





Microbiological food safety challenges in the Indian dairy industry

Theme: Session 2: Microbiological Sampling and Testing: Food Safety Management

FSSAI-ICMSF-CHIFSS Symposium On Microbiological Food Safety Sampling And Testing In Food Safety Management

भाकुअनुप ICAR Delivered By:

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Emergence of microbiological food safety concerns and challenges

Major trends

- Increased awareness among consumers on food safety and quality
- Food consumption patterns:- minimal processed foods , preference for processed ready to eat foods
- □ Use of new additives /ingredients and development of new products
- **Shift in agricultural production , manufacturing and distribution practices in food chain**
- **International travel and trade transportation of infectious agents**
- **Globalization of food supply chain**
- Detection, reporting and surveillance system
- □ Acquisition of virulence and antibiotic genes by pathogenic bacteria-Emergence of antimicrobial resistance in bacteria (AMR)
- Microbial adaptation and enhanced survival of pathogens in food







Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health

Risk profiling of pathogens in milk and milk Products

Organism	Shed directly in milk#	Contaminant of raw milk##	Survives pasteurization	Severity of illness §	Dairy products implicated in food- borne illness
Aeromonas spp.	×	✓	×	Serious	+
Brucella spp.	✓	✓	×	Severe	+
Clostridium botulinum	×	✓	*	Severe	+
Clostridium perfringens	×	✓	1	Moderate	+
Corynebacterium spp.	✓	✓	×	Serious	+
Coxiella burnetii	✓	✓	×	Serious	+
Cryptosporidium	×	✓	×	Severe	+
Mycobacterium avium subs. paratuberculosis	×	✓	×	-	-
Mycobacterium bovis	\checkmark	✓	×	Severe	+
Shigella spp.	×	✓	×	Serious	+
Streptococcus spp.	\checkmark	✓	×	Serious	+
Yersinia enterocolitica	×	✓	×	Serious	+
Bacillus cereus	×	✓	✓	Moderate	++
Staphylococcus aureus	✓	✓	×**	Moderate	++
Campylobacter jejuni / coli	×	✓	×	Serious	++
Salmonella spp	×	✓	×	Serious	++
Enterobacter sakazakii	×	✓	×	Severe^	++
Pathogenic E. coli	×	×	×	Severe	++
Listeria monocytogenes	✓	1	×	Severe^	++

Transmission through udder; mastitis etc; ## via faeces, the environment etc; *Neurotoxin is heat labile; ** Enterotoxin is heat stable; ^ for vulnerable populations; § based on ICMSF (2002) severity ranking; + Reported, but rare; ++ More commonly associated with food-borne illness; – No data/unknown; ✓ = yes; X = no















COMMON SOURCES OF FOOD POISONING



Source: Centre for Disease control and prevention







Outbreaks associated with *Campylobacter jejuni*

Year	Country	Cases	Product	Causative agent	Cause	Ref.
2003	USA	13	Raw milk	C. jejuni	Unpasteurised milk	(Peterson, 2003)
2000	Austria	38	Unpasteuriz ed milk	C. jejuni	Unpasteurised milk distributed by a local dairy	(Lehner <i>et al.,</i> 2000)
1998	Hungary	52	Raw milk	C. jejuni	Unpasteurised milk	(Kalman <i>et</i> <i>al.,</i> 2000)
1996	UK	33	Unpasteuriz ed milk	C. jejuni	Educational farm visit, exposure to raw milk	(Evans <i>et al.,</i> 1996)
1992	USA	50	Raw milk	C. jejuni	Consumed at church	(CDC 2002)
1995	UK	110	milk	C. jejuni	Inadequately pasteurized milk from a local dairy	(Fahey <i>et al.,</i> 1995)

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Major Outbreaks associated with Salmonella spp.

