Public health microbiology

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Chapter one

Introduction to public health microbiology

Outline of presentation

- Objective
- Definition of public health bacteriology
- Public health microbiology core functions
- History of microbiology in food
- Taxonomy , role & Significance of microorganism in foods, , water, milk and beverages
- Source of food and water contamination & types of microorganisms
- Summary

Objective

- After the end this chapter , students should be able to :
  - Define public health microbiology
  - Explain the core functions of public health microbiology
  - Describe the history of microbiology in food

Objective cont’d…

- Describe the taxonomy, roles and significance of microorganisms in food , water, milk and beverages

- Identify sources of contamination and types of microorganisms

Introduction to Public Health Microbiology

This subject deals with :-

1. Microorganisms in food , water and beverages
2. Inspection and sampling of foods, water and beverages
3. Microbiological examination of foods, water and beverages
4. Methods for microbiological analysis of food water and beverages

Public Health Microbiology Laboratory Core Functions

- Disease prevention, control, and surveillance;
- Integrated data management;
- Reference and specialized testing;
- Environmental health and protection;
- Food safety;

Core functions cont'd…

- Laboratory improvement and regulation;
- Policy development;
- Emergency response;
- Public health-related research;
- Training and education; and
- Partnership and communication

Disease prevention, control, and surveillance

- Provide accurate and precise analytical results
- Rapidly recognizing and preventing the spread of communicable diseases
- Serve as a center of expertise for the detection and identification of biologic agents
- Provide specialized tests for low-incidence, high-risk diseases
- Detect epidemiologic shifts; and detect newly emerging Pathogens
- Provide population surveillance, or screening for public health community

Reference and Specialized Testing

- Serve as the state's primary reference microbiology laboratory to :-
  - Test for unusual pathogens;
  - Confirm atypical laboratory test results;
  - verify results of other laboratory tests;

Specialized Testing cont'd…

- Provide toxicology testing,
- Provide oversight for quality assurance;
- Test epidemiologically significant specimens
- Provide reference diagnostic testing to private sector laboratories

Environmental Health and Protection

- Conduct scientific analyses of environmental samples
- Analyze environmental and biological specimens and detect, identify, and quantify toxic contaminants
- Ensure laboratory services that support assurance of clean air and water in the state,
- Provide environmental chemistry testing including organic and inorganic compounds

Environmental Health and Protection

- Provide or ensure analysis of environmental samples
- Measure toxicants to determine conclusively the extent of exposure to environmental hazards.
- Provide industrial hygiene/occupational health testing

Food Safety

- Test specimens in food borne illness outbreaks to identify causes and sources.

- Analyze food specimens to detect, identify, and quantify toxic contaminants.

- Ensure, radiation-control studies to monitor radioactive contamination of water, milk, shellfish, and other foods.

Laboratory Improvement and Regulation

- Coordinate and promote quality assurance programs
- Serve as the standard of excellence for local and private laboratory performance
- Exercise leadership and authority for laboratory regulation and training.
- Provide analytical support of federal, state, county, and local regulations and laws.

Policy Development

- Provide scientific and managerial leadership in developing state and federal public health policy

- Participate in developing standards for all health-related laboratories

Emergency Response

- Provide laboratory support for state and national disaster preparedness plans.
- Ensuring the capacity to quickly and accurately handle a substantial volume of tests during an emergency situation;
- Providing a rapid response system for hazardous contaminants in waste.

Public Health-Related Research

- Evaluate and implement new technologies and analytical methodologies.

- Ensure the laboratories provide state-of-the-art, cost-effective, and timely analytical and diagnostic services.

Training and Education

- Provide, or facilitate, training courses and workshops for laboratory staff for providing quality services
- Provide short- and long-term training opportunities
- Provide continuing education in management and leadership development for those in administrative positions.

HISTORY OF MICROROGANISMS IN FOODS

- Two major era in food science at the pre scientific era
- Food gathering period
  - The period from human origin, 1milion years - 8000 yrs ago were reported
  - Human were considered as Carnivores
  - Food were cooked for the first time
  - While plant food recognized later.

HISTORY OF MICROROGANISMS IN FOODS

- **Food processing period**
  - 8000-10000 years ago and now
  - Food spoilage and poisoning

Major events	Year	Place
Spoilage of prepared food dated	6000 B.C	
first evidence of beer manufactory	as far back as 7000 B.C	ancient Babylonian
the first to bread live Stock and diary men, and among the first to make butter.	-	Sumerians
usage of salt from Dead Sea for the preservation of food.	-	Jews
Preparation of Wines.	3500 B.C	Assyrians
Preparation and consumption of Fermented sausage.	as far back as 1500 B.C	ancient Babylonians and ancient Chinese
practice of smoking to preserve food	around 1000 B.C.	-
Few advances were apparently made towards understanding of food poisoning and food spoilage	between the time of the birth of Christ and A.D. 1100.	
Ergot poisoning cause 40,000 deaths	943 A.D	France

Taxonomy, Roles And Significance Of Microorganisms In Food And Beverages

- Many of the new taxa have been created as a result of the employment of molecular genetic methods alone or in combination with some of the more traditional methods including:
  - DNA homology and moles % G + C content of DNA.
  - 23 s, 16s and 5s rRNA sequence similarities
  - Oligonucleotide cataloging

- Numerical taxonomic analysis of total soluble proteins / battery of morphological and biochemical characteristics

- Cell wall analysis

- Serologic markers

- Fatty acid analysis

Bacterial taxonomy con’td…

- The most important genera known to occur in foods are listed below in alphabetical order.
- **Bacterial group**

Acitinobacter	*Enterobacter*	*Pediococcus*
Aeromonas	*Erwinia*	*Proteus*
Alcaligenes	*Escherchia*	*Pseudomonas*
Alteromonas	*Flavobacterium*	*Psychrobacter*
Bacillus	*Hafnia*	*Salmonella*
Brochothrix	*Lactococcus*	*Serratia*
Campylobactyer	*Lactobacilus*	*Shewanella*
Carnobacterium	*Leuconostoc*	*Shigella*
Citrobacter	*Listeria*	*Staphylocoous*
Closteridum	*Micrococous*	*Vagocoous*
- *Enterococous*	*Pantoea*	*Yersinia*
Cornibacterium	*Moraxella*	*Vibrio*

Molds group

Alternaria	*Cladosporium*	*Mucor*
Aspergillus	*Colletotrichum*	*Penicillium*
Aureobasidium	*Fusarium*	*Rhizopus*
Botrytis	*Geotrichum*	*Trichothecium*
Byssochlmys	*Monilia*	*Wallemia*
Zeromyces		

Yeasts group

Brettanomyces	*Issatchenkia*	*Schizosaccharomyces*
Candida	*Kluyveromyces*	*Torulaspora*
Crptococcus	*Picha*	*Trichosporon*
Debaryomyces	*Rhodotorula*	*Zygosaccharomyces*
Hanseniaspora	*Saccharomyces*	

Protozoa

Cryptosporidum parvum	*Girdia lambilia*
Entamoeba histolytica	*Toxoplsam gondii*

Incidence and types of Microorganisms in foods

- Basic description of microorganisms in foods
  - Bacteria are prokaryotic unicellular mircoorganisms
  - Their cells have a relatively rigid cell wall that maintain their characteristics shape
  - Morphological features of interest are size, shape, grouping of the cell and possession of features such as endospores , flagella or capsule
  - Can also be described by their staining reactions

- On the basis of morphology, bacteria are classified as
- Cocci
  - A spherical cell
  - Reproduce by binary fission
  - On completion the division of the cells may remain attached and form either a cluster as in the case of micrococcos and staphylococus or chains as in streptococuss

- Micrococci (cocci that form cluster
  - Non pathogenic but are important in food spoilage
  - Are resistant to heat (some survive 74ºc for 60 minute)
  - Grow at relatively at low water activity
  - Tolerate high concentration of sodium chloride
  - Are also resistant to freezing and drying

- staphylococci (cocci that form cluster)
  - Includes some pathogenic strains
  - During growth in foods , the often produce exo toxins which provoke food poisoning .
  - The exo toxins are heat stable and are not destroyed by ordinary cooking
  - Grow at relatively low water activity and in the presence of high concentration of sodium chloride
  - Both micrococci and staphylococci are gram positive cocci arranged in clusters
  - Unlike the staphylociocci , the microcci utilize dextrose oxidatively and are able to grow on ammonium phosphate water

- Streptococci (cocci in short and long chains)
  - Are gram positive cocci arranged in short or long chains
  - Are lactic acid producers and tolerate low Ph values
  - Grow only in highly enriched media
  - Are resistant to heat, freezing and drying
  - May survive pasteurization of foods
  - The enterococcus group consists mainly streptociossu fecalis and S.faecium
  - Entrococci serve as indicator organism in some laboratory

- Leuconostoc
  - Are micro aerophilic cocci
  - Produce lactic acid as well as gas
  - Tolerate high concentration of sugar and sodium chloride
  - Are important in food spoilage
- Pediococci
  - Are cocci that occur singly and in pairs and are important agents in the spoilage of beers

- Rods
  - They are cylindrical or rod like
  - They may occur in chains and some are motile
  - basic features in the classification
    - Gram staining reaction
    - production of spores

- Non spore forming gram positive rods
  - Lactobacilli
    - Are lactic acid producers
    - Tolerate low PH values
    - Are micro aerophilic and the often form the predominant part of the micro flora in vacuum packed products
    - Some strains are heat resistant and they may survive pasteurization of food

- Spore forming gram positive rods
  - Are divided in to two genera
    - Bacillus
    - Clostridium

- Bacillus species are aerobes/facultative anaerobes and catalase positives
- Clostridium species are obligate an aerobe and catalse negative
- All species of both genera are able to produce spores

- Spore forming gram positive rods
  - Spores can resist may form of adverse conditions and can survive for more than 100 years
  - Survive massive dose of irradiation and are extremly resistant to chemicals and heat
  - Spores are killed at 121 oc within 20 minutes
  - the most important spore forming bacteria in food microbiology are Clostridium Botulinum , C.perfiringes and bacillus Cereus

- Non spore forming gram negative rods
  - Non spore forming gram negative rods of importance in food microbiology are aerophilic and catalse positive
  - Ingeneral they are much more sensitive to heat , dehydration , freezing , low PH & low water activity than the cocci and the gram positive rods
  - Some of the gram negative rods are psychrophilic and are responsible for the spoilage of food kept low temperature

- Non spore forming gram negative rods
    - the most important spoilers are Pseudomonas,Achromobacter,Flavobacter and Proteus
    - The gram negative rod also includes pathogens such as , salmonnela shigella , vibrio e.t.c.
    - Several of them are inhabitants of the intestinal tract of man and animals and these are used widely as fecal indicators
    - Most commonly used in this way are the coliforms

- Fungi
  - Yeasts
    - May be desirable(in food industry as leavening of bread , production of alcohol, glycerol e.t.c.) or undesirable inputs
    - Several types of yeasts are food spoilers but few are known to be pathogenic to man

- Fungi
  - Moulds
    - Play important role in foods
    - Some are desirable because they produce products that enhance the flavor of foods
    - Some other are capable of producing mycotoxins which poses public health hazards
    - Most are considered mesophilic, (25-30oc)
    - Some other are psychrophilic.
    - Few are aerobes and can grow over a wide range of pH values but the majority are favored by an acid pH

- The most important moulds in relation to foods are
  - Asperigilli
  - Penicillia
  - Mucor and rhiazopus
  - Geotrichum /dry mould
  - Sporotricum

Primary Sources of Microorganisms In Foods And Beverges

- There are eight environmental source of organisms to foods

1. **Soil and water**

- Soil is a very rich environment in microbes and is a major source of contamination of foods and water
- Bacilus , clostriduim , enterobacter, escharcia micrococcus , alkaligens , flavobacterium , pseudomonas , proteous , aerobacter ,moulds and yeasts are kind of organisms that contaminate food and water from soil **.**
- **Water** is also important source of food contamination

2 .Plants and plant products

- Many soil and water organisms contaminate plants.

