a	State Animal Husbandry Department
	Retail	State capitals from top 10 producer states+ 5 metros	Meat (drum-stick)	43	43	300	150	n.a	n.a	FSSAI
Layer (chicken)	Slaughter -house	Top 10 producer states	Ceca	34	34	60	60	n.a	n.a	State Animal Husbandry Department
	Farm	All states; 2 districts in each state	Eggs	50	50	375	n.a	n.a	n.a	State Animal Husbandry Department
	Retail	All states	Meat (spent)	43	43	200	200	n.a	n.a	FSSAI

n.a: not applicable

Phase

Surveillance framework: Crops

Example

Sector/ specie	Location type	Geographic location	Sample types	Sample size (per quarter per state)						Sample collector
				E. coli	*Entero-coccus* spp.	*Salmon-ella* spp.	*Campy-lobacter* spp.	*Aeromo-nas* spp.	*Vibrio* spp.	
Crops	Retail	State capitals from top 10 producer states+ 5 metros	Tomato	60	60	150	n.a	n.a	n.a	Indian Council of Agricultural Research (ICAR)
	Retail	All states	Coriander	38	38	600	n.a	n.a	n.a	ICAR
	Retail	All states	Water-Melon	60	60	1500	n.a	n.a	n.a	ICAR

Phase 2

Phase 2

n.a: not applicable

Testing method(s)

Method for bacterial isolation, identification and characterization	• Bacterial isolation by growth on **selective media** • Identification and characterization by **biochemical analysis**
Standard method for AST and	• **Disk diffusion** may be the first step; for reporting of **zone of inhibition** • **Minimum Inhibitory Concentration** (MIC) method is ideal – Recommended for large antibiotic molecules – Labs with necessary infrastructure may prefer MIC
AST Interpretation/Cut-off values	• **CLSI, EUCAST or VETCAST** • Use of **WHONET** recommended

Laboratory network(s)

Number of laboratory facilities/ network	• Lab network of **FSSAI** supported by **State Animal Husbandry** and **State Fisheries Departments** • **Phase 1:** few labs in the state covering each sector strengthened • **Phase 2**: deeper network of labs at district level developed • **Reference centres/agencies** to be engaged for sample collection at district level • **Regional or national level reference laboratory** for genotypic/sequence analysis to be institutionalized

Data analysis and reporting

- **Sampling information**
  - Date of sample collection
  - Sampling strategy/design
  - Type of sampling
  - Sampling population
  - Sampling size
  - Sample source (e.g.: farm, retail, feed etc.)
  - Sample type (e.g., meat, skin, ceca etc.)
  - Sample location (e.g. districts, states etc.)

- **Bacteria specific information**
  - Date of isolation
  - Date of AST testing
  - Type of AST method used
  - Code of the isolate
  - Bacterial identification - species and serovar
  - AST profile
  - Raw data: MIC /Zone inhibition diameters
  - Proportion of susceptible isolates
  - PCR or sequence data obtained

- **Data harmonization**
  - **Quarterly online reporting of ABR** surveillance data food animals, preferably using WHONET
  - All data provided to a centralized database for e.g., **National Reference Laboratories** (to be designated in India)
  - **Data uploaded into GLASS** by government designated National Focal Points for each sector and correlated (in future)

- An **annual report** made available in the public domain

Surveillance of antibiotic residues in food-animal products

Approach taken

1. Identify target bacteria and antibiotics
2. Identify sampling location, sample type, sample size, collector
3. Testing method(s)
4. Laboratory network(s)
5. Data analysis and reporting

Target antibiotics (consideration set)