Year	Country	Cases	Product	Causative agent	Cause	Ref.
2000	USA	38	Pasteurized Salmonella milk typhimurium		Likely contaminated containers or milk contact surfaces after pasteurisation because of environmental conditions in plant	(Olsen <i>et</i> <i>al,</i> 2004)
2003	USA	62	Raw milk	Salmonella typhimurium	Unpasteurised milk at dairy/petting zoo	(Mazurek <i>et al.,</i> 2004)
1998	UK	40	Pasteurized milk	Salmonella	Pasteurisation failure	(Brown, 1998)
1999	Washing ton	17	Raw-Milk Cheese	Salmonella typhimurium DT 104	Infection due to consumption of un- pasteurised milk	(Villar <i>et</i> al.,.1999)

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Outbreaks associated with Listeria monocytogenes

Year	Country	Reported cases	Causes
	PASTEURIZED MIL		
1986	Austria	28	Consumption of raw milk
2006-07	USA	5	At the plant where the milk was
1997	USA	54	processed, inspections revealed no evidence of improper pasteurization
CHEESE			
2003	France	18	
2003	Sweden	15	
2002	Canada	17	Cheese was made from the
2001	Soft cheese	45	contamination
2000	USA	13	
1995	Switzerland	57	
2013	USA	22	Ricotta Salata Cheese
2014	USA	5	Soft cheese made from pasteurized milk
BUTTE	R AND BUTTER PRODU		
2003	UK	17	Listeria isolated from a dairy drain / butter







Pathogenic bacteria isolated from different Indian foods

BFJ	Type of food	Bacteria		
114,0	Milk	Listeria monocytogenes, Yersinia enterocolitic Bacillus cereus, Srteptococcus feacalis, Escherichia coli		
670	Meat	Bacillus cereus, Escherichia coli Stahylococcus aureus, Vibrio parahaemolyticus		
	Beef sample	Escherichia coli 0157:H7		
	Sweets	Salmonella Newport, Salmonella enteritidis		
	Dahi (yogurt), Khoa	Escherichia coli, Enterobacter aerogenes Salmonella Newport, Salmonella enteritidis, Fecal coliforms		
	Prawns	Vibrio parahaemolyticus		
	Cooked and uncooked rice	Bacillus cereus		
	Poultry	Campylobacter jejuni, Salmonella bornum		
	Fish	Staphylococcus, Escherichia coli		
	Samosa	S.aureus		
Table III.	Batatawada	S.aureus		
Pathogenic bacteria	Tamarind	Salmonella, Staphylococcus, Shigella		
Indian foods	Butter milk	Yersina enterocolitica		

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Common foodborne pathogens and their percentage of contamination in Indian foods

Type of food	Bacteria	% of contamination	Foodborne
Milk	B.cereus	16-50	uiseases in muia
	L.monocytogenes	6	
	Yersinia	5-59	
	Aeromonas	7	
	Vibrio	8	671
Meat	Salmonella spp	3-5	
	Staphylococcus spp	21	
	E.coli	9-14	
	Aeromonas	13	
	B.cereus	35	
Poultry	C.jejuni	41	
	Salmonella spp	11	
	Aeromonas	28	
Fish	E.coli	7	
	Vibrio	16-32	
	Shigella	4	
Seafoods	Salmonella spp	1	
	Vibrio spp	1	
	Listeria	1	
Beef	E.coli 0157:H7	60	
Rice	B.cereus	28-46	
Vegetables	B.cereus	24	
	Coliforms	75	
	E.coli	75	
	Listeria	12	
	C.jejuni	3	Table IV.
Lassi	B.cereus	5	Common foodborne pathogens and their
Khoa	Staphylococcus spp	20-36	percentage of
	Salmonella spp	5	contamination in Indian
	E.coli	9	foods

10/10/2018

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Source: Vemula *et al.*, **2010** 10







Two stage enzyme assay for rapid detection of *L. monocytogenes* in milk



Stage 2: Confirmatory detection of *L. monocytogenes*



Results Validated with ISO procedure

Conventional method -ISO: 11290 Part-1:1996 (5-7 days Protocol)

IP Status: Patent Reg. No. 1357/DEL/2013







Two stage enzyme assay for detection of *E. coli* in milk







Results Validated with ISO procedure

Conventional method -IS:5887 Part-I:1976 (4-5 days Protocol)

IP Status: Patent Reg. No. 2214/DEL/2014







Rapid detection of coliform in milk





Results Validated with ISO procedure

Conventional method –ISO:4832:2006 (4-5 days Protocol)

IP Status: Patent Reg. No. 2214/DEL/2014

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Two stage enzyme assay for *Enterococci* in milk