- However, only a relatively small number find the plant environment suitable to their overall well-being .

- Those that persist on plant products do so by virtue of a capacity to adhere to plant surfaces.
- so that they are not easily washed away and because they are able to obtain their nutritional requirements.

- The Lactic acid bacteria and some yeast are the most notable examples.

- Other plant pathogens are Cornybacterium, Curnobacterium, Pseudomonas Xanthomonas and fungal pathogens.

3. Food Utensils

- When vegetables are harvested in containers and utensils, some or all of the surface organisms on the product can contaminate the contact surfaces.
- As more and more vegetables are placed in the same containers, a normalization of the flora would be expected to occur.
- In a similar way, the cutting block in meat market along with cutting knives and grinders are contaminated from initial samples and this process lead to a build up of organisms, thus ensuring a fairly constant level of contamination of meat borne organisms.

4. Intestinal tracks of humans and animals

- This flora becomes a water sources when polluted water is used to wash raw food products.
- The intestinal flora consists of many organisms that do not persist as long in water as do others.
- A good example is Salmonella species.
- Many of The Enterobacteriace group are expected in the fecal wastes, along with intestinal pathogens including some protozoan pathogens.

5. Food Handlers

- The micro flora on the hands and other garments of handlers generally reflects the environment and habitat of the individuals and the organisms in questions may be those from soils, waters, dust and other environmental sources.
- The nasal cavities, the mouth, the skin and those from the gastrointestinal tract that may be a source of food contaminants/ entry to foods

6. Animal Feeds

- Salmonella is the notorious organisms that can be transmitted to poultry and other farm animal through animal feeds.
- Incase of some silage it is a known source of Listeria monocytogens to diary and meat animals.
- The organisms in dry animal feed are spread throughout the animal environment and may occur in the animal hides.

7. Animal Hides

- In the case of milk cows, the type of organisms found in raw milk can be a reflection of the flora of the udder when proper procedures are not followed in milking and the general environment of such animals.

- From both the udders and hide, organisms can contaminate the general environment, milk containers and the hands of handlers.

8. Air and dust

- Most of the organisms that are listed above may be suspended in the air and dust in the food processing rooms.

- The gram positive bacteria can persist long. Some fungi also occur in air and dust along with some yeast.

Summary

- Public health microbiology deals with microbiological analysis of water , food and beverages

- The core function of public health microbiology laboratory is
  - Providing a comprehensive laboratory service for public health problems
  - Improving and regulating laboratory services

Summary

- Bacteria , moulds , yeast , protozoa are the known microorganisms for contamination of foods

- Soil, water, air , plant and plant products , animal hides , food utensils , intestinal tract of human and animals and food handlers are the source of contamination

Chapter two: Factors that affect and favor microbial growth in foods

Outline of presentation

- Objective
- Requirement for growth of microorganisms
- Parameters of food that affect microbial growth
  - Intrinsic factor
    - PH
    - Moisture content
    - Oxidation /reduction potential
    - Nutrient content
    - Antimicrobial constituents
    - Biological structure

- Extrinsic factor
  - Temperature
  - Relative humidity
  - Gases in the environment
- Summary

Objective

- After the end of this chapter, the student should be able to :
    - Explain the intrinsic and extrinsic factors that affect and favor microbial growth in food

Intrinsic And Extrinsic Factors Of Food That Affect Microbial Growth

Requirements for growth of microorganisms

- Nutrients
  - Water
  - Carbon, Nitrogen
  - Organic growth factors
  - Minerals
- Favorable environment
  - Temperature
  - PH
  - Gas mixture
  - No inhibitors
- Time

What parameters of foods affect microbial growth?

- Intrinsic parameters
    - pH
    - moisture content
    - oxidation-reduction potential
    - nutrient content
    - antimicrobial constituents
    - biological structures
- Extrinsic parameters
    - temperature of storage
    - relative humidity
    - gases in the environment

I. The intrinsic factors

- These are inherent parts of the plant or animal tissues. It includes:
- PH:
  - Measure of hydrogen ion concentration ($-\log[H^+]$)
  - Most organisms grow best around pH 7
  - Bacteria have narrowest pH range, then yeasts, then molds
  - Pathogens tend to have narrowest pH range
- Organic acids are more inhibitory than inorganic acids
  - Why? --> they can penetrate cells more easily

- Foods usually in acid --> neutral pH range:
  - fruits -->vegetables-->meats, milk
- Buffer capacity reflects resistance to pH change, rate of pH change
- Effects of acids on organisms
  - energy required to maintain cell's internal pH
  - enzyme activity affected
  - proteins, DNA, other molecules denatured
- Longer lag, less rapid growth
- Most microorganism grow best at PH of 6.6-7.5, few grow at PH of 4.0.

Table 2.1. Depicts the PH ranges of groups of microbes that grow in food, beverages.

Microorganisms	Lower PH range	Higher Ph Range
Molds	0.0	11.0
Yeasts	1.5	8.6
Lactic acid bacteria	3.2	10.5
Staphylococcus aureus	4.0	9.8
Acetobacter spp	4.0	9.2
Salmonella spp	4.1	8.9
Escherichia coli	4.4	9.0
Yersina enterocolitica	4.6	9.0
Clostridium botulinium	4.8	8.4
Bacillus cereus	4.7	9.3
Clostridium perferegens	5.5	8.8
Campylobacter spp	5.8	9.0
Vibiro spp	6.6	9.0

- Bacteria tend to be more fastidious in their relationship with PH than molds and yeasts.

- The pathogenic bacteria being the most fastidious one.

- The PH range described above should not be taken as an exact boundary for microbial growth, by itself alone, because the actual values also depends also on other growth parameters of the microorganism .

- In the presence of 0.2M Sodium chloride , Alcalegiens feacalis has been shown to grow over a wide PH range than in the absence of sodium chloride or 0.2 M sodium citrates.

- Some foods, fruits, soft drinks, vinegar and wines fall below the point at which bacteria normally grow.
- Fruits generally undergoes mold and yeast spoilage and this is due to the capacity of these organisms to grow at PH values less than 3.5 which is considerably below the minimum PH for most food spoilage and all food poisoning bacteria.

- Most meats and sea foods have a final PH of about 5.6 and above, which make these products susceptible to bacteria, mold and yeast spoilage.
- Most vegetables on the other hand have a higher PH values than fruits and consequently vegetables should be subjected to more bacterial spoilages than fungal spoilages.

- With respect to the keeping quality of meats, meat from fatigued animals spoils faster than from rested animals, and that is a direct consequence of final PH attained up on completion of rigor merits.

- Up on the death of a well rested meat animals, the usual 1% glycogen is converted in to lactic acids which directly causes a depression in PH values from about 7.4 to 5.6, this could vary from animals to animals. The PH range values of beef after rigor mortis is thought to be 5.1-6.2.

Table 2.2. Showed the approximate PH values of diary meat, poultry and fish products.

Products	PH	Products	PH
Diary product			
Butter	6.1-6.4	Butter milk	4.5
Milk	6.3-6.5	Cream	6.5
Cheese	4.9-5.9	Meat and poultry	
Beef ground	5.1-6.2	Ham	5.9-6.2
Chicken	6.2-6.4		
	Fish add	**Shellfish**	
Most fish	6.6-6.8	Clams	6.5
Crabs	7.0	Oysters	4.8-6.3
Tuna fish	5.2-6.1	Shrimp	6.8-7.0
Salmon	6.1-6.3	White fish	5.5

- Regarding to fish, which attains a PH of 5.6 has better keeping qualities than fish with PH of 6.2.-6.6.
- Some foods are characterized by inherent acidity others obtain their acidity due to the action of certain microorganisms which is called biological acidity.
- This type of acidity is observed in fermented milk and other foods.

- The natural or inherent acidity of foods may be thought of as nature's ways of protecting the respective plants or animals tissues from destruction by microorganism.

- It is of interest that fruits should have PH values below those required by many spoilage microorganisms.

- The biological function of the fruits is the protection of the plant's reproduction body, the *seed.*

- The PH of the living animal favors the growth of most spoilage organisms, other intrinsic parameters come in to play to permit the survival and growth of organism in the animal.

- The source of acidity does not have effect on the keeping quality of the foods.

- Foods that able to resist change in PH than others are called buffered foods.
    - E.g Meat is highly buffered than vegetables due to low protein content of vegetables that lack buffering capacity to resist changes in PH by the growth of microorganism.

What are the effects of PH on microbial cells?

- Have effect on the enzymatic function
- Effect on the transport of nutrients in to the cells

- The amino acid decarboxylase that have optimum PH of 4.0 and almost no activity at PH of 5.5, that causes a spontaneous adjustment of PH towards neutrality when cells are grown in acidic range.

- Bacteria such as Clostridium acetobutylicum raises substrates PH by reducing butyric acid to buthanol.

What are the effects of PH on microbial cells?

- Enterobacter aerogens produces acetoin from pyrovic acid to raise the PH of it’s growth environment.

- When amino acids are decarboxylated , the increase in PH occurs from the resulting amines.

- When grown in alkaline range, a group of amino acids deaminases , that have optimum activity at about PH 8.0, causes the spontaneous adjustment of ph towards neutrality as a result of the organic acid that accumulate.

- With respect to the transport of nutrients, the bacterial cells tend to have a residual negative charge.
- Nonionic compound therefore can enter cells , but not ionized one.
- At neutral or alkaline PH organic acids do not enter , while at acid PH values, these compounds are nonionised and can enter the negatively charged cells.
- If ionic characteristic is affected it result in denaturation of membranes and transport enzymes.

- The morphology of some microorganisms could be changed by change in PH
  - Eg. The length of Hypahe of Penicilum chrysogenum could be decreased if the PH values are above 6.0. Hydrogen ion may favor fermentation process while Potassium ion could suppress.

- The metabolism of glucose in yeast cells in acidic medium markedly stimulated by high concentration of potassium ion.

- With respect to temperature PH of substrate becomes more acids as temperature increases.

- Concentration of salts has a definite effect on PH growth rate curves, were the addition of 0.2 M Sodium chloride broadened the PH growth range of Alcalegens.

- An adverse PH make cells more sensitive to toxic agents and young cells are more susceptible than older cells; the lag phase could be increased.

b. Moisture content

- Desiccations or drying are one of the oldest means of preserving foods.
- It is due to a direct consequence of removal of moisture, without which microorganism can not grow.
- The water activity of the microorganisms is defined by the water activity in the environment (aw).
- It is defined by the ratio of water vapor pressure of food substrate to the vapor pressure of pure water at the same temperature.

- Aw = P/Po

- P- Vapor pressure of solution, Po – vapor pressure of solvent usually water.

- This idea is related to relative humidity (RH).

- RH= 100X Aw

- Pure water has an aw of 1.00; 22% NaCl solution has an aw of 0.86 and saturated solution of sodium chloride has an aw of 0.75.

- The aw of most fresh foods is above 0.99.
- In general bacteria require higher values of aw for growth than fungi.
- Gram-negative bacteria has higher requirements than gram positive.

- Most spoilage bacteria do not grow below aw of 0.91 while spoilage molds can grow at aw as low as 0.8.

- Food poisoning bacteria can grow as low as aw of 0.86 Staphylococcus aureus).

- While Closterdium botulinum does not grow at aw below 0.94.

Effect Of Low Aw On Microbial Growth In Foods

- The general effect of lowering aw below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population.
- This may result in lowering of water that required for metabolic activities.
- Most chemical reaction needs an aqueous environment.

