



# ANTIBIOTIC RESIDUES IN MILK: A PUBLIC HEALTH CONCERN

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## ● INTRODUCTION

- “Veterinary drugs” any substance applied or administered to any food producing animal, such as cattle, poultry, fish or bees, whether used for therapeutic, prophylactic or diagnostic purpose or for modification of physiological function or behavior (IDF, 1997)
- “Antibiotics” low to medium molecular weight compounds exhibiting a variety of chemical and biological properties.



## Table . Livestock Wealth of India

Species	Population (Millions)	World position
Buffalo	105.3	I (57%)
Cattle	199.1	II
Goat	140.5	II
Sheep	71.5	III
Camel	0.51	VI

❖ Highest milk producer in the world (15%)





**Table. Net work of veterinary services in India**

<b>Villages</b>	6,41,169
<b>Veterinary Hospitals and Polyclinics</b>	8,732
<b>Veterinary Dispensaries</b>	17,830
<b>Veterinary Aid Centres and Mobile Veterinary clinics</b>	25,195
<b>Registered veterinarians</b>	~ 54,000
<b>Para- veterinarians</b>	~ 60,000
<b>Disease investigation support</b>	250 State & 6 Central Govt Lab.
<b>Veterinary Biological Production Institutes (vaccines)</b>	26
<b>Total of Animal Disease Reporting Units</b>	51,973
<b>One disease reporting unit catering(Av)</b>	12 Villages



## MILK



Milk a highly perishable commodity is susceptible to contamination :

- Pesticides
- Toxic heavy metals
- Myco-toxins antibiotics and other veterinary drugs
- Microorganisms:
  - ✓ Pathogens and spoilage



## ANTIBIOTICS IN MILK

- ❑ Cornerstone of prevention and treatments of ailments (1940s)
- ❑ Feed additives – to enhance growth and food efficiency (1950s)
- Synthetic, natural or semi-synthetic sources
- Mode of action: Some of the antibiotic's mode of action is not fully understood; some have more than one mode of action



## ➤ Groups of antimicrobials:

- ❑  $\beta$ -lactams (penicillin, ampicillin, cephapirin etc)
- ❑ Tetracyclines (chlortetracycline, oxy-tetracycline)
- ❑ Sulfonamides (sulfadimidine, sulfadimethoxine)
- ❑ Macrolides (erythromycin, spiramycin)
- ❑ Aminoglycosides (gentamycin, neomycin, streptomycin)
- ❑ Quinolones (enrofloxacin, sulfadiazine)
- ❑ Miscellaneous (chloramphenicol, colistin, novobiocin)





- Milk and milk products - staple food over the globe
- Single potential source of certain residues and contaminants
- Consumed by most vulnerable group:
  - ❑ Infants'
  - ❑ Young children
  - ❑ Elderly

Hence, concentrations of anti-microbial residues in dairy foods must be kept low as practical possible



## SOURCES, OF ANTIBIOTICS IN MILK AND MILK PRO

### ➤ Antibiotics used:

- ❑ Prevention and treatment of diseases (major mastitis)
- ❑ Maintenance of stable milk supply
- ❑ Feed additives

### ➤ Mode of drug administration:

- ❑ Intramammary
- ❑ Intramuscular
- ❑ Intravenous
- ❑ Oral



➤ **Commonly used anti-microbial:**

- ❑  **$\beta$ -lactams**
- ❑ **Tetracyclines**
- ❑ **Aminoglycosides**
- ❑ **Sulfa drugs**

➤ **Sub therapeutic use of anti-microbial (feed additives) do not normally cause residue problem in milk and milk products**

➤ **Antibiotics appears in milk during treatment and after cessation of treatment.**



## Reasons of appearance of veterinary drug residues in m

- ❑ **Indiscriminate use**
- ❑ **Non-adherence to withdrawal period.**
- ❑ **Economic reasons/ignorance**
- ❑ **Lack off medication records.**