Results Validated with ISO procedure

IP Status: Patent Reg No 119/DEL/2012

Conventional method :IS 5887 Part-2: 1976 (3-4 days Protocol)

Stage 2: Confirmatory detection of *Enterococci* in milk







Incidence of Aflatoxin M1 in Milk samples



10/10/2018







Risk profiling of dairy pathogens : ICMSF 2002









FSSAI Microbiological criteria approved for Milk & milk Products

Micro-organisms

- 1. Total Plate Counts
- 2. Coliform
- 3. Yeast and Mold Counts
- 4. Staph aureus
- 5. Bacillus cereus
- 6. E. coli
- 7. Salmonella
- 8. Listeria monocytogenes
- 9. Enterobacter sakazakii
- 10. SRC

Risk Assessment

Hazard identification
Hazard characterization
Exposure assessment
Risk characterization

Risk management

Risk evaluation
Option assessment
Option implementation
Monitoring and review

Risk communication Interactive exchange of information option concerning risks

Codex Alimentarius Risk Assessment frame work

Generation of scientific data on hygiene/ safety indicators in milk and milk products through National surveillance network Project







Mastitis and its impact in India





Source of loss	Loss per cow(\$)	Percent of total(%)
Reduced production	121.00	66.0
Discarded milk	10.45	5.7
Replacement cost	41.73	22.6
Extra labour	1.14	0.1
Treatment	7.36	4.1
Veterinary services	2.72	1.5
Total	184.40	100

• In India annual losses in dairy industry due to mastitis has been reported approximately 1670 Cr / 231.2 Million USD (Jingar, et al, 2017)







Microorganisms involved in mastitis

Environmental pathogens	Contagious pathogens	Others
Escherichia coli (40%)	Staphylococcus aureus (40-70%)	Staphylococcus epidermidis (1.3%)
Klebsiella pneumonia	Streptococcus agalactiae (8-10%)	Staphylococcus simulans (1.0%)
Arcanobacteriumpyogenes	Streptococcus dysgalactiae (1.6%)	Staphylococcus chromogens (0.7%)
Yeast spp.	Corynebacterium spp.	
	Mycoplasma spp. (5-12%)	



Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings





Fresh milk

Fresh, Frozen, Preserved

Fresh, Frozen, Preserved

Lab

Lab

Lab

Inspiring Trust, Assuring Safe & Nutritious Food Ministry of Health and Family Welfare, Government of India

Types of mastitis and Diagnostic methods for its identification

Sub-clir	nical	Cli	nical		Chronic	
Test	Identification of mastitis milk	Identification of pathogen	Time of detection	Test location	Sample type	
California mastitis test	V	Х	Minutes	Farm	Fresh milk	
Somatic cell count	V	Х	Minutes	Lab	Fresh milk	

Availability of diagnostic for early detection of mastitis will be a great help in sustainability of dairy farm business and controlling AMR

Days

Hours

Hours

٧

v

٧

Bacterial culture

MULTIPLEX PCR

ELISA

٧

Х

V







Global trends of antimicrobials use in livestock

- 63151 tones in 2010
- 67% increase in 2030 •
- **100%** increase in **BRICS** countries









Antibiotic consumption is growing rapidly...









Existing diagnostic for detection of <u>Antimicrobial Residues</u> in milk

Name of	Concept /	Manufact	Time per	Cost of the	Antibiotic	Application	
test	principle	urer	test	test	tested		
Delvo test	Microbial inhibition based	Gist brocades/ DSM	2 :30 min	Rs. 150 per test	Broad spectrum	Qualitative screening test use in few multinational companies / bigger dairy farms in India	
DPA based assay	Microbial inhibition based / Color change	NDRI Karnal	3.0-3.15 h	Rs. 50 per test	Broad spectrum	Qualitative screening test (semi-quantitative test) are in	
Paper strip based assay	Spore germination / enzyme / color change	NDRI Karnal	1.00 h	Rs. 75 per test	Broad spectrum	use in few multinational companies / bigger dairy farms in India	
Penzym Test	Enzyme colorimetric	Neogen Corporatio n	15 min	Rs. 200 per test	β -lactams	Not in use	
Tetra sensor	Antigen antibody based	Different companies	5-10 min	~Rs. 200 for one antibiotic	Individual antibiotics / group	Used in research institutions for quantitative estimation, rarely in big dairies	
Snap Test	Receptor binding assay	IDEXX	10 min	Rs. 300 per test	Family specific	Not in use	
Diagnostic for Mastitis Antimicrobial Posiduos							