Water activity (aw)

- Measure of water available to the organism
- Defined as p/p0 where p = vapor pressure
- Relative humidity (RH) = 100aw
- Aw of fresh foods usually > 0.99

- Bacteria most restricted, then yeasts, then molds

- Spoilage organisms require aw> 0.91; pathogens >0.95

- See synergistic effect of stress conditions

- Various solutes tie up water
  - degree of inhibition: salt > sugar > glycerol

- Organisms tolerant of low aw:
  - halophilic bacteria osmophilic yeasts
  - xerophilic molds
- Response to low aw
  - organism concentrates osmoregulators
  - salts sugars
  - amino acids polymers
- Effects of low aw
  - longer lag, slower growth
  - impaired transport
  - loss of membrane fluidity

Oxidations/reduction potential (Eh)

- Oxidation = loss of electrons; reduction = gain of electrons
- O/R potential = ease with which electrons are lost or gained
- Potential also reflects availability of free oxygen
  - Low potential = anaerobic; high potential = aerobic
- Organisms classified as:
  - Aerobic ;Facultative
  - Anaerobic; Microaerophilic

- Ranges for microorganisms:
  - Molds are usually aerobic
  - Yeasts usually aerobic or facultative
  - Bacteria from strict anaerobes to strict aerobes
- O/R potential determined by:
  - Characteristic O/R of food
  - Resistance to change (poising capacity)
  - Oxygen tension and access of atmosphere
  - Reducing agents in foods: -SH groups, ascorbic acid, sugars (low pH --> high Eh)

Nutrient Content

- Required by all organisms: water, carbon, nitrogen, minerals
- Organic growth factors needed to varying degrees:
  - Gram + > Gram - > yeasts > molds
- How easily are energy sources metabolized?
  - sugar > alcohol > amino acids > complex molecules
- How easily are nitrogen sources metabolized?
  - Amino acids > Proteins
- B vitamins required by many bacteria

Antimicrobial constituents

- Protective mechanism for living plant/animal?
- Numerous examples
  - Spices: essential oils (eugenol in cloves)
  - Milk: lactoferrin, conglutinin, lactoperoxidase
  - Eggs: lysozyme
  - Fruits: organic acids, hydrocinnamic acid derivatives

Biological structures

- protective structures (may be primary defense)
- skin of fruits (tough protection for seeds)
- shells of eggs, nuts

II. Extrinsic parameters

Temperature

- Growth rate vs. Temperature:
  - Rate is highest at optimum temperature
  - Growth slows but may not stop at freezing point
  - Rate falls sharply at temperatures above optimum

- Survival vs. Temperature:
  - Assume organisms survive freezing
  - Organisms killed by exposure to high temperatures
  - Temperature range for best growth: 15 - 450 C

- Groupings based on preferred temperature range:
  - Psychrophiles vs. Psychrotrophs
  - Mesophiles
  - Thermopiles vs. Thermoduric
- Storage temperature is key factor in stability of perishable foods

Relative humidity (RH)

- Should maintain internal aw without adding or losing moisture
- Temperature usually inversely proportional to RH
- Retard surface spoilage by low RH

Gases in the environment

- Controlled or modified atmosphere storage: increased CO2

- Retard fungal rot in fruits
  - Decrease pH of meats as carbonic acid forms

- Gas mixtures studied to extend shelf life of meats
  - Floral shift from Gram - to Gram + (more resistant)
  - Shift from aerobes to facultative or anaerobic species
  - Will competition among organisms be changed?
  - Will pathogens survive where spoilage organisms don't?

Some general principles to keep in mind

Growth rate
Bacteria > yeasts >mold

Optimum to stress condition
Bacteria > yeasts >mold

-Pathogens – tolerate stress poorly

Summary

- Organisms require nutrient , favorable environment and time to grow

- Two factors which affect and favor microbial growth in food

  - Intrinsic factors
    - pH
    - moisture content
    - oxidation-reduction potential
    - nutrient content
    - antimicrobial constituents
    - biological structures

  - Extrinsic factors
    - temperature of storage
    - relative humidity
    - gases in the environment

Chapter Three : Incidence and Types of Microorganisms in Food

Outline of Presentation

- Objective
- Incidence of microorganisms in foods
- Types of microorganisms in different food items
  - Meat and meat products
  - Poultry
  - Sea foods
  - Vegetables
  - Dairy products
  - Others
- Summary

Objective

- After completion of this chapter ,students will be able to :
  - Recognize the incidence of microorganisms in foods
  - Describe type of microorganisms in different food

Incidence and Types of Microorganisms in Food

- The number and types of microorganisms present in a finished food products are influenced by:
  - The general environment from which the food originally obtained
  - The microbiological quality of the food in it’s raw or unprocessed states
  - The sanitary conditions during handling and processing
  - The adequacy of subsequent packaging, handling and storage conditions in maintaining the flora at low level

- Ideally fresh foods are sterile and we can prepare many foods free from microbes , but practically it is difficult in mass production.

- The number of microorganism in a fresh food products may reflects the overall conditions of raw product quality, processing, handling storage and so forth.

- In many conditions due to a number of variables, it is difficult to know the minimum microorganism that is attained in good production conditions.

Types of microorganisms in different food items Fresh Red Meats

Table 3.1 The relative percentage of organism in red meats that meet the specified numbers.

Products	No. of samples	Microorganism	Target %
Raw	735	APC Log 6 or less	76%
Beef	735	Coliforms Log2 or <	84%
Patties	735	E.coli log 2 or <	92
		S.aureus log 2/<	85
		Presence of salmonel	0.4
Fresh	1830	APC 6.7 or less	79%
Ground	1830	S.aureus	3 or less
Beef	" "	Salmonella	2
Frozen	604	APC log 6 or less	67
Ground beef	"	E.coli <2.7	85
Patties	604	" " >3	9
Fried	107	APC <3 Log or less	78
		Absence of Enteroco	
		Coli forms, S.aureus	100%
Comminuted	113	Coli forms 20 or less	42
Pork	67	E.coli 2 or less	88
Fresh break fast saus	183	APC $<10^7$	91

- Communited meats have higher number of microorganism than non communited meats due to
    - Commercial ground meats generally consists of triminings from various cuts, consequently contains more microorganism.
    - Ground meats provides increased surface area
    - The commercial establishments are hygienically poor that increases microbial load.

- Bacilli and Clostridia are found in meats of all kinds, spores in fresh and cooked pork trimngs and canned pork (low number).

- The Significance of spores in meats is due to the problems encountered in the heat destruction of these forms in the canning industry.

- *Erysileothrix rhusiopathiae* was isolated from about 34 % of retail pork samples in Japan and 4-54 % of pork lions in Sweden.

- The incidence of C. perfrengens in many American foods showed recovery rate of
    - 16.4% of raw meats , poultry, and fish tested
    - 5 % of spices ,
    - 3.8 % of fruits and vegetables,
    - 2.7% of commercially prepared frozen foods,
    - 1.8%for home prepared foods
- Member of Enterobacteriacce have been found in common fresh and frozen beef, pork and other foods.

- The following Enterobacteriacce are also identified:
    - E.coli biotype-I
    - Pantoea agglomerrans
    - Serratia liquefaciens
    - Citrobacter frundii
    - Klebsella pneumonia
    - E. hafniae
    - Enterobacter clovae

Soy –Extended ground meats

- The additions of soy proteins at level of 10-30 % to grow meats patties is fairly widespread in the fast food industry.

- When ground beef or chicken was extended with 10 or 30 % soy, APC of these products increased over unextended control when it is stored at 4 ^{0}cfor 8-10 days

Ground meat cont'd…

- Coliforms was also high
- APC was high at30 % soy level than 10%.
- The mean time to spoilage at 4 degree centigrade for the beef soy blend was 5.3 days compared with 7.5 days for the unextended ground days.
- 10%, 20%, and 30 % soy APC increased significantly with both time and concentration of soy in the blend.

Mechanically Deboned Meat/MDM/, poultry and fish

- During deboning process small quantities of bone powder become part of the finished products.

- According to the USDA regulation ,
  - Calcium content should not be > 0.75 %
  - Protein of minimum of 14 %
  - Fat should not be more than 30 % .

- Coli form usually Salmonella, C.preferenges)counts of the commercial MDM produces ranged from 460 to more than 1100/gm .

MDM cont'd…

- APC of hand boned lamb breast was found to be 680, 000 compared with MDM which has 650, 000 /gm that aged for one week (prolonged).

- Commercial samples of mechanically deboned fish contain ten fold higher numbers of organisms than conventionally processed fish.

Hot Boned Meats

- In conventional processing of meats (cold boning) carcasses are chilled after slauther for 24 hours and processed in the chilled states (post rigor).

- Hot boning (hot processing) involves the processing of meats generally with in 1-2 hours after slauthers (prerigor) while the carcasses is still hot.

- Generally speaking the microbiology of hot boned meats is comparable with that of cold boned meats but some differences may exist.

- Ham made from hot boned meat have a high APC at 37 ^{o}c than the cold boned hams. (67 % of staphylococci versus 47 % respectively)

- Mesophiles counted at 35 ^{o}c were significantly is higher in hot boned than cold boned cuts.

- Hot and cold boned beef
  - Initially contain low bacterial count

  - After 14 days of storage period , the hot boned meats contain higher numbre of counts than cold boned meats.

- Sausage made from hot boned pork significantly higher counts of mesophiles and lipolytics than cold bonded products but not psychrophiles.

- A numerical taxonomy study of the flora from hot and cold bonded beef both at the time of processing and after 14 days of vacuum storage at 2 ^{o}c reveled no statically significant differences in the flora.

- More Staphylococci and bacilli were observed before processing but high Streptococci and Lactobacilli seen after storage conditions

- Restructured lamb roast made from 10 % and 30 % MDM and hot boned meats result in good quality in terms of microbial load (uncooled).
- 30 % of MDM had generally more number of coliforms and fecal coliforms.
- This is due to: contamination of shanks and pelvic regions during slauthering and evisceration.
- Cooking reduced cell counts in all products to less than 30 per gram.

- Hot boning is often accompanied by prerigor pressurization, which consists of application of around 15000 psi for two minutes.

- This process improves muscles color and overall shelf appearance and increase tenderization.

- It appears not to have any effect on the microbial flora.

- Electrical stimulation of carcasses may result in toughing and tenderization; some suggest that it could reduce APC.

Organ and Variety Meats

- These include: Liver, Spleen, and Tongue
- Have higher PH and glycogen content (6.1-6.5)
- Have less number of microorganisms
- Mainly:
  - Gram positive cocci,
  - Cornyforms,
  - Aerobic spore formers,
  - Morexsella,
  - Accetinobacter and Pseudomonas species

- Vacuum packaged beef and pork livers and kidneys had lower counts after seven days (7-14 days) storage at 2 ^{o}c or 7 days at 5 degree centigrade than stored in air or PVC film.

Vacuum Packaged Meats

- Placing meats in plastics or pouches followed by removal of air using vacuum machines.
- It has longer shelf life
- Microbial quality is good
- Oxygen is removed largely and the remaining could be utilized by aerobic bacteria resulting in an increase in cabondioxde that inhibit the flora.

- Refrigerated fresh meats $\xrightarrow{\text{Oxygen permeable packaging}}$ Gram negative spoilage $\rightarrow$ PH and foul odors

- Pseudomonas species are the commonest bacterial agents and their growth is directly proportional to oxygen concentration .

- Storage life of vacuum packaged beef is inversely related to film permeability.

- It is estimated that 15 weeks at zero oxygen film, 2-4 weeks with highly permeable film (920 cc O 2/m2/24 hrs at 25 degree centigrade and RH of 100%).

- Growth of lactic acid bacteria is favorable because of increase in carbon dioxide and low Eh (O-R) which result in low PH that create unfavorable environment for most food born pathogen and gram negative bacteria.

- Microbial load of vacuum packaged cooked meats - a decrease and / absence of the microbial agents.

- When nitrites are present in vacuum packaged meats, lactic acid bacteria could dominate

- B. thermosphata is sensitive to nitrites, so they are absent in vacuum treated with nitrites.

Sausage, Bacon, Bologna and Related Meat products

- Many spices and condiments have high microbial counts.

- The lactic acid bacteria and yeasts in some composition products are usually contributed by milk solids.

- Incase of pork sausage natural , casings have been shown to contain high number of bacteria.

Related meat cont'd…

- It may range as:
  - Log 4.48-7.77 in salt packaged casings
  - Log 5.26-7.36 in case of wet packaged casings
- Many of the bacteria is Bacillus species followed by Clostridia and Pseudomonas species .