### ➤ **Other Factors:**

**Type of antibiotics; dose administered, physio-pathological conditions of udder, carrier/vehicle employed, milk yield, time interval between treatment and milking.**



# SIGNIFICANCE OF ANTIBIOTICS IN MILK AND MILK PRODUCTS

**Why public is concerned about antibiotics**

**Public health :**

- ❖ Allergic reaction
- ❖ Toxicity
- ❖ Hypersensitivity
- ❖ Carcinogens/ mutagens
- ❖ Alter gastrointestinal micro-flora
- ❖ Food should be completely antibiotic free



## ➤ Dairy industry:

### ☐ Interfere with starter metabolism

- Inhibition of lactic acid production.
- Off flavours
- Poor quality product

### ☐ Economic loss



## ANTIMICROBIAL USE IN ANIMALS: EFFECTS ON ANTIBIO- RESISTANCE EMERGENCE

- The problem of antimicrobial resistance knows no boundaries.
- Drug-resistant microbes of all kinds can move among people and animals, from one country to another—without notice.
- The threat of antibiotic resistance is growing at an alarming pace, perhaps more rapidly in developing countries.



- It has been observed that farms using AGPs had more resistant bacteria in the intestinal of farm workers and animals than in those for similar people and animals on farms not using AGPs.
- Bacterial resistance to antimicrobial drugs is one of the most serious concern to global public health.
- Deaths caused by a single multi-resistant species, MRSA, might be more than those caused by AIDS.
- The results showed not only colonization of the chickens with tetracycline-resistant and other drug-resistant *Escherichia coli* strains but also acquisition of resistance in *E. coli* in the intestinal flora of the farm family.





## Reasons of antibiotic resistance threat among bacteria than microorganism:

- ❖ The abuse of antibacterial drugs is much higher than that of antifungal or antiviral agents; the later ones are seldom self-prescribed, wrongfully used as prophylaxis, or have agricultural usage;
- ❖ Genetic characteristics and abilities to rapid evolution toward resistance as compared to viruses, fungi, and protozoa: haploidy, horizontal gene transfer mechanisms, extrachromosomal elements
- ❖ Much more abundant than viruses, fungi, and protozoa as microbiota of humans, which increases exponentially the exposure of the former to antibiotics each time they are used clinically, creating more chances of resistance to emerge and be selected;
- ❖ Bacterial diseases are also more abundant, at least for treatment purposes, increasing also the exposure to antibacterial drugs, perhaps with the exception of malaria. Therefore, although microbial resistance in general is posing grave problems for public health, it is not possible to view all resistance from the same perspective.



## EVIDENCE FOR ANIMAL-TO-HUMAN SPREAD OF ANTIBIOTIC RESISTANCE:

- Low-dose, prolonged courses of antibiotics among food animals create ideal selective pressures for the propagation of resistant strains.
- Spread of resistance may occur by direct contact or indirectly, through food, water, and animal waste application to farm fields. It can be augmented greatly by the horizontal transfer of genetic elements such as plasmids via bacterial mating (conjugation).



## Antibiotic Resistance Transmission through Food Chain

- It is complex transmission routes between farms and consumers and the frequent transfer of resistance genes among host bacteria
- Reports have demonstrated a broader linkage of resistance genes through the farm-to-fork food chain. A resistance-specifying bla<sub>CMY</sub> gene was found in all resistant isolates of *Salmonella enterica* serotype Newport originating from humans, swine, cattle, and poultry. The host plasmid, which conferred resistance to nine or more antimicrobials, was capable of transmission via conjugation to *E. coli* as well



- The complexities of the modern food chain make it challenging to perform controlled studies that provide unequivocal evidence for a direct link between antibiotic use in animals and the emergence of antibiotic resistance in food-borne bacteria associated with human disease.