Diagnostic for Mastitis, Antimicrobial Residues

and Resistant bacteria in dairy settings







Impact of Antimicrobial Resistance

•700,000

Annual deaths currently

•10 million

Projected deaths in 2050

•\$ 100 trillion

Loss to global economy till 2050

•3.5%

Reduction in global GDP in 2050

AMR is now

- Economic problem
- Food security risk
- Development Issue
- Political challenge

UNGA, WHO. FAO, OIE, G8, G20, G77, ASEAN, OPEC, EU

Implementation of National

Action Plan on AMR











ANTIMICROBIAL RESISTANCE- A THREAT

The Bad Bugs

Extended spectrum β-lactamase producers









Factors leading to antimicrobial resistance (AMR) in dairy animals



Diagnostic for Mastitis, Antimicrobia Residues and Resistant bacteria in dairy settings







	WHO PRIORITY LIST OF PATHOGENS global action plan on AMR
	giobal action plan on Arriv
Priority 1 CRITICAL	 Carbapenem-resistant Acinetobacter baumannii Carbapenem-resistant Pseudomonas aeruginosa, 3rd generation cephalosporin-resistant, ESBL-producing carbapenem-resistant Enterobacteriaceae
Priority 2: HIGH	 <i>Enterococcus faecium</i> vancomycin-resistant <i>Staphylococcus aureus, Vancomycin resistant Helicobacter pylori</i> Fluoroquinolone-resistant <i>Neisseria gonorrhoeae</i>
Priority 3 MEDIUM	 Streptococcus pneumoniae Penicillin-non-susceptible Haemophilus influenzae Ampicillin-resistant Shigella spp.
	Diagnostic for Mastitis, Antimicrobial Residues

Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings







AMR – where to look and what to look?









Proposed draft guidelines on integrated surveillance of antimicrobial resistance

CODEX ALIMENTARIUS COMMISSION



Food and Agriculture Organization of the United Nations



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Agenda Item 5

CX/AMR 17/5/6 September 2017

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

AD HOC CODEX INTERGOVERNMENTAL TASK FORCE ON ANTIMICROBIAL RESISTANCE

Fifth Session

PROPOSED DRAFT GUIDELINES ON INTEGRATED SURVEILLANCE OF ANTIMICROBIAL RESISTANCE

Prepared by the Electronic Working Group led by the Netherlands and co-chaired by Chile, China and New Zealand

Codex members and Observers wishing to submit comments at Step 3 on the proposed draft Guidelines (Appendix I to this document) should do so as instructed in CL 2017/82-AMR available on the Codex webpage/Circular Letters 2017:

http://www.fao.org/fao-who-codexalimentarius/circular-letters/en/.

Report of the electronic working group for the drafting of the Guidelines on integrated monitoring and surveillance of Antimicrobial Resistance







Target microorganisms and resistance determinants

10.6 Target microorganisms and resistance determinants

Bacterial species should be chosen considering public health aspects, including the epidemiology of foodborne diseases, and should include both foodborne pathogens and indicator organisms of commensal bacteria.

Salmonella is a key foodborne pathogen and should therefore be included in an integrated monitoring and surveillance programme. Other foodborne pathogens like Campylobacter should also be strongly considered, as well as other pathogens depending on national or regional situation and risks (e.g. *Staphylococcus*, *Clostridium* or *Vibrio*).

Indicator organisms of commensal intestinal bacteria may contaminate food and can harbour transferable resistance genes. Commensal *E. coli* and *Enterococcus* spp should be used as indicators of Gram negative and Gram positive intestinal flora.

Whenever possible the monitoring and surveillance programme should include genetic and/or phenotypic analysis of particular isolates that may be a public health concern such as ESBL- AmpC and carbapenemase-producing strains.