- Processed meats such as bologna and salami (liver sausage) may be expected to reflect the sum of ingredient make up with regard to microbial number and types.

- The microflora of frankfurters mainly consists of :

    - Gram positive organism (micrococci, bacilli, lactobacilli mycobacterium, Streptococci and some yeasts).

- The flora of vacuum packaged sliced bacon consists of largely of
    - Catalse positive cocci such as micrococci,
    - Coagulase negative Staphylococci,
    - Catalase negative bacteria(lactobacillus Leukonostocs and group D streptococci)

- Soul food consists of high number of organism (close contact with the intestinal tract flora and other parts of the animal) APC of 7.9 or more for most soul food products.

Poultry

- Whole poultry tend to have lower microbial count than cut up poultry.
- Most of the organisms on such products are found on the surface so surface counts per centimeter square are more valid than counts on the surface and deep tissues.
- Surface count of bacteria increased significantly from log3.3/ cm^2 to 4.08/cm^2 before and after cutting.
- Poultry represents an important food source of salmonella to humans.

Poultry cont'd…

- Out of 50 frozen comminuted turkey meat samples examined 38 % yielded Salmonella species.
- Unprocessed and processed turkey carcasses yield Salmonella species of 11.5% & 26.8 % respectively
- Microbial flora of fresh poultry consists of
  - Largely pseudomonas and other gram negative bacteria,
  - Corynforms and yeasts.

Seafood

- The incidence of microbes on seafood depends greatly on the quality of water from which these animals are harvested.

- Assuming good quality of water, most of the organism are picked up during processing.

Seafood cont'd…

- Microbiological contamination of most hand cooked fillets –
  - During handling prior to packaging
  - Notified by count of log 5.6 to the morning to log 5.9 at night in one processing.
- Frozen seafood and other frozen products have generally lower microbial counts than the comparable fresh products.

Seafood cont’d…

- APC of geometric mean for 240 frozen samples and 357 fresh products was log 3.54-4.9 & 4.8-8.43 respectively.

- Plate counts are generally higher in seafood when it is incubated at 30 ^{0}c than 35 ^{0}c (log 5.5& 5.9 for 35 and 30 ^{0}c respectively).

Vegetables

- Incidence of microorganism in vegetables is determined by :
  - Sanitary quality of the processing
  - Microbiological condition of the raw products during processing
- 40-75 % of the microbial flora of peas, snap beans and corn was consists of mainly streptococci and lactic acid cocci and some Cornybacteria species .

Vegetable cont'd…

- After processing the Staphylococci may dominate due to hand contamination.
- Frozen vacuum pouch pack vegetables consists of type A and B Clostridium botulinium spores.
- The spore formers
  - Can survive pasteurization temperature
  - may be changed to vegetative forms at Refrigerator temperature
  - It has also photolytic proprieties.

Diary Products

- The microbial load of raw milk - the cow's udder and hide and milking utensils are the source of microorganism

- Under proper handling of milk, the dominating bacteria is gram positive.

- Yeasts, molds and gram negative bacteria may be found along with lactic acid bacteria, (heat sensitive - killed by pasteurization process).

Dairy cont'd…

- In raw milk :
  - Psycotropics spore formers
  - Mycobacteria could be present
  - Bacilli and Clostridium are also common.
- 69 % of raw milk from 51 samples showed Mycobacteria and Nocardia and soil is reach with these microorganism.

- However, Pseudomonas is common in soil, but not found to be milk contaminants.
- Commercial cream cheese may have log 2.69 flora mainly lactic acid bacteria.
- Outbreaks of diseases was observed due to
    - Consumption of raw milk,
    - Certified raw milk,
    - Home made ice cream containing fresh eggs,
    - Dried and pasteurized milk.

Delicatessen and related Foods

- Foods such as salads sandwiches and other related food are known as Delicatessen.
- These foods could be involved in food poisoning outbreaks.
- Mostly these foods could be contaminated during processing by hand.
- Staphylococci aureus is the dominant organism found in these foods.

Delicatessen cont'd…

- The prior microbial load is reduced during heating but later *S.aureus* can dominate later, possibly after contamination.
- Coliforms, yeasts and molds could also involved . The APC should be smaller than log 5 per gram of these foods.
- Geometric mean APC of 1187 samples of refrigerated biscuits dough shown 34000 per gram of organism mainly coliforms, Staphylococci aureus, E.coli and some Fungi.

Frozen meats Pies

- Meats products that are incorporated with ingredients, this may increase the total number of microorganism.
- The total number of microorganism reflects the overall quality of ingredients, handling and storage conditions.
- The total counts should not be more than log 5 per gram of these foods.

Dehydrated foods

- These foods usually have microbial load of < log 5 /gram of foods.
- Checkennoode, chicken rice, beef noode, vegetables, mushrooms, pea, onion, tomato, Gelatin and etc.
- Mainly facilitative anaerobes could be present
- Powdered milk and eggs have increase number of organism
- Fruit juice concentrates has also high counts compared with fresh non concentrated foods.

Enteral Nutrients Solutions (Enteral foods)

- These are foods solution or liquid that is administered by tube
- Available as powdered products that need reconstitutions.
- It is administered mainly for weak patients and some times for routine uses.
- Administration is by continuous drip from eternal feeding bags.

Enteral foods cont'd…

- It may consist of complete diets (proteins, peptides, CHO etc.)
- It may have 10^{8} microorganisms per ml of these foods, at time of infusion, in hospitals,
- $9x\ 10^{3}$ per ml -$7x\ 10^{4}$ microorganisms /ml in commercial ENS solutions.
- Staphylococci epedermidis, Cornybacterium, Pseudomonas, Citrobacter and Acinetobacter species are common.
- Addition of Potassium sorbets may reduce microbial growth significantly in ENS foods.

Summary

Table 3.2 Types of Microorganism in Different Food Items

Type of Microorganism	**Food items**
Bacilli and clostridia	Meat of all kinds
Coliforms	Soy- extended ground meat
Staphylococci and bacilli	Before processing of hot boned meat
Streptococci and lactobacilli	After storage of hot boned meat
Gram positive cocci, *Cornyforms,* *Aerobic sporformers,* *Morexsella,* *Accetinobacter and Pseudomonas species.*	Organ and variety foods
Pseudomonas	Vacuum packed meat
Salmonella	Poultry
Streptococci , Lactic acid cocci, some *corynbacteria species*	Vegetables
Yeast, mold , gram negative bacteria along with lactic acid bacteria	Dairy products
Staphylococcus aureus	Delicatessen and related foods
Staphylococcus epidermids , cornybacteria , citrobacter and acinetobacter species	Enteral food

Chapter 4: Microbial Spoilage of Food

Outline of presentation

- Microbial Spoilage of Food
- Major groups of food products : their safety and quality
- Spoilage of fruits and vegetables
- Spoilage of Fresh Beef, Pork and Related Meats
- Spoilage of Poultry

Outline cont'd…

- Spoilage of Fish and Shell Fishes
- Spoilage Of Misllaneous Foods
- Spoilage of cereals, Flour and Dough products
- Spoilage of Diary Products

Objective

- After completion of this chapter , students will be able to :
  - Categorize major groups of food products
  - List the causes for food spoilage
  - Identify safety and quality of major food products

Objective cont'd…

- List the nutritional content of major food products
- Identify the favorable conditions for microbial growth in food
- List the different types of microorganism responsible for spoilage of different food products

Microbial Spoilage of Food

- Spoiled food - Foods that has been damaged or injured so as to make it undesirable for human consumption.

- Some causes of food spoilages:-
  - Insect damage
  - Physical damage / injury like bruising and freezing
  - Enzyme activity or microorganisms

Spoilage cont'd…

- Microbial spoilage of food is not deliberately planned to destroy foods but as the normal function of these organisms.
- 20 % of all fruits and vegetables harvested for human consumption are lost through microbial spoilage by one or more of 250 market diseases.
- Bacteria, Yeasts, and molds are the commonest agents of microbial spoilage, but not viruses or Mycoplasma.

Spoilage of fruits and vegetables

Spoilage of vegetables

- The average water content of vegetable is about 88 %.
- Carbohydrate =8.6% Fats= 0.3 %
- Proteins= 1.9% ASH = 0.84 %
- vitamins, Nucleic acid and other plant constituents < 1 %.

- Vegetables support the growth of molds, yeasts and bacteria and be spoiled by these agents.

Vegetable spoilage cont,d…

- Growth of spoilage microorganism is favored by:
  - Higher water content in vegetables
  - Low carbohydrates and fats,
  - Wide PH range
- The high oxidation reduction potential (O-R) and lack of high poising capacity favor aerobic and facultative types of microorganisms than anaerobic microorganisms.

Vegetable spoilage cont'd…

- Erwinina are associated with plants and vegetables in the natural growth environment.

- The common spoilage pattern displayed by these organism is called bacterial soft rot.

- The following microbes could be agents of spoilage:-

Bacterial agents

- Bacterial soft rots:
  - Commonest causative agents
    - Erwinina carotorra
    - Pseudomonas marginalis ,
  - Secondary bacterial agents.
    - Bacillus species
    - Clostridium species

Spoilage cont'd…

Mechanisms of spoilage

Breakdown of pectin → soft, mushy consistency → Bad odour - water soaked appearance

The commonest vegetables affected:

- Onions , Garlic, Asparagus, Beans
- Carrots, Beats, Lettuce, Spinach
- Potatoes, Cabbages, Cucumbers, Peppers, water melons

Spoilage cont'd…

- Because of early and relatively rapid growth of theses bacteria , molds are poorly spoiled???.
- The outer plant barrier has been destroyed by pectinase producers; non pectinase producers enter the plant tissues and ferment simple carbohydrate .
- The nutrients (nitrogen compounds, vitamins, minerals) are adequate to sustain the growth of the invading organism until the vegetables are destroyed or consumed.
- The maladors produced is probably due to the direct results of volatile compounds (NH_3**,** Volatile acids) produced by the microorganisms.

Spoilage cont'd…

Microorganisms $\longrightarrow$ Decarboxylate $\longrightarrow$ Amines $\longrightarrow$ $\uparrow$ PH
In acidic medium $\xrightarrow{\hspace{3em}}$ Amino acids

- Compounds not attacked until late spoilage by soil flora
  - Aromatic compounds
  - Poryphyrins

- Causes a rot of potatoes /" **black leg"**
  - E. castorova pratoseptica ,
  - E.castorova pr.castorom and E.chrysanthemi

Fungal Agents

- Spoilage conditions are initiated
  - Preharvest
  - Postharvets conditions.
- The largest number of market fruits and vegetables spoiled after harvesting (fungi invades bruised and damaged products).

Table 4.1 Fungal agents that affect vegetables

Fungal agent	Parts of vegetable affected	Damage resulted
Botrytis	flowers of Strawberries	gray mold rots
Colletotrichum	epidermis of banana	banana anthracnose.
Gloeosporium	lenticels of apples	lenticels rots

Gray Mold Rots

- Cause:
  - Botrytis Cinerea : this may produce gray mycelium that is formed by high temperature and high humidity.
- Affected vegetables:
  - Asparagus, Onions, Garlic, Beans, Carrots
  - parsnip, tomatoes, lettuces, cabbage, peppers etc.

Sour rot (oospora rot, Watery soft rot)

- Cause: Geothriucm candidum
    - Widely distributed in soil and on decaying fruits and vegetables
- Affected vegetables:
    - Onion, garlic, beans, (green beans, Limu, wax)
    - Carrots, lettuce, cabbages, cauliflowers,

Drosophila melaanogatster (fruit fly)

Decaying fruits ---- Fruit fly ----- Health fruits &
vegetables Spores & vegetables
mycelial

- **Rhizopus soft rot**
  - Cause: Rhizopus stolonifer:
  - This makes vegetables soft and mushy .
  - Cottony growth of the mold with small black dots of sporangia often covers the vegetables.

- **Affected plants:**
  - Beans, carrots, sweet potatoes, potatoes, cabbages
  - cauliflowers, Broccoli, pumpkins, water melons, tomatoes.
- Transmissions:
  - D. Melanogaster which lay eggs in the growth cracks on fruits and vegetables through wounds and skin breaks.