# DECTION METHODS

- **Microbial inhibitor tests;**

  - Delvo test**

  - Charm II Test**

- **Demerits:**

  - Specificity, accuracy, sensitivity, cost and time**

- **Hence, urgent need to develop precise accurate, sensitive, economic and fast screening test**

**Table : Sensitivity limits (in µg/kg) of different microbial tests for various anti-microbial**

Substance (group)	<i>B. stearothermophilus</i> var. <i>calidolactis</i>		<i>Streptococcus salviarius</i> ssp. <i>thermophilus</i> Valio T101
	Improved tube diffusion method	Delvotest SP (3h)	
Benzylpenicillin	3	3	5
Ampicillin	6	6	25
Amoxicillin	6	6	40
Cloxacillin	25	25	200
Ceftiofur	40	50	20
Oxytetracycline	750	1000	400
Tylosin	20	50	200
Erythromycin	10	200	75
Spectinomycin	750	3000	1500
DH/streptomycin	250	1500	400
Gentamicin	50	200	1500
Sulfadimine	500	150	>5000
Dapsone	2	2	>2000
Chloramphenicol	5000	7500	1500



## INCEDENCE OF ANTIBIOTIC RESIDUES IN MILK AND MILK PRODUCTS

- Very few published reports are available on incidences of veterinary drug residues in milk and milk products
- History of data on incidence of antimicrobial residues in USA in milk loads showed rise and fall

**Table . Incidence of antimicrobials in milk loads**

Year	% +ve milk samples
1960	5
1970s	7
1991	0.1
1995	0.06
2001	0.1



# MICROBIAL INHIBITORY SUBSTANCES (ANTIBIOTIC RESIDUES) IN COW AND BUFFALO MILK

(ICAR Funded project)

## Objectives:

- ❖ To survey the use of different antibiotics in milch animals.
- ❖ To investigate the nature of antibiotic residues and their frequency of appearance in milk samples commercial, tankers and farm.
- ❖ To investigate relationship between antibiotic, residues and inadequate curdling of milk during *dahi* and yoghurt manufacture.





## SURVEY ON THE USE OF ANTIBIOTICS IN DAIRY ANIMALS

- Majority of the farmers get their animals treated with antibiotics by veterinary surgeons/ stockmen
- Farmers seldom treat dairy animals with antibiotics by themselves.
- The common antibiotic used are tetracycline, gentamycin, ampicillin, amoxycillin, oxytetracycline, cloxacillin and penicillin due to the lower cost .
- Some doctors also use enrofloxacin, lincomycin, streptomycin and chloramphenicol for treatment of certain diseases



- The major drugs used for the treatment of mastitis are  $\beta$ -lactams or in combination with other drugs such as streptomycin. Oxytetracycline is also used for treatment of mastitis by some doctors.
- All the veterinary doctors and majority of the stockmen are aware of the need to discard the milk from animals treated with antibiotics during the treatment and 2-3 days after cessation of the treatment. However, most of the farmers are not
- As the usage of antibiotics in crossbred animals is maximum for the treatment of mastitis, milk should be screened particularly for  $\beta$ -lactam and oxytetracycline residues as they are the major drugs used for the treatment.



**Table : Incidence of antibiotic residues in individual animals milk**

<b>Source of milk</b>	<b>No.of samples analysed</b>	<b>No.of positive samples</b>	<b>Beta lactam positive</b>	<b>Tetracycline positive</b>	<b>Streptomycin positive</b>	<b>Gentamycin positive</b>
<b>Cow</b>	<b>125</b>	<b>3</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>0</b>
<b>Buffalo</b>	<b>87</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>



**Table 15: Antibiotic residues in tanker milk samples**

<b>Milk</b>	<b>No.of samples analysed</b>	<b>No.of positive samples</b>	<b><math>\beta</math>-lactam positive</b>	<b>Tetracycline positive</b>	<b>Streptomycin positive</b>	<b>Gentamycin positive</b>	<b>Unidentified</b>
<b>Tanker</b>	<b>385</b>	<b>15</b>	<b>13</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>2</b>

*Fifteen tanker milks received at commercial dairy in Andhra Pradesh from a route of buffalo tract was also analysed for antibiotic residues. None of the sample was tested positive for the drugs.*