Tests for virulence factors, AMR genes, gene transferability and gene sequencing can also be applied.

http://www.fao.org/fao-who-codexalimentarius/sh-roxy/it/?lnk=1&url=https%253A%252F%252 Fworkspace. fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-804-05%252FWD%252Fam05_06e.pdf







CARBAPENEM RESISTANCE IN FOOD ANIMAL ECOSYSTEM

The European Food Safety Authority (EFSA), 2013 in association with the Panel on Biological Hazards (BIOHAZ) states that

- Carbapenemases are now seen as a new and potentially emerging problem in food-producing animals
- •All isolates of <u>Salmonella spp. and E. coli</u> should be screened for Carbapenem resistance
- Isolates which are resistant to 3rd or 4th generation cephalosporins should be subjected to phenotypic testing and characterization of the carbapenemase genes
- Carbapenem resistance in dairy cattle and raw milk samples should also be addressed because of specific use of cephalosporins in dairy cattle, and to the risk posed by potential consumption of raw milk







AMR surveillance in India

- > WHO Global Antimicrobial Resistance Surveillance System (GLASS)
- > MoHFW /NCDC : AMR surveillance network (10 labs)
- ICMR : AMR Surveillance Network (4 institutions/6 labs)
- INFAAR: Indian Network for Fishery and Animals Antimicrobial Resistance (13 institutions)







Prevalence of Antibiotic resistant bacteria in animals Indian scenario

PLACE	SAMPLE	ISOLATES	RESISTANCE		RESISTANCE	REFERENCE
	TYPE		MDR positiv e	Carbapene m positive	MECHANISM	
West Bengal	Mastitis milk	8 (<i>E. coli</i>)	5	1	Horizontal transmission from human or environmental sources	Ghatak <i>et al</i> ., 2013
	Bovine mastitis	7 (E. coli)	3	-	Emergence of antimicrobial resistance among bovine mastitis pathogens	Bandyopadhyay <i>et al</i> ., 2015
	Mastitis milk	50 gram negative (Enterobacteriaceae)	24		Extensive use of β-lactam antibiotics	Das <i>et al</i> ., 2017
Odisha	Poultry (fecal) Cattle (milk)	252 64 (<i>E. coli</i>)	16 2	4 1	Indiscriminate antibiotics use	Kar <i>et al</i> ., 2014
Hyderabad	Meat, Egg, Raw milk	22 (E. coli)	6	1	Widespread use of antibiotics	Rashmeed <i>et al.,</i> 2014
Kolkata	Mastitis milk	291 (Klebsiella)	23	4	Cross transmission between the human and animal	Koovapra <i>et al.</i> , 2016
Haryana (NDRI)	Raw milk,	139 (<i>E. coli</i>)	11	-	Transmission and acquisition of antibiotics resistance gene by plasmid and mobile genetic element	Amarjeet <i>et al.</i> , 2018







Diagnosis for AMR cont..

Method / name of test		Based on	Reference	Time	Cost	Application
Determinati on of Susceptibilit y / resistance	Disk diffusion assay	Growth based with pure isolates	Reference method (as per CLSI / EFSA guidelines)	Overnigh t 10-12 hr.	~ Rs. 500- 1500/ test	R&D institutes / referral centers / regulatory agency / veterinary hospitals
	Dilution test					
	E test					
	Automated AST systems	Convenientional / microdilution / automated plates	Vitek System, Micronaut Phoenix	4-12 hrs	Rs.2000- 3000/ test	R&D institutions and laboratories only
Detection of metabolic activity	Carba NP test	Colour from red to yellow Due to pH shift	Growth based enzyme assay with pure isolates	30-60 min	Rs. 250 per test	Different settings of dairy food chain / dairy farms/ veterinary hospitals / milk reception docks etc.
	Nitrate reductase analysis; Resazurin microplate assay	Color change of growth medium due to pH change due to bacterial growth				
Genotypic Methods		PCR; Microarray and other Modifications	Different Biotechnological companies	3-4 hours	5000- 10000 per	Clinically / research laboratories / not suitable for different
9/24/2018		Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings			sample	setting in dairy supply chain 34