- **Phytophora rot**:
  - Occurs in a blight and fruits, rot of market vegetables.

  - Affected vegetables and plants:
    - Aspargus, onion, Garlic, Cantaloupes, water melons, Tomatoes, egg, plants, peppers.

Anthracnose:

- Characterized by spotting of leaves, fruits and seed pods.

- Cause:
  - Colletotrichum coccodes and other species.
    - Considered as a weak plant pathogens.

    - Live from seasons to season on plant debris in the soil and on the seed of various plants like tomatoes.

- Warm and wet weather is a contributing factor for transmission.

- Beans, cucumbers, water melons, pumpkins, Squash, tomatoes and pepers are mostly affected.

Spoilage Of Fruits

Fruits

- Average water content is 85 %
- Average carbohydrate content is about 13 %
- Has generally less water but more carbohydrates than vegetables.
- Mean content of protein, fats, and ashes is 0.9%; 0.5%; 0.5% respectively.
- Consists of vitamins and other organic compounds.
- Support the growth of yeasts and molds (their nutrient content)
- The PH of fruits is below the level that generally favors bacterial growth but molds and yeasts can grow and spoil them.

Spoilage of fruits cont'd…

- Except Erwinia rot, bacteria are of no known importance in the initiation of fruit spoilage.
- Many types of yeast are capable of attacking the sugars found in fruits and bringing about fermentation with the production of alcohol and carbon dioxide.
- Due to faster growth of yeasts than molds, they often proceed molds in spoilage process of fruits.
- High molecular weight compounds of fruits are thought to be destroyed by molds than yeasts, during which the sources are depleted the structural polysaccharides and rinds could be destroyed.

Spoilage of Fresh Beef, Pork and Related Meats

- Up on slaughter of a well rested beef animal the following series of events takes place
- It’s circulations ceases
- The ability to re synthesis ATP is lost
- Lack of ATP result in combination of actin and myosin to form actomyosine that lead to stiffing of muscles
- Oxygen supply falls, resulting in a reduction of the O-R potential.

Spoilage of fresh meat cont’d…

- The supply of vitamins and antioxidant is reduced that facilitate process of rancidity.

- The nervous and hormonal regulation may ceases, their by causing the temperature of the animal to fall , these also solidify fats

- Respiration may ceases and ATP synthesis is stopped.

Spoilage of fresh meat cont'd…

- Glycolysis ---- lactic acid accumulation --- depress PH (from 7.4 to 5.6)---- protein
  denaturation & cathepsins produced ---
  complete rigor mortis.

- The reticuloendothelial system ceases to scavenge that allow growth of microorganisms.

- Different metabolites may accumulate and protein could be denatured.

Spoilage of fresh meat cont'd…

- All theses events may require 24-36 hrs at usual temperature of holding slaughtered beef.
- The normal flora of these meats may come from:
  - Lymph nodes
  - Stick knife used for exsanguinations
  - Hide of the animals
  - Intestinal tract, dust, handlers
  - Cutting knives, storage beans and others.

Bacterial agents of meat spoilage

- Bacterial group that involve spoilage of the meats:
  - Clostridium perfregens
  - The Enterobacterace group
  - Pseudomonas
  - Alcalegens
  - Acetinobacter
  - Moraxella
  - Aeromonas

Fungal groups

- Among fungal agents that spoil meat
  - Thaminidium, Mucor, Rhizopus = Causes Whiskers in the meats
  - Cladosporium common causes of “black spot”
  - Penicilum species causes green patches
  - Sporotichum and Chrysosporium species produces “**white spots**”

Spoilage Of Vacuum Packaged Meats

- Vacuum packaged meats may undergo long-term refrigerator spoilage.
- The predominant organisms are *Lactobacilli*, *B. Thermosphacta*.
- Other organism may spoil depending on:-
  - Weather the product is raw or cooked
  - Concentration of nitrates present
  - Relative load of psychrothropic bacteria
  - Oxygen permeability of the packages
  - Product PH

Vacuum packaged cont'd…

- Cooked or partially cooked meats and dark cutting meats have higher PH than raw and light cutting meats.

- The dominant microorganisms in vacuum packaged meats held at 2 degree centigrade for six weeks, are;

    - Yersina enerocolitica, Serratia species, Shewanella and lactobacilli.

Vacuum packaged cont'd…

- Lactobacil and Enterobacteriace will dominated at 6th & 8th week respectively in vacuum packaged dark cutting meats stored at PH 6.6 between 0-2 degree centigrade.

- Use of high barrier oxygen film in vacuum packaged beef loin steaks after 12-24 hrs may consists of hetrofermentative Lactobacilli (Lactobacillus cellulobiose, Aeromonas, Enterobacter, Hafna, B.thermophatica and pseudomonas species

Vacuum packaged cont’d…

- Addition of nitrites generally inhibits B. thermophitica and Psychrotrophic Enterobactreace. The lactic acid bacteria may dominate as they are insensitive to nitrite.

- The presence off odors and off flavor in vacuum packaged meats, poultry and sea foods indicate the production of volatile compounds by the spoiling agents.

Vacuum packaged cont'd…

- Volatile compounds that are mainly generated by Lactobaccli, B. thermphatica, Pseudomonas and others are:
  - Acetoin, short chain fatty acids, isovaleric acids, isobutric acids, 2,3 butanediol , 3-methyibuthanol, 3-methylpropanol .
- Addition of glucose may prolong the shelf life of vacuum packaged meats.

Spoilage of Frankfurters, Bologna Sausage and Luncheon meats

- These meats are prepared from various ingredients which may contribute growth of microorganism.
- Bacteria and yeast are very common agents and some molds.
- Three forms of meats spoilage described:-
  - Slimy spoilage
  - Souring
  - Greening

1. Slimy spoilage

- Occurs outside the casings, that occurs discrete Colonies in early stages, which later coalesces to form uniform layers of gray slime.
- Yeats , Lactic acid bacteria, the Enterococcus , Bacilus thermophatca, produces both slimness and greening.
- Slime formation is favored by moist surface and confined to outer casing.
- Removal of these materials with hot water is effective (The product is unchanged).

2. Souring

- Found underneath the casing.
- Usually caused by Lactobacilli , Enterococci, and related bacteria.
- Milk solids are source of these agents.
- Caused by Fermentation of lactose and other sugars with production of acids.

Souring cont'd…

- Sausage usually contains more varied flora than other processed meats due to different seasoning agents employed.
- B.thermopatca is very common agent.
- When the product is moist and stored under high humidity bacteria and yeasts can spoil.
- Mold spoilage likely occurs only if the surface dry.

3.Greening

Two types of greening occur in stored and processed meats, one by hydrogen peroxide and other by hydrogen sulphide gas.

- H2O2 commonly found in frankfurters and vacuum packed meats and cured.
  - Generally occurs after anaerobically stored meats product is exposed to air.

Greening cont'd…

- Up on exposure to air H2O2 formed and reacts with nitrosohemochrome to produce greenish oxidized poryphyrin.

- Low oxidation reduction potential also contribute for it's formation.

- Lactobaciilus, Leuconostos, Enteroccous, can produce greening.

Greening cont'd…

- H_2O_2 producers such as Lactobaccilus species
    - Resistant to sodium nitrite as high as 200 PPM
    - Can grow at 2-4 % sodium chloride but not at 7 % sodium chloride.
- The green product is not harmful for consumption.

- The second types of greening occur in fresh red meats at 1-5 ^{o}c and stored in gas impermeable packs.
- It is accompanied by conversion of myoglobin to sulpmyoglobin.
- Psudomonas species are the commonest agents.
- H_2S producing Lactobacclius is also isolated from vacuum packaged Fresh beef and found to produce H2S at PH of 5.4-6.5.

- Oxygen transmission rate of 1 ml -300ml / m2 oxygen can prevent greening.
- Yellow discoloration of vacuum packed meat is usually caused by Enteroccous, Caseliflavous as small spots.
- Product stored 4.4 degree centigrade, this may flourish at UV source.
- The agents could be E. casseliflavus, E. mundi, that survive at 71 degree centigrade for 20 minutes but not for 30 minutes

Spoilage of beacon and cured hams

- Smoking and brining make less susceptible to bacterial spoilage.

- Moldiness is the commonest spoilage that is mainly by Aspergillus , Alternaria, Fusarium, Mucous, Rhizopus, Penicilium due to high fat concentration and low activity of water.

- However, Enterocouus , Lactobacclius, and Micrococous capable of spoilage of becons.

Spoilage of beacons cont'd…

- Microccous and Lactobacclius tend to sour vacuum packed becons.

- Staphylocoous can spoil vacuum packed becons if the salt concentration is low and the temperature of storage is more than 20^{o}c

- Bacillus, Pseudomonas, Lactobacillus Proteious, Micrmisouus and Clostridium can sour cured ham.

Spoilage of Poultry

- Slimness is common features in the outer surface of cuts and carcasses.
- The visceral cavity is often display sour odors called visceral taint.
- Pseudomonas, Acetinobacter, Flrobacteria, Cornybacteria and Yeast are the commonest agents.

Spoilage of poultry cont'd…

- Fungi are less important in poultry spoilage except antibiotics are employed to suppress bacterial growth. Mainly molds (Candida, Rhodotorula and Torula)

- Poultry spoilage is mainly restricted to the surface as the tissue is sterile or have less bacteria that can not grow at low temperature.

- High humidity may favor Pseudomonas infection.

Spoilage of Fish and Shell Fishes

- Salt water and fresh water fishes contain high proteins and nitrogenous constituents.
- The amount of carbohydrate is almost nil.
- The total nitrogen to protein ratio is about 0.7:0.87.
- The nitrogen compounds in fish are not only in the form of proteins but also non protein nitrogen compounds such as amino acids, volatile nitrogen (ammonia, creatine), taurine, Betahine, uric acid, Carnosine and histamine.

Spoilage of fish cont’d…

- The internal flesh of healthy live fish is sterile but the outer surface slime, gills, and intestine.
- Penicillin, Aurobasidium and Aspargillus are agents that spoil fish (molds).
- Among yeasts: Candida, Cryptocouus sppeices, Saccharomyces, Sproboromyces, Trichosporium.
- Acetobacter, Aeromonas, Bacillus, Enterobacter, Vibro, Shewanella Psudomonas and Esherchia are common bacterial agents.

Spoilage of fish cont'd…

- Fresh iced fish are invariably spoiled by bacteria next to fungal agents.
- The early symptoms organoleptic spoilage may be noted by examining the gills for the presence of off odurs.
- If feeding fish is not eviscerated soon, intestinal bacteria goes to cavity that is aided by protolytic enzymes.

Spoilage of fish cont'd…

Shell Fishes

a. Crustaceans:

- Shrimp, lobsters, crabs and Cray fishes.

- Spoilage could dependent on handling and chemical composition. (Has only 0.5 % carbohydrate unlike fishes)

b. Mollusks:

- Oysters, Clams, Squid and Scallops have high carbohydrate but low nitrogen.

- They have also high free arginine, Aspartic acids, and Glutahmic acids.

- Serratia, Psudomonas, Proteious, Closteridum Bcaillus, Escherichia, Enterbacter, Shewanella, Lactobacliuus are common agents.

- Due to high glycogen concentration, the spoilage of mollsucum shell fish is fermented.

- PH measurement is the best indicators of spoilages
  - PH: 6.2- 5.9 is good
  - PH: 5.8 is offodours
  - PH: 5.7-5.5. : Musty
  - PH: Less than or equal to 5.2: Sour or putrid.

Spoilage Of Misllaneous Foods

- Eggs: has three parts externally
  1. Waxy shell membrane
  2. The shell
  3. Inner shell membrane

- The inner part has lysozyme in white egg which is active against gram positive bacteria.

- Egg white has also avidin that will form a complex with biotin and make this vitamin unavailable for microorganism.