**Table: Occurrence of antibiotic residues in market milk**

<b>State</b>	<b>No. of samples examined</b>	<b>No. of positive samples</b>	<b><math>\beta</math>-lactam positive</b>	<b>Tetracycline positive</b>
<b>Andhra Pradesh</b>	<b>110</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>Karnataka</b>	<b>231</b>	<b>2</b>	<b>2</b>	<b>0</b>
<b>Kerala</b>	<b>112</b>	<b>0</b>	<b>0</b>	<b>0</b>
<b>Tamilnadu</b>	<b>197</b>	<b>2</b>	<b>2</b>	<b>0</b>



**Table : Antibiotic residues in composite milk**

<b>Milk</b>	<b>No.of samples analysed</b>	<b>No.of +ve samples</b>	<b><math>\beta</math>-lactam +ve</b>	<b>Tetracycline +ve</b>	<b>Streptomycin +ve</b>	<b>Gentamicin +ve</b>
<b>Organised</b>	<b>93</b>	<b>5</b>	<b>4</b>	<b>1</b>	<b>0</b>	<b>0</b>
<b>Unorganised</b>	<b>89</b>	<b>2</b>	<b>1</b>	<b>1</b>	<b>0</b>	<b>0</b>



**Table . Incidence of antibiotic residues in milk in Southern India**

Type of milk	%+ve samples	No of samples analysed
Market milk	0.74(6)	806
Tanker milk	3.9(15)	385
Organized farm	4.7(6)	128
Unorganized farm	1.6(2)	125



## EFFECT OF HEAT PROCESSING ON ANTIBIOTICS STABILITY

- Antibiotics are heat stable and it is affected by;
  - ❑ Concentration
  - ❑ Chemical nature
  - ❑ Composition of dairy products
  - ❑ Processing parameters
- These are more heat stable in milk as compared to buffer and water
- Pasteurization found ineffective to destroy veterinary drugs
- Penicillin more stable in buffalo milk than cow milk





**Table : Effect of heat treatments on destruction of penicillin**

Treatment	Type of milk	Percent destruction
Pasteurization	Whole milk	7
	Skim milk	11
Boiling	Whole milk	19
	Skim milk	24

# FOOD SAFETY REGULATION



- **National and international organisations**
  - ❑ **Drug residue free milk and milk products**
  - ❑ **Proper use and quantitative estimation**
- **Codex Alimentarius Commission(CAC) in 16<sup>th</sup> session (1985) established the Codex Committee on residue of “Veterinary drugs” in foods (CCRVDF)**



**Table . MRLs (Codex, EU) and tolerances (USA) of residues of anti-microbial drugs in milk ( $\mu\text{g} / \text{kg}$ )**

Substance(-group)	MRL Codex	MRL (EU)	USA	PFA*
<b><math>\beta</math>-lactams</b>				
Penicillin	4	4	5	4
Ampicillin	-	4	10	
Amoxicillin	-	4	10	
Cloxacillin	-	30	10	
Oxacillin	-	30	50	
Ceftiofur	100	100	50	
Cephapirin	-	10	20	



<b>Tetracyclines</b>				
Chlortetracycline	100	100	300	
Oxytetracycline	100	100	300	100
Tetracycline	100	100	300	
<b>Sulfonamides</b>				
Sulfadimidine	25	100	10	25
Sulfadimethoxine			10	
Sulfamerazine			10	
Sulfathiazole			10	



<b>Macrolides</b>				
Erythromycin		40	50	
Spiramycin	100	200		
Tylosin		50	50	
<b>Aminoglycosides</b>				
Gentamicin	200	100	30	200
Neomycin	500	500	150	
Spectinomycin	200	200	30	
DH / Streptomycin	200	200	125	200



❖ India, many dairies do not include testing of milk for antibiotic residues

❑ Non existence of food laws for the same

❑ Lack of awareness on detection tests

❖ Dairy industry should initiate action to monitor antibiotic residues in dairy foods