- Un-organized milk production system
- In sufficient awareness about clean milk production, hazards and risks involved (HACCP system)
- Inadequate power supply/ or cold chain
- Poor linkage between milk producers and dairy processers because of active role of middle man
- No regulatory intervention during milk production and reception of milk at dairy units
- Lack of rapid field levels kits for monitoring of different microbial contaminants in milk for conducting risk assessments work and regulatory compliance are lacking
 Development of Sector wide food safety
- Manual handling leading to post pasteurization contamination
- Level of automation during processing

Development of Sector wide food safety guidelines (GAP/GDP/GHP/GMP) during entire dairy supply chain involving all stake holders for regulatory compliance









The Government established the Food Safety and Standards Authority of India (FSSAI) under the Food Safety and Standards Act, 2006 (FSSA) with the mandate to lay down science based standards for food products and to regulate their manufacture, storage, distribution, sale and import, to ensure availability of safe and wholesome food

Main features of Integrated food law

- Harmonization with international standards such as CODEX
- > Shift from a regulatory regime to self compliance
- To lay down scientific standards and ensure availability of safe food
- Single reference point for all issues related to food safety and standards
- Clear procedures for food recall

Key Microbial Food safety Challenges

- Awareness creation
- Capacity building
- Infrastructure creation
- Building Research & Development Capacity
- Certification of Raw Material
- Traceability system







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Awareness Creation

Poor general awareness towards the hazards associated with unsafe food practices and the best practices to be followed



Effective awareness creation programs carried be out bv the need to Governmental agencies for smooth transition from the current food safety laws to the proposed system, specifically medium keeping the small and bv enterprises abreast of the salient features of the law and practical issues that are likely to be faced by the manufacturers and their solutions

Capacity building programme for enhanced food safety at different stages in dairy supply chain (FBO/ regulators)







Infrastructure creation

- > One of the critical links in the successful implementation of FSSAI is food testing laboratories.
- > Under the new law the manufacturers need to get their products tested every month and keep a certificate.
- Hence, building up a sufficient number of accredited laboratories is of paramount importance

Establishment of referral centre /BSL-2 laboratory for food safety monitoring / FSSAI standard compliance









Insufficient technical expertise and skilled manpower for implementation of legislation at the grass root level





Massive efforts are required for capacity building in order to successfully implement the proposed FSSAI at the grass root level

- Well evolved training programs need to be conducted for the state, district and block level enforcement agencies
- The programs would have to equip the implementing officers with knowledge on international standards of food safety and quality thus enabling regulators to make judicious decisions relating to food contamination

Microbiological food safety challenges in the Indian dairy industry

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- of the major sources \geq One of contamination food in systems during the primary occurs production stage - which is kept out of the ambit of the FSSAI
- Successful holistic \geq and of implementation Food safety system would require an extensive campaign that encourages implementation of Good Agricultural Practices (GAP) at the farm level

Certification of Raw Material

Exclusion of primary producers from the purview of the law thus putting the onus of preventing food hazards on the manufacturers or processors











Problems in traceability of product especially in the upstream processors of the food chain – from the farm gate to the processing unit



Organized manufacturers should be encouraged to take pro-active steps to ensure that GAP is adhered to by their suppliers, and a traceability system including geographic application is placed at the back-end thus reducing the risk of food contamination







Concluding remarks

- ✓ Generation of scientific data on hygiene/ safety indicators in milk and milk products through National surveillance network project.
- ✓ Establishment of codex cells for development of food safety standards based on risk analysis
- ✓ Development of food safety guidelines (GAP/GDP/GHP/GMP) during entire dairy supply chain involving all stake holders
- Compulsory implementation of QMS and HACCP for dairy industry to ensure domestic as well as export standard compliance
- Capacity building programme for enhanced food safety at different stages in dairy supply chain (FBO/ regulators)
- ✓ Establishment of referral center /BSL-2 laboratory for food safety monitoring / FSSAI standard compliance
- Stronger interaction between industry and R & D institutions for enhanced innovation and know how transfer leading to better productivity in dairy sector
- Adoption of new technologies developed by R & D institutions by FSSAI for their regulatory compliance
- $\checkmark\,$ Development of rapid diagnostics for addressing the issues of residues , mastitis , AMR and dairy pathogen detection in dairy food chain









Diagnostic for Mastitis, Antimicrobial Residues and Resistant bacteria in dairy settings