Misllaneous Foods cont'd…

- However, the yolk materials at PH 6.8 is suitable for growth of microorganism.
- Freshly laid eggs is sterile but latter can be infested by bacteria.
- Rotting is a condition were bacterial spoilage of eggs occurs.
- Green rot: Happened by Pseudomonas florescence
- Colorless rot: by other Pseudomonas and Aeromonas species.

Misllaneous Foods cont'd…

- Pink rots: by Pseudomonas species
- Red rots**;** by Serratia species
- Custard rots; by Proteoius vulgaris and
  Proteious intermedium
- **Pin spots** - mold spoilage of eggs,
  - caused by Penicilium, Cladosporium species)

Misllaneous Foods cont'd…

- Mustiness - caused by Pseudomonas species.
- Ovaflavoproteins and ovatrasferins are antimicrobial agents that is found in eggs.
- It is effective for both gram positive bacteria and Yeasts.

Spoilage of cereals, Flour and Dough products

- The soil, storage environment and processing unit may be the source of microorganisms for spoilage of these products.

- These types of foods may consist of high amount of carbohydrates and proteins but low activity of water that inhibit spoilage process.

- Bacillus and molds could grow if activity of water is altered in the flour.
- Spoilage of fresh refrigerated dough, butter , milk, biscuits, , dinner pizza dough is usually caused by mainly Lactic acid bacteria.
- The activity of water of bakery products is low so only molds,(Rhizopius species) grow, and the condition is called “Bread mold”.
- Home made bread spoilage is termed as ropiness that is caused by Bacillus subtils.

Spoilage of Diary Products

- Due to chemical compositions, diary products such as milk, butter, creams, cheese are susptible to microbial spoilage.
- Yeasts can grow in milk as it is an excellent medium for growth.
- Non –pasteurized milk contain bacteria depending on milking process cleaning and handling of milk stencils.
- Enterocouus, Lactocouus, Sterptocoous, Leukonostoks, Lactobacilli, Mycobacterium, Pseudomonas, Bacillus are the causative agents.
- Pasteurization process eliminates all but thermoduric bacteria (Sterptocous, Lactobacillus, spore formers Bacilli species may result in curdling and liquefaction of milk.

Butter spoilage could be:-

1. Surface taint or putridity that is caused by Pseudomonas putrificans (4-7 degree centigrade) for 7-10mdays exposures.

Isovaleric acid is responsible for the odor.

2**. Rancidity**: This is due to hydrolysis of butter fats and liberation of free fatty acids by Pseudomonas and fungi.

- Malty flavour: By Lactocoous lacti

Spoilage of Sugars, Candies and Spices

- These products are rarely affected by microorganism, if properly handled.
- However, under high humidity microorganisms may spoil them.
- Torulla, Sacaromyces are good spoiling agents. They may causes inversion of sugars (eg. Lukonostocs spp. sucrose to dextrans, a challenge to sugar factories).
- Candies may be spoiled with molds and explosion may be able to occur by Clostridium species.
- Propylene treated spices are less spoiled.
- Similarly, beers, wines could be spoiled by bacteria and yeasts.

- **Beers**:
  - Ropiness (oily stream) this may lead to sourness by acetobacter (Ethanol to acetic acids)

- ***Turbidity***:
  - Happened by Zymonas species, Yeasts, Pseudomonas species.

- **Wines:**
  - spoilage happened by Candida valida and called wine flowers

- ***Tourine diseases***:
    - Caused by facultative anaerobes.

- **Malolactic fermentation:**
    - Caused by degradation and fermentation of malic acids and tartaric acids to lactic acids and carbon dioxides.

Spoilage of Canned Foods

- Flippers, Spriters, Soft swell and hard swell is common characteristics of spoilage of canned foods.
- Theses could be due to microorganism or hydrogen that swells from food.
- Leakage types spoilage may result in leakage of content.

Summary

- Spoiled food - damaged/injured food
- Causes of food spoilage :-
  - Insect damage
  - Physical damage
  - Enzyme activity
- Bacteria ,yeast and molds – commonest agents of microbial spoilage , but not viruses and Mycoplasma
- Bacterial soft rot – common spoilage pattern by Erwinina
- Different fungal agents affect different parts of vegetables

Summary cont'd…

- Fruit spoilage is initiated by yeasts and molds ,but not bacteria except *Erwinina*
- Bacteria and fungi are responsible for spoilage of meat
- Three forms of meat spoilage :-
  - Sliming
  - Souring
  - Greening
- Fungi are less important in poultry spoilage
- Bacteria , yeasts and molds are responsible for fish spoilage

Chapter Five : Microbial Indicators of Food Safety and Quality

Outline of presentation

- Objective
- Microbial indicators of food safety and quality
- Organisms associated with product quality
- Microbial product as an indicator of food quality
- Indicator of food safety
- Limitation of coliforms as indictor of safety

Outline cont'd…

- Comparison of coliforms and entrococcus as indicator of sanitary quality
- Analysis of milk and water
- Spoilage of milk
- Milk standards
- Laboratory tests for milk analysis
- Microbiology of water
    - Method of microbial detection

Objective

- After completion of this chapter ,students should be able to :
  - Describe the criteria of Indicator *organism for food safety and quality*
  - *Identify the organisms & their product associated with food quality*
  - *Describe the criteria for indicator of food safety*
  - *Differentiate the intestinal origin organism to be used as safety indicators*
  - *Recognize the lowest possible number of coliforms to indicate quality food with their limitation*

Objective cont'd…

- Compare coliforms and enterococcus for indicator of food quality
- Recognize the nutritional content of mild and favorable condition for microbial growth.
- Classify milk based on bacterial load
- Describe microbiological analysis of milk and water .
- Perform different tests for milk and water analysis
- Comply with the standard operational procedure for milk and water analysis.

Microbial Indicators of Food Safety and Quality

- Indicator organism may be employed to reflect the microbiological quality of foods relative to product shelf life or their safety from food borne pathogens.

- ***Criteria to be an indicator organism.***
    - They have to be present in most foods and it has to be detected.
    - Their growth and number should have a direct negative correlation with quality of the product.
    - They should be easily detected and enumerated with proper identification.

Criteria cont'd…

- Should be enumerable in short period of time
- Their growth should not be affected by other product flora.

- The most reliable indicator organism is product specific.
- The following table showed organism and metabolic products that is useful as indicator of product quality.

Organisms associated with product quality

- ***Table 1. Organisms associated with product quality***

Organisms	**products**
Acetobacter	Fishes
Bacillus species	Bread dough
Closteridunium species	Hard cheeses
Lactic acid bacteria	Wines and Beers
Lactocoocus lacti	Raw Milk
Psudomonas species	Butter
Yeasts	Fruit juices

Microbial product as an indicator of food quality

- ***Table 2. Microbial product as an indicator of food quality***

Metabolites products	**Foods**
Cadaverine and Putriescine	vacuum packaged beef
Ethanol	Fishery products and apple juice
Diacetyl	Frozen juice
Histamine	Canned Tunna
Lactic acids	canned vegetables
Trimethylamine (TMA)	Fishes
Volatile fatty acids	Butter

Indicator of Food Safety

- Microbial indicators are more often used to asses food safety and sanitation than quality.

- A food safety indicators should have the following criteria:

    - Be easily and rapidly detected

    - Be easily distributed from other food flora

    - Have a history of constant association with the pathogen as indicator organism

- Always present when the pathogens of concern present
- Posses growth requirements
- Absent from foods that are free of the pathogens, except in minimum numbers

- Theses criteria may be applied on most food that may be vehicle or food borne pathogens

- In the historical use of safety indicators the pathogens of concern were assumed to be of intestinal origins that may result in direct or indirect fecal contamination .
- E.coli was used as the first indicator organism with the following additional criteria:-
  - The bacteria specifically found only in the intestine
  - Found in high number in faces
  - They should resist the external environment
  - Should be detected easily and even when they are found in small numbers

- Coliforms notably E.coil and E. aerogens are used as indicator organisms as they grow in the presence of bile salts unlike gram positive bacteria, i.e. selective isolation is possible from various sources , they ferment lactose with gas production that make presumptive determination.

- However, their identification is complicated by the presence of atypical strains.

Detection and Enumeration

- Various methods have been discussed previously (direct count, broth culture methods, membrane filter methods etc.)

Distribution of Coliforms

- The intestinal tract of, most warm blooded animals is the primary habitat of E.coli, while E aerogens is mainly found in vegetation and occasionally on the intestine.

- It is also suspected in air, dust hands, in many foods, but their number is insignificant from the point of Public health importance.

Coliform criteria and standards

- The presence of large number of coliforms and E.coli in food is undesirable , in addition it is impossible to eliminate from fresh and frozen foods.

- **The basic questions regarding Nos are:**
- What is the lowest possible No. in a properly harvested, collected , handled and stored foods/ water

- At what quantitative level do coliforms and E.coli indicate unsafe products?

- The following criteria have been used in water, diary products and other foods:
  - Not more than 10 ml of pasteurized milk, milk products
  - Not over 10 /ml of certified raw milk and not over 1/ml of certified pasteurized milk .
  - Not more than 10 / ml for precooked or partially cooked frozen foods.
  - Not more than 100 / ml for crab meats
  - Not more than 100 /ml for custard filled items.
- The international commission on the microbiological specification for foods have different coli forms criteria.

Limitation of Coliforms as indicator of safety

- Coliform tests for diary products are not intended to indicate fecal contamination but do reflect overall diary farm and plant sanitation.

- Some coliforms found in vegetation like Enterobacter-So no sanitary Significance in vegetation but showed process problems

- For Poultry products not good sanitary indicators as Salmonella species may exist.

- Enterococci may be used as Quality and Safety Indicators of water due to:
    - As theses species do not grow in water
    - Found in less numerous number in human faces
    - Die slower than E.coli
- Therefore some authors suggests the simultaneous use of E.coli and Enteroccous as indicator organism particularly in water.
- In frozen foods Enterocoous is used better indicator of food sanitary quality , but not in fresh foods.
- Bifdobacteria bifdium , used also as fecal pollution indicators of water.

Summary

- Microbial indicators are often used to assess food safety and sanitation than quality
- Previously intestinal originated bacteria was used as a safety indicator for food quality
- Criteria to be an indicator organism
    - Present in most foods and has to be detected.
    - Growth and number should have a direct negative correlation with product quality.
    - Easily detectable and enumerated in short period of time.
    - Growth should not be affected by other flora.

Summary cont'd…

- The most reliable indicator organism is product specific.
- There are specific organisms and metabolic products which are used in indicator system.
- Salmonella is a good indicator of poultry production.
- Enteroococci is used as a quality and safty indicator for water and frozen foods.

Summary cont'd…

- Limitation of coliforms as indicator or safety
    - Coloforms tests reflect overall quality of farm and plant sanitation but not intended to indicate fecal contamination.
    - Coliform may not have sanitary significance in vegetation

Chapter Six : Food Born Diseases

Outline of Presentation

- Objective
- Food borne illnesses
- Bacteria involved in food borne illnesses
  - Clostridium
  - Bacillus cerus
  - Listeria monocytogens
  - Staphylococcus aureus

Outline cont'd…

  - Vibrio
  - Salmonella
  - Shigella
  - Escherichia
  - Yersenia enterocolitica
  - Plesiomonas shigeloids
  - Mycotoxins
- Summary

Objective

- After the end of this chapter , students will be able to :
  - Differentiate food intoxication and food infection.
  - Describe micro organisms responsible for food borne illnesses.
  - List and analyze the factors that affect microorganisms of food borne illnesses.
  - Identify the original sources of microorganisms and the type of food stuffs which are highly vulnerable for microorganism of food borne illnesses.

Food born illnesses

- Food borne illness is estimated to affect 10% of the population annually.
- Estimates have placed the annual cost of treatment and work hours lost at more than $3 billion.
- When we discuss food poisoning, we must distinguish between food intoxication and food infection.
- Food intoxication
- Food infection

???? Definition

- Among microbes mainly bacteria are involved in food borne illness.