❖ Train at least one quality control person on various aspects of antibiotics screening tests



## FSSAI- main regulatory authority

- Food Safety and Standards Act- 2006 (MoH&FW)
- The various provisions which have direct or indirect implication for AGPs include
- **FSSA, 2006- Section 16(2)(b)**
- The Food Authority specify the limits for use of food additives, crop contaminants, pesticide residues, **residues of veterinary drugs**, heavy metals, processing aids, mycotoxins, **antibiotics and pharmacological active substances** and irradiation of food



### FSSAI, 2006- Section 16(3)(b)

- The Food Authority shall also search, collect, collate, analyze and summarize relevant scientific and technical data particularly relating to **incidence and prevalence of biological risk, residues of various contaminants, identification of emerging risks among many others** also.

### FSSAI, 2006- Section 21(1)

- No article of food shall contain insecticides or pesticides residues, **veterinary drugs residues, antibiotic residues, solvent** residues, pharmacological active substances and micro-biological counts in excess of such tolerance limit as may be specified by regulations.





## Food Safety and Standards Regulations- 2011 (Section 2.3.2)

- Specifies limits of antibiotics and other pharmacologically active substances in sea foods (Shrimps, Prawns, Fish & Fishery products)



## Drug and Cosmetics Rules, 1945

### Rule 97- on 17.01.2012

- Container of a medicine for treatment for food producing animals shall be labeled with the withdrawal period of the drug for the species on which it is intended to be used.
- Specific withdrawal period shall not be less than seven days for eggs or milk, twenty eight days for meat from poultry and mammals including fat and offal, five hundred degree days for fish meat.



## National Policy for containment of Antimicrobial Resistance (AMR)

- ❖ Reducing antibiotic selection pressures by instituting appropriate control measures.
- ❖ Promotion of discovery of newer and effective antimicrobials based on current knowledge of resistance mechanisms.
- ❖ Rapid and accurate diagnosis of infections and infectious diseases.



## ● **Points for Act**

- Establish government commitment and support
- Establish a national alliance to control AMR
- Institute a surveillance system to capture AMR
- Promote rational use of antimicrobial agents
- Strengthen infection prevention and control
- Support research in newer antimicrobials
- Educate, Train and Motivate stake holders
- Establish quality system & national registry
- Join with industry for the co-development of antimicrobial agents



- **Inter-Sectoral Co-ordination Committee**
- Develop and promote guidelines to minimize and contain AMR arising from use of antimicrobials in food producing animals
- Develop a list of critically important antimicrobials (CIA) for human health as well as for treatment of food producing animals in order to guide risk management strategies.
- Provide technical support and capacity building in monitoring AMR through research projects, training activities and reference services



## Inter-Sectoral Co-ordination Committee- Cont...

- Establish and perform pilot studies to address lack of data for risk assessment.
- Measures for improving animal health to reduce/restrict antibiotic use and to create awareness through IEC campaigns.
- Develop regulations & advisories considering the Indian scenario on use of antimicrobials with continuous supervision, audit & feedback
- Ban in a phased manner the non-therapeutic/ growth promoter use of antimicrobials to **prevent the development of AMR.**



## CONCLUSIONS

- ❖ There is no doubt that human misuse and overuse of antibiotics are large contributors to resistance, particularly in relation to bacteria associated with human infection.
- ❖ Interventions in medical, veterinary settings and the community are clearly needed to preserve the efficacy of antibiotics.
- ❖ Antibiotic contaminated milk should not be used for consumption
- ❖ Milk from antibiotic milch animals should not be mixed with good quality milk
- ❖ Largest milk producer with low milk production
- ❖ Potential candidate for export of milk products
- ❖ **Challenge:** Open global market demands international standards for food safety requirements
- ❖ Indian dairy industry should be proactive
- ❖ Dairy development agencies should conduct awareness programme targeted towards farmers and para-veterinary staff