	Aquaculture	Poultry - Broilers	Poultry - Layers	Dairy
Phase 1	**Penicillins**-Amoxicillin, Ampicillin **Fluoroquinolones-** Ciprofloxacin, Enrofloxacin **Tetracyclines**- Oxytetracycline Tetracycline **Quinolones-** Oxolinic Acid **Cephalosporins**- Cephalexin **Trimethoprim** **Amphenicols-** Chloramphenicol **Nitrofurans**	**Penicillins**-Amoxicillin **Fluoroquinolones**- Enrofloxacin **Tetracyclines**- Oxytetracycline Tetracycline **Aminoglycosides**- Gentamicin **Cephalosporins-** Ceftriaxone **Macrolides-** Azithromycin, Erythromycin **Polymixins**-Colistin	**Penicillins**-Amoxicillin **Fluoroquinolones**- Enrofloxacin **Tetracyclines**- Oxytetracycline Tetracycline **Aminoglycosides**- Gentamicin **Cephalosporins-** Ceftriaxone **Macrolides-** Azithromycin, Erythromycin **Polymixins**-Colistin	**Penicillins-** Amoxicillin **Fluoroquinolones**- Enrofloxacin **Tetracyclines**- Oxytetracycline Tetracycline **Aminoglycosides**- Gentamicin **Cephalosporins**- Ceftriaxone **Sulfamethoxazoles**
	• In addition to the above, residues of antibiotics which may be used widely at sector or regional level and those which are allowed/not allowed by the FSSAI should also be tested.			
Phase 2	• Include **more antibiotics** and **increase the scope of sampling** • **Test crops** of antibiotic residues (e.g.,, tetracycline, streptomycin, fluoroquinolones, nitrofurnatoin based on reported use)			

Surveillance framework: Fisheries

Example

Sector/ specie	Location type	Geographic location	Sample types	Sample size (per quarter per state)	Sample collectors
Aquaculture	Fish farms	Phase 1: • Top 4 producer states with 2 districts in each state Phase 2: • All states; with 2 districts in each state	Meat	Phase 1: • 2% of farms in each district with minimum 5 samples per farm site Phase 2: • 5% of farms in each district with minimum 5 samples per farm site	State Fishery Departments + Locally trained collectors
	Retail	Phase 1: • State capitals of top 4 producer states + 2 metro cities Phase 2: • State capitals of top 10 producer states + 5 metro cities	Meat	Phase 1: • 75 retail points with minimum 1 sample per site Phase 2: • No. of retail points or sample per site may be increased	FSSAI +Locally trained collectors

Surveillance framework: Broiler poultry

Example

Sector/ specie	Location type	Geographic location	Sample types	Sample size (per quarter per state)	Sample collectors
Broiler (chicken)	Farms (contract and non-contract)	Phase 1: • Top 4 producer states with 2 districts in each state +one low-producing state for control Phase 2: • All states; with 2 districts in each state	Meat	Phase 1: • 2% of farms in each district with minimum 5 samples per farm site Phase 2: • 5% of farms in each district with minimum 5 samples per farm site	State Animal Husbandry Departments + Locally trained collectors
	Backyard farms#	Phase 1: • Top 4 producer states with 2 districts in each state +one low-producing, state for control Phase 2: • All states; with 2 districts per state	Meat	Phase 1: • 25 farms with 1 sample per farm site Phase 2: • No. of farms or sample per site may be increased	State Animal Husbandry Departments + Locally trained collectors
	Processing units/ slaughter-house	Phase 1 • Top 4 producer states Phase 2: • All states	Raw/ Processed meat	Phase 1: • 1 unit per state with minimum 5 samples per site Phase 2: • No. of units per state or number of samples per site may be increased	State Animal Husbandry Departments + Locally trained collectors
	Retail/wet market	Ph 2: • State capitals of top 10 producer states + 5 metro cities	Meat	Phase 1: • 75 retail points with minimum 1 sample per site Phase 2: • No. of samples per site may be increased	FSSAI +Locally trained collectors

Could be main cities with consumer markets in a state

Testing method(s)

Standard method for residue testing to be used	• High-performance liquid chromatography • Liquid chromatography–mass spectrometry

- **Since residue surveillance is expensive, consider qualitative estimation first**
- **Consider using ELISA for qualitative testing**
- **Quantification can be done on select samples only**

Laboratory network(s)

Number of laboratory facilities/network that need to be designated	• Lab network of **FSSAI** supported by **State Animal Husbandry** and **State Fisheries Departments** • FSSAI can be the **regional or national level reference lab** for coordination with states and districts – **Phase 1**: at least 1 or few labs in the **state** strengthened (for e,g., NABL-accredited labs). – Institutional labs or Private labs may be explored. – **Phase 2**: network of labs at **district** level developed • **Reference centres/agencies s**hould be engaged for sample collection at district level

Data analysis and reporting

- **Sampling information**
  - Date of sample collection
  - Sampling strategy/design
  - Type of sampling
  - Sampling population
  - Sampling size
  - Sample source (e.g.: farm, retail, feed etc.)
  - Sample type (e.g., meat, skin, ceca etc.)
  - Sample location (e.g. districts, states etc.)