Bacteria involved in food born illnesses *Clostridium botulinum*

- *Clostridium botulinum* food intoxications - Most debilitating and life-threatening food intoxication .
- Two other forms of botulism:
    - Wound infection
    - Infantile botulism (children < one year).
- Based on the serological characteristics of the neurotoxin - *C. botulinum* is divided into seven groups (A, B, C, D, E, F, and G)
- Type A,B,E are the concerns of food industry

Boutulinum cont'd…

- 200 known outbreaks of botulism was observed in the early 1900s, of which 10% was due to commercial products.

- The botulinum toxin is LETHAL in very small quantities;

  - One tablespoon of pure botulinum toxin potentially could cause the death of about $2x10^9$ individuals.

Boutulinum cont'd…

- Implicated foods by botulunim:
  - Home-canned string beans
  - Vegetables
  - Home-canned meats and fish
  - Foil-wrapped baked potatoes
  - Sautéed onions, garlic in oil
  - Fermented green peppers

Symptoms of Boutulinum

- Appear in 12 to 24 hours after a meal
- But, may vary from a few hours to several days.
- Initially, some vomiting and perhaps diarrhea, but no fever.
- Blurred or double vision,
- Difficulty in swallowing and speaking
- Loss of muscle coordination.
- Patient will be conscious to the very end
- Death is due to respiratory failure

Boutulinum cont'd…

- The only effective treatment is injections of polyvalent antiserum.
- In the past, the mortality rate was 50% or higher, but in recent times it has dropped to about 10%.
- *C. botulinum* is :
  - Motile
  - Gram positive,
  - Anaerobic,
  - Spore-forming rod.
  - Can be controlled through temperature, pH, aw, and salt .

Boutulinum cont'd…

- There were about 50 known botulism outbreaks due to acidified food with pH < 4.6(acid-tolerant microorganisms (yeast and molds) were detected.
- Nitrites are used in cured meats to prevent botulinum toxin development.
- Dairy products are not known to be involved in botulism outbreaks. (due to the competing lactic acid bacteria producing an acid environment as well as such compounds as nisin and pediocin)

Boutulinum cont'd…

- The distribution of *C. botulinum* spores differs from states to states depending on the soil and water types.

- The spores of Type A and B are more heat resistant (D10 at 225°F is about 5 min) than Type E spores (D10 at 176°F is about 5 min)

- The botulinum toxin is heat labile (20 min at 176°F or 5 min at 185°F).

Clostridium perfringens

- Is responsible for food intoxication
- Meats, stews, sauces, meat pies, and casseroles are involved.
- During cooking oxygen is driven off and while vegetative cells may be destroyed, the spores not only survive, but are stimulated to germinate.
- As the food passes though temperature ranges at which the bacterium can grow, during refrigeration and later during reheating, this bacterium can grow rapidly, reaching very high numbers ($1x10^6$ or more/G).

Clostridium perfringens cont'd …

- When ingested, the stomach acids may be neutralized by the food, allowing the vegetative cells to survive.
- As the food enters the small intestine, the cells begin to multiply and sporulate.
- The sporulating cells lyse, releasing an enterotoxin.

Clostridium perfringens cont'd …

- The incubation period is about **13 hours**, but it can be shorter or longer.
- Symptoms include diarrhea, abdominal cramps as well as headache and nausea.
- Some patients may develop a fever, pass bloody stools, vomit, or experience dizziness.
- Younger patients have milder symptoms than older patients.

Clostridium perfringens cont'd …

- *C. perfringens* is :
  - Gram positive, Anaerobic
  - Non-motile, spore-forming rod.
  - Found in soil, thus is easily introduced into food.
  - Growth range is between 60 and 126°F
  - A very short generation time (about 10 minutes) at higher temperature limits.
  - PH growth range is between 6 and 7 and aw between 0.95 and 0.99.

Bacillus cereus

- Causes two types of gastroenteritis, depending on the type of food involved.
- The types of gastroenteritis not describe ???
- This bacterium when found in:
  - Meats,
  - Vegetables
  - Sauces

B. Cereus cont'd…

- Symptoms include :
  - Stomach cramps
  - Profuse watery diarrhea
  - Sometimes nausea and vomiting.

- Recently food infections involving this bacterium have been reported.

- Such outbreaks were associated with a high number of *B. cereus* cells (> $1x10^5$/G), implying the food must have been temperature abused.

B. Cereus cont'd…

- This bacterium is :
  - Motile, Gram positive
  - Aerobic, spore-forming rod.
  - Found in most soils, thus it has easy access to food.
  - Can be controlled by keeping food colder than 50°F or warmer than 131°F.

Listeria monocytogenes

- *Listeria monocytogenes* has been known since the early 1900s to cause infections, but it was not until quite recently that it was involved in food borne infections.
- The first outbreak occurred in 1981 in Nova Scotia.
- The incriminated food was coleslaw.
- Cabbage used for the coleslaw came from a farm where a few sheep had died of listeriosis.

Listeria monocytogenes cont'd…

- There were 34 cases in this outbreak, all pregnant women.
- These cases ended in abortion, stillbirth, or serious infection of the newborn.
- The second outbreak implicated pasteurized milk in Massachusetts in 1983.
- There were 49 individuals involved with compromised immune system, of whom seven were pregnant women.

Listeria monocytogenes cont'd…

- The third outbreak took place in California in 1986 and involved 86 individuals, half of whom were pregnant women.
- The implicated product was Mexican-type soft cheese.
- In all three cases, serotype 4b was involved.
- Persons with suppressed immune systems, including pregnant women, are susceptible to the infection.

Listeria monocytogenes cont'd…

- Healthy individuals only experience flu-like symptoms, severity depending on the individual's resistance and the number of microorganisms involved.

- *L. monocytogenes* is widely distributed in nature.

- It has been found associated with cattle, poultry, and birds.

- As many as 25% of seagulls are suspected of excreting this bacterium.

Listeria monocytogenes cont'd…

- This bacterium will persist in soil and in water, but the greatest danger is that it will grow at temperatures as low as 32°F.

- In some foods at 43°F, it has been found to double in number every 24 hours, while at 32°F, it may double every seven days.

- This bacterium is heat sensitive and readily destroyed by pasteurization, but because it is so widely distributed in nature,
- It is frequently reintroduced into cooked foods.

Staphylococcus aureus

- Causes food intoxication.
- This bacterium produces 6 serologically different enterotoxins (A, B, C, D, E, and F), which are heat resistant.
- The types of foods involved. :
  - Meats, especially cured meats,
  - Barbecued chicken,
  - Various salads, and bakery products with custard or cream fillings

Staphylococcus aureus cont'd…

- Symptoms usually appear in two to five hours after a meal, but they may appear sooner.
- The patient will experience nausea, vomiting, abdominal cramps, and diarrhea.
- The illness is self-limiting within a day or two and not fatal, unless complications arise.
- Severity of the symptoms depends on the amount of toxin ingested and the individual's resistance.

Staphylococcus aureus cont'd…

- *S. aureus* is a Gram positive, facultative coccus, forming a cluster of cells on a microscopic slide.
- Growth and toxin production can be controlled with proper refrigeration (<50°F), hydrogen ion concentration (pH <4.6), or water activity (aw <0.85).
- The source of this bacterium is human carriers and minor infections, such as pimples, and boils.
- Carriers, who can represent 25% or more of the population, will have this bacterium in the nasopharynx.

Vibrio cholerae

- Causes very severe diarrhea (watery stool), resulting in dehydration, loss of electrolytes, shock, and death within days.
- Symptoms appear within one to two days.
- Treatment requires antibiotics and the replacement of lost fluid and electrolytes.

Vibrio cholerae cont'd…

- The organism is transmitted by polluted water and can be carried by vegetables and other foods that are not cooked.

- Sporadic cases can occur from people eating inadequately cooked crabs or raw oysters.

- This bacterium is a Gram negative, comma-shaped, motile (single polar flagellum), aerobic rod.

Vibrio Parahaemolyticus

- Does not cause nearly as severe an illness as *V. cholerae*;
- Cause - diarrhea, abdominal cramps, nausea, vomiting, and to lesser extent headache, chills, fever, and bloody stool.
- Annually there appear to be two or three outbreaks.
- Foods involved include crabmeat, processed lobster, boiled shrimp, cooked oysters, and raw oysters.
- Incubation period is 12 to 24 hours, but exceptions frequently occur.

Vibrio Parahaemolyticus cont'd…

- Infectious level is thought to be 1 x 10^5 cells.
- This is a marine organism, growing well in the presence of 1% to 3% salt.

[

- Its growth range is 50 to 109°F, minimum aw of 0.94, and pH range 5 to 11.
- The bacterium is Gram negative, straight or curved, motile, facultative rod.
- Although the cells are sensitive to low temperatures, salt appears to protect the cells from damage.

Vibrio vulnificus

- **Was recognized initially in the 1970s as causing frequently fatal wound infections.**

- **Since then it was noted that this bacterium is a marine organism that is different from *V. cholerae* and *V. parahaemolyticus*, living in estuarine waters.**

- **Ingestion results in an infection and septicemia with 60% mortality.**

Vibrio vulnificus cont'd…

- **The bacterium is sensitive to cold temperatures, becoming non-culturable when exposed to 39°F.**
- **It is Gram negative, straight to curved, motile (single polar flagellum), aerobic rod.**
- **Growth has been observed at 77 to 104°F.**

Salmonella species

- Have been known since the late 1800s to cause "**hog cholera**", which was transmittable to people.

- The illness is an infection due to the ingestion of live *Salmonella* cells.

- Incubation period
  - May be 14 hours
  - But may be shorter or longer depending on :
    - Number of cells ingested
    - Virulence of the serovar
    - Natural resistance of the individual.

Salmonella cont'd…

- Symptoms include diarrhea, abdominal cramps, fever, nausea, and vomiting.
- Infection depends on the virulence of the serovar.
- Most serovars are very invasive and upon entering the blood stream can cause infection of any internal organ.
- Annually there are about 60 outbreaks with 2,500 cases and 10% mortality due to *Salmonella* infections.

Salmonella cont'd…

- The microorganism gains entry to food from persons recovering from an illness, who show no symptoms and feel absolutely healthy, but are still shedding the bacillus.
- The initial contamination level of food need not be high to cause an outbreak, since this microorganism will grow in most foods at temperatures between 50 and 117°F.
- There are some serovars that will grow at temperatures as low as 41°F.
- The food itself exerts some effect on the growth of this pathogen.

Salmonella cont'd…

- The pH range for these microorganisms is between 4.5 and 9.0, but minimum pH will depend on the nature of the acid used.

- Growth occurred at pH 4.05 when HCl or citric acids were the acidulants, pH 4.40 when lactic acid was used, and pH 5.40 when acetic acid was employed.

- *Salmonella* cells require relatively high water activity for growth, with aw 0.92 being the lower limit.

Salmonella cont'd…

- There are some 1,700 serovars that are distinguished from one another by
  - Serology (specific antigen/antibody reaction)
  - Biotyping (substrate utilization)
  - Phage typing (susceptibility to virus destruction)
  - Colicin typing (proteinaceaous bactericidal compounds produced by *Escherichia coli*)
  - Plasmid fingerprinting (electrophoresis of extracted plasmids).

Salmonella cont'd…

- The more useful characterization of a serovar is the plasmid fingerprinting since the plasmids determine antibiotic resistance, substrate utilization, adhesion to surfaces, ability to form pilli, and so on.

- A very useful observation is that only certain plasmids will coexist in a cell.

- The bacillus is a Gram negative, motile or non-motile, aerobic rod.

Shigella species

- *Shigella* species is responsible for bacillary dysentery, where in severe cases the stool contains blood and mucus.
- Outbreaks involving this microorganism are primarily a problem in crowded urban institutions.
- There are several species in this group that cause varying degrees of dysentery.

Shigella species cont'd…

- Transmission is from person to person or by contaminated salads.
- The incubation period is from 14 hours to a few days.
- Symptoms are primarily diarrhea and abdominal cramps.
- This microorganism is a Gram negative, non-motile, facultative rod.
- Upon ingestion, it will travel to the small intestine where it attaches itself to epithelial cells, causing ulceration.