- **Antibiotic specific information**
  - Date of testing
  - Methodology of residue testing
  - Antibiotics for which residue testing was carried out
  - Sample type in which residue was found
  - Antibiotic(s) whose residue was/were found
  - Amount of antibiotic residue(s) detected in sample
  - Analyzed data (comparison as per available MRLs; national and international)

- **Data harmonization**
  - **Quarterly online documentation** of residue surveillance data
  - All data provided to a **centralized database** for e.g., National reference laboratory(to be designated in India)

- An **annual report** made available in the public domain

Surveillance in environmental samples

Approach taken

1. Identify target bacteria and antibiotics
2. Identify sampling location, sample type, sample size, collector
3. Testing method(s)
4. Laboratory network(s)
5. Data analysis and reporting

Target bacteria

Phase 1 (1-3 years)	*E. coli* After 1-2 years, consider including: *Enterococcus* spp. ESBL producing *Enterobacteriaceae* ESBL-coding genes *bla*CTX-M, *bla*SHV and *bla*TEM Plasmid mediated quinolone resistance genes *qnr*A, *qnr*B, and *qnr*S
Phase 2 (4-5 years)	**Surveillance of AMR:** Carbapenem-Resistant *Enterobacteriaceae,* specifically *Klebsiella* spp., *Salmonella* spp. **Surveillance for Antibiotic Resistant Genes (ARGs):** Sulfonamide resistance genes (*sul I* and *sul II*), Carabapenemase resistance genes (VIM and NDM) Integrase coding genes (int1)

Target antibiotics (consideration set!)

	Surveillance of ABR	Surveillance of Antibiotic Residues (AR)
Phase 1 (1-5 years)	For *E. coli* • **Fluoroquinolones:** Ciprofloxacin • **3rd generation cephalosporins:** Cefotaxime • **Carbapenems:** Imipenem • **Penicillin:** Amoxicillin • **Aminoglycopeptides:** Gentamicin • **Polymyxins:** Colistin • **Tetracyline** • **Sulfonamides:** Cotrimoxazole For ESBL-producing *E. coli:* • **Beta-lactams**	**Point source(s) and Non-point source(s)** • Ciprofloxacin • Sulfamethoxazole • Oxytetracycline • Azithromycin • Amoxicillin • Chloramphenicol Antibiotics to be tested will be site-specific and will also depend on rate of breakdown of and the concentration of antibiotic use
Phase 2 (6-10 years)	For *Klebsiella* spp. : • **Sulfonamides and trimethoprim:** Cotrimoxazole • **Fluoroquinolones:** Ciprofloxacin • **4th generation cephalosporins:** Cefepime • **Carbapenems:** Imipenem • **Polymyxins:** Colistin For *Salmonella* spp. : • **Fluoroquinolones:** Ciprofloxacin • **3rd generation cephalosporins:** Ceftriaxone • **Carbapenems:** Imipenem	**Point and Non-point source(s)** Additional antibiotics to be added as per ongoing research

List of antibiotics sourced from pathogen–antimicrobial combinations in the 2015 WHO GLASS Manual for Early Implementation

Surveillance framework: Point sources

Example

Sampling location	Geographical areas	Sample types	Sample Size (per quarter, per state)	Sample collectors	ABR, AR, ARG
SURVEILLANCE UNDER GOVERNMENT MANDATE					
Slaughter houses (govt. approved)	All states	Effluent water (composite sample)	• All units • Minimum 5 samples per site	3rd party (ABR), SPCB (AR)	ABR AR
Dairy, meat, fish processing	All states	Effluent water (composite sample)	• All units • Minimum 5 samples per site	3rd party (ABR), SPCB (AR)	ABR AR
Pharmaceutical manufacturing plants	All states	Effluent water (composite sample)	• All antibiotic manufacturing plants and formulators • Minimum 5 samples per site	3rd party (ABR), SPCB (AR)	ABR AR
Common Effluent treatment plants (CETP)	Pharmaceutical hotspot districts (e.g. Tonsa, Patancheru, Baddi, Ankleshwar, Cuddalore, Thiruvallur, Solan, Shrikullam, Vizag,)	Effluent of direct discharge	Phase 1: • 1 major CETP in each pharmaceutical hotpot districts • Minimum 5 samples in each CETP site Phase 2: • + 10% of samples taken above to be tested for ARG	3rd party (ABR, ARG), SPCB (AR)	ABR AR ARG

Surveillance framework: Non-point sources

Example

Sampling location	Geographical areas	Sample types	Sample Size (per quarter, per state)	Sample collectors	ABR, AR, ARG
ROUTINE SURVEILLANCE					
Rivers/ Reservoirs*	Based on size and religious importance of rivers such as Ganges, Yamuna, Narmada, Caveri, Mandakini, Kshipra	Stratified grab samples (horizontal and vertical stratification)	Minimum 5 samples from river/reservoir per season;	SPCB+ technical support from private institutions	ABR AR ARG
Ground-water	Urban areas; peri-urban areas; areas near healthcare clusters, pharmaceutical manufacture, industrial clusters; drinking water source of All state capitals (Phase 1) All states with five districts in each state (Phase 2)	Samples from groundwater wells near clusters	Minimum 5 samples	Groundwater Control Board + technical support from potential private Institutions	ABR AR

*Sampling to be done quarterly and also after major festivals at bathing places, industrial locations, ceremonial sites. Control sample can be obtained from uppermost reach.

Testing method(s)

Method for bacterial isolation, identification and characterization	• Bacterial isolation by growth on **selective media**; all isolates to be preserved • Identification and characterization by **biochemical analysis**
Standard method for AST and AST Interpretation/Cut-off values	• **Disk diffusion** may be the first step; for reporting of **zone of inhibition** • **Minimum Inhibitory Concentration** (MIC) method is ideal – Recommended for large antibiotic molecules – Labs with necessary infrastructure may prefer MIC • **CLSI, EUCAST** • Use of **WHONET** recommended
Residue testing methods	• **Enzyme-Linked Immunosorbent Assay (ELISA)** • 10% of samples with positive results from ELISA further validated using **HPLC/LCMS**

Laboratory network(s)

- **Environment regulators (Pollution Control Boards) t**o take lead in the **antibiotic residue surveillance** in environment

- For AMR surveillance, **environment regulators** to be initially supported by **technical expertise from third party** (animal husbandry or fisheries departments; research consortia; laboratories in universities; medical colleges or other research institutions etc.)

- **Referral Labs to be designated**
  - 1% of all samples tested should go to referral labs

- **Nodal centre** for **quality assurance** and **quality control**

Data analysis and reporting

- **Sampling information**
  - Date of sample collection
  - Sampling strategy/design
  - Type of sampling
  - Sampling area
  - Sampling size
  - Sample source
  - Sample type
  - Sample location; Consider using GPS coordinates.

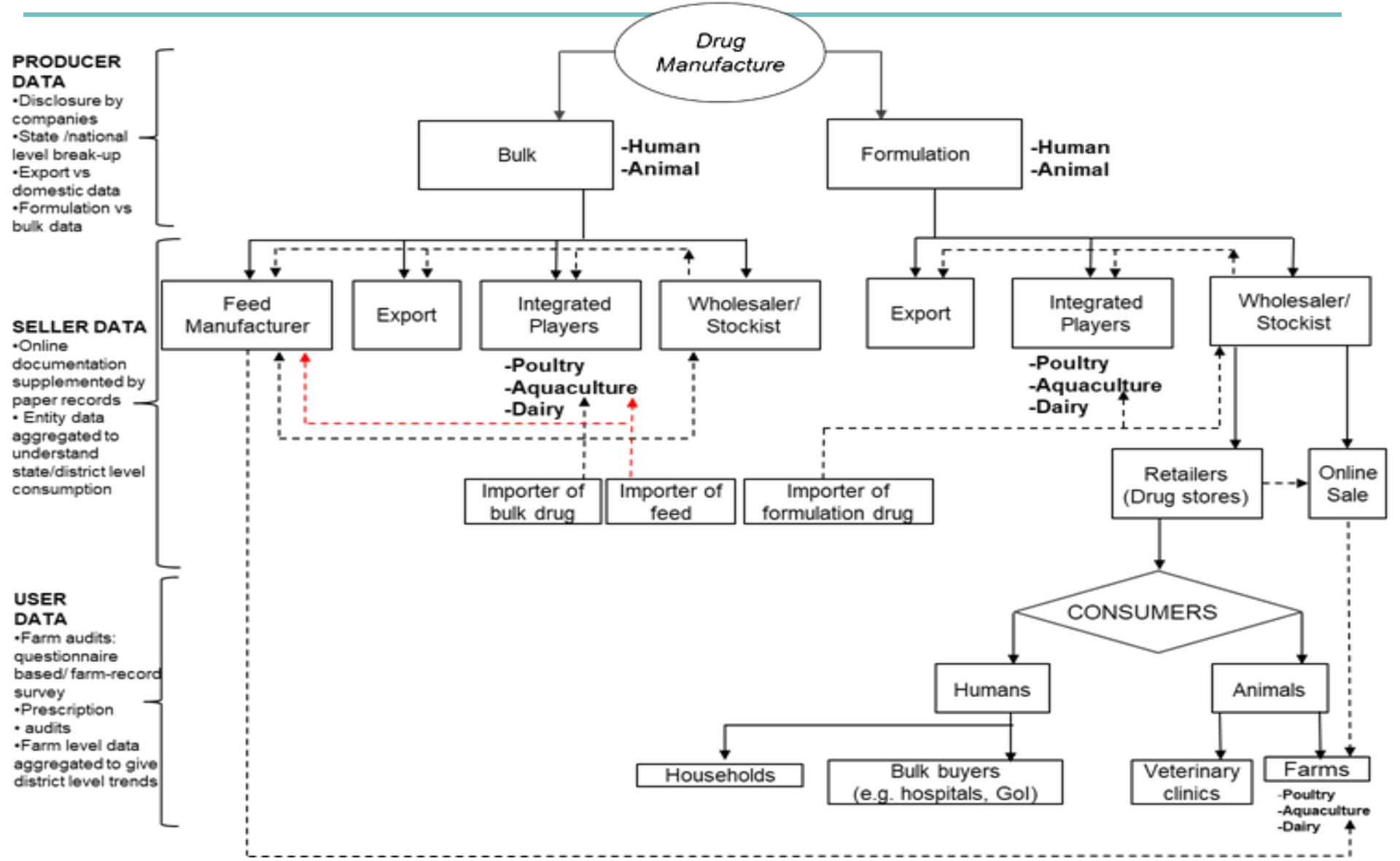
- **Bacteria specific information**
  - Date of isolation
  - Date of AST testing
  - Type of AST method used
  - Code of the isolate
  - Bacterial species isolated and its serovar
  - Antibiotics used for AST
  - Raw data: MIC or Zone of inhibition
  - Analyzed data: Proportion of susceptible isolates.

- **Data harmonization**
  - **Quarterly online reporting of** surveillance data ABR in environment
  - All data provided to a centralized database for e.g., **National Reference Laboratories** (to be designated in India)
  - Quarterly reporting through the **WHONET**

- An **annual report** made available in the public domain

Surveillance of antibiotic use

Sources of antibiotic sale and consumption data – human vs animals



Antibiotic use surveillance framework

Level	Goal	Phase	Scope	Sources for data collection	
PRODUCER	Quantitative estimation	1-2	National	• Manufacturer (govt. or private) – Bulk and/or formulations – Humans and/ or animals – Domestic market	All Antibiotics
SELLER/ DISTRIBUTOR	Quantitative estimation	1	District	• Importer • Seller – Wholesaler data for antibiotic class – Wholesaler data for antibiotics sold per sector – Feed Manufacturer	
		2	State		
USER	Qualitative estimation	1	District	• Farmer, vet etc.: Questionnaire-based surveys • Pharmacist: Tracking invoices/questionnaire based surveys	
		2	District	• Farmer, vet etc. – Questionnaire-based surveys – Registry for antibiotic used/prescribed – Weekly vial collection method • Pharmacist – Tracking invoices – Questionnaire based surveys – Registry for antibiotics sold	

Thank you!

For information, contact:

Amit Khurana
Programme Director
Food Safety and Toxins, CSE
k_amit@cseindia.org

Rajeshwari Sinha
Deputy Programme Manager
Food Safety and Toxins, CSE
s_rajeshwari@cseindia.org