Escherichia coli

- Is the predominant microorganism of the normal flora in the intestine of a warm-blooded animal.
- Because of this association, *E. coli* is used as an indicator of fecal pollution of drinking water.
- Is also used as an indicator of fecal contamination of clam flats and for that matter any food product.

Escherichia coli cont'd…

- This microorganism is Gram negative, motile, facultative rod.
- It will not grow at a minimum temperature of 46°F, in a maximum salt concentration of 8%, and a minimum pH of 5.6.
- More recently it was noticed that *E. coli* could cause four different syndromes of gastrointestinal disturbance.

E.Coli cont'd…

- It is now known that there are four distinct types:
  - enteropathogenic (EPEC),
  - enteroinvasive (EIEC),
  - enterotoxigenic (ETEC),
  - enterohemorrhagic (EHEC).

Entropathogenic e.coli

- 15 different serotypes in the entropathogenic group, having distinct O antigen.

- causes infantile diarrhea and is associated with fever, vomiting, and abdominal cramps.

- Also causes nausea, vomiting, diarrhea, abdominal cramps, headache, fever, and chill in adults.

- The stool is watery but without blood.

- Incubation period is from 12 hours to 3 days, and the infectious dose is 1×10^6 or more cells.

Enteroinvasive e.coli

- Causes dysentery type symptoms, resembling those of *Shigella* infection.
- These symptoms are the result of the bacterium invading the epithelial mucosa of the intestine.
- Incubation period is 8 to 24 hours.
- Outbreaks occurred in hospitals where mothers and their babies developed the infection.
- Similar outbreaks occurred on cruise ships, where the incriminated food was cold buffets.
- There are 9 serotypes in this group with different O antigen and all are non-motile.

Entrotoxigenic e.coli

- Primarily known as travelers' diarrhea.
- The initial outbreak appeared to be due to O148:H28 serotype, but now there are at least 17 serological (O antigen) serotypes in the ETEC group.
- The microorganisms produce two toxins:
  - Heat-sensitive enterotoxin that is related to cholera toxin,
  - Heat-resistant, non-antigenic enterotoxin of small molecular size

Entrotoxigenic e.coli cont'd…

- The incubation period is about 24 hours, but could vary from a few hours to two days.

- The infectious dose is 1×10^8 or more.

- Symptoms include watery diarrhea, fever, nausea, and abdominal cramps.

Enterohemorrhagic *E. coli*

- Represented by a single serotype: O157:H7.
- This microorganism is responsible for at least three different syndromes.
  - **Hemorrhagic colitis** - sudden sever , stomach cramps, which are followed a day later by watery, grossly bloody diarrhea.
  - **Hemolytic uremic syndrome** - kidney failure, primarily in children.
  - **Thrombic thrombocytopenic purpura** - the nervous system, causing blood clots in the brain.

Yersinia enterocolitica

- *Yersinia enterocolitica* was initially noticed by investigators examining children with enteritis in the early 1940s.
- By 1980 several thousand isolates were obtained from adults and chinchillas.
- Symptoms in people include fever, abdominal cramps, and diarrhea.
- Septicemia was reported with healthy infants and adults with predisposing illnesses (diabetes and pneumonia).

Yersinia enterocolitica cont'd…

- Cause other infections:
    - Skin infection
    - Eye infection,
    - Endocarditis
    - Thyroid disorder,
    - Glomerulonephritis
    - Liver disease,
    - Respiratory infection, and muscle abscesses.

Yersinia enterocolitica cont'd…

- The first reported case involved a person who drank from a mountain stream.
- Cats and dogs were found to harbor this microorganism, along with wild animals and rodents.
- From very early on it was suspected that food was the transmitting medium.
- The microorganism is a Gram negative, motile, aerobic rod.

Yersinia enterocolitica cont'd…

- It will grow best at room temperature, but also will grow at 39°F.
- The pH range for this bacterium is 6.8 to 9.0.
- Two other species that can also cause similar symptoms are :
  - *Y. pseudotuberculosis*
  - *Y. pestis.*

Campylobacter jejuni

- *Campylobacter jejuni* causes acute gastrointestinal infection.
- This microorganism was first recognized in 1938 as causing infectious infertility and abortion in sheep and cattle.
- In 1938 two outbreaks of acute gastroenteritis occurred involving 151 persons in two prisons.

Campylobacter jejuni cont'd…

- Symptoms include diarrhea, vomiting, abdominal cramps, headache, and fever.
- As time passes, the stool becomes watery and later bloody.
- Incubation period is 2 to 5 days, and illness may last one week.
- The microorganism is Gram negative, microaerophilic, slender, curved, motile rod.

Campylobacter jejuni cont'd…

- Temperature growth range is between 77 and 109°F and pH range is 5.5 to 8.0.
- Foods involved include milk, poultry, eggs, pork, and beef.
- The bacterium appears to be spread by the food handler, while the source may be healthy or sick cats and dogs.
- Wild birds are suspected of being the source of *C. jejuni* in fresh water.

Plesiomonas shigelloides

- *Plesiomonas shigelloides* has been involved in several outbreaks of gastroenteritis.

- Symptoms include nausea, vomiting, fever, chills, headache, diarrhea.

- Incubation period is about 1 to 2 days.

- Raw oysters, salted fish, and cooked crabs have been implicated in infections.

Plesiomonas shigelloides cont'd…

- The source of this microorganism includes freshwater fish, reptiles, crustaceans, mammals, and birds.

- The bacterium is a Gram negative, facultative, halotolerant, motile rod.

- It can grow between 46 and 122°F and pH 4.0 to 9.0.

- It has also been observed to grow in nutrient medium in presence of salt to 3% concentration.

Mycotoxins

- During the digestion of substrates, fungi secrete enzymes into nutrients in order to break down complex compounds into simpler compounds that can be taken up by the fungi and used as nutrition.

- These digested nutrients produce secondary metabolic byproducts called mycotoxins that are released to give the fungi a competitive edge over other microorganisms and fungi.

Mycotoxins cont'd…

- Unfortunately, mycotoxins cause avariety of symptoms - cold/flu-like symptoms, sore throats, headaches, nose bleeds, fatigue, diarrhea, dermatitis, and immune suppression.

- Some mycotoxins may also be carcinogenic and teratogenic.

Mycotoxins cont'd…

- Molds that have been known to potentially produce these toxins are
  - Acremonium, Alternaria, Aspergillus,
  - Chaetomium, Cladosporium, Fusarium,
  - Penicillium, and Stachybotrys.
- Currently, it is unknown exactly what conditions promote the growth of mycotoxin production.

Types of Mycotoxins

Aflatoxin.

- This mycotoxin is primarily produced by Aspergillus species.
- It is one of the most potent carcinogens known to man and has been linked to a wide array of human health problems.
- The FDA has established a maximum allowable level of total aflatoxin in
  - Food commodities of 20 parts per billion (ppb)
  - Milk products is 0.5 ppb.

Ochratoxin

- This mycotoxin is primarily produced by species of Penicillium and Aspergillus.
- It can be damaging to the kidneys / liver, and it is a suspected carcinogen.
- There is also evidence supporting it’s role in impairing immune system function.

Tricothecene

- The toxin is produced by Stachybotrys species and Fusarium species and - use as a biological weapon.
- Can severely damage the entire digestive tract and cause rapid death due to internal hemorrhaging.
- It has also been implicated in human disease such as infant pulmonary hemosiderosis.

Suspected Human Mycotoxocosis

- Diseases caused by mycotoxins are called mycotoxicoses and can be acute, chronic, or subchronic.

- Acute cases of mycotoxicosis are rare and limited to developing countries.

- Conversely, long-term exposure to low levels of mycotoxins is an insidious, widespread problem that scientists are called to address world wide.

Ochratoxin and nephropathy

- OTA is suspected to be involved in the etiology of human nephropathies and tumors of urinary organs.
- The persistence of OTA in the human body is prolonged as it has a blood half-life of 35 days after a single oral dosage, due to unfavorable elimination toxicokintetics

Aflatoxins and hepatocellular carcinoma

- Hepatocellular carcinoma (HCC) is one of the most widespread malignancies.

- It has the fourth highest mortality rate worldwide and is estimated to cause approximately half a million deaths annually.
- Geographic areas of interest include Asia, southern China, and sub- Saharan Africa.
- An increasing incidence of HCC has also recently been observed in the US.

Fumonisin And Oesophagel Cancer :

- In areas where humans consume corn as the staple food, fumonisin contamination has been linked with esophageal cancer.

Various corn based foods

- Fumonisin B1 (FB1) is the most common fumonisim.
- High health risk stem from corn simultaneously contaminated with FB1 and combination of mycotoxins .
- it is reported in China, Indonesia, Korea, Brazil, Ghana, Thailand and Southesat Asia
- Wine and Beer: The ocurance of OTA in wine was demontarted for the first time by Zim Dick in a survey on 133 wines from retail outlet in Switerland.

Fruits and Fruits Juice

- Fruits and vegetables are another important source of food for human but contaminated by molds that produce mycotoxines.

- A common mycotoxines in apple juice Citrinin produced by Penicilium expansum and OTA biosynthesized by Aspergilus carbonarius.

Coffee and Cocoa products

- Coffee may contain OTA produced by Aspergilus ochraceus or A. carbonarus.

- Poor drying of coffee may be the cause of mycotoxocosis.

Spices

- Spices are another foods that have the potential to be highly contaminated especially by AFB1.

- However, their toxicological importance is below to their quantitative inclusion in the diet.

Viral and parasitic food born infection

???

Interventions and Practices Considered

Diagnosis

- Differential diagnosis:
  - Evaluation for signs and symptoms
  - Differentiation between food born illness and other syndromes
  - Duration
  - Associated foods
  - Evaluation for underlying medical conditions

- Microbiology testing:
  - Stool cultures
  - Blood cultures
  - Direct antigen detection tests and molecular biology techniques
  - Botulinum antitoxin and botulism immune globulin

Treatment

- Supportive care
- Oral and/or intravenous hydration, with fluid and or electrolyte replacement
- Gastric lavage
- Antibiotics

Surveillance and Reporting

- Report potential food borne illness
- Contact local or state health department with specific notifiable disease
- Report increases in unusual illnesses, symptom complexes, or disease complexes to public health authorities
- Follow the most current information on food safety

Summary

- Food intoxication & food infection
- A,B and serogroup of C.Botulinum are the primary concern of food industry.
- Clostridium Perfiringes and S.Aureus are responsible for food intoxication.
- Bacillus Cerus causes gastroenteritis due to enterotoxin
- Person with suppressed immune system and including pregnant women are susceptible to food bone infection by L. Monocytogenes

- Food boner infection by S.Aureus is a self limited
- Serogroup o:1 and non 0:1 of v.cholera are responsible for responsible for food borne infection by vibrio.
- V.Parahemolyticus grow well in the presence of 1-3% salt concentration
- Salmonella requires high water activity for growth
- E.Coli is an indicator organism for fecal contamination
- Types of Mycotoxins
    - Aflatoxin
    - Ochratoxin
    - Tricothecene

- If food borne infection happens, the following should be done :
  - Differential diagnosis
  - Microbiological diagnosis
  - Surveillance and reporting the results.

Chapter Eight : Food Preservation and Storage

Outline of presentation

- Objective
- Food preservation
  - Physical method of preservation
  - Chemical method of preservation
  - Biological processes
  - Emerging methods of preservation

Objective

- Up on completion of this chapter, the students should be able to:
  - Describe food preservation
  - Categorize methods for food preservation
  - Explain physical ,chemical and emerging technique of food preservation
  - Identify specific preservation method for different food item.

Food preservation

- Deals with the practical control of factors capable of adversely affecting the:
  - Safety, nutritive value,
  - Appearance, texture,
  - Flavor, and keeping qualities of raw and processed foods.
- A number of food products have differing constituents resulting in deterioration by a number of agents
- Natural processes of drying, cooking, salting, pickling, fermenting, and freezing for food preservation were used previously .

Food preservation cont'd…

- Food preservation methods involve the use of:
- Physical method
  - Heat,
  - Refrigeration, freezing,
  - Dehydration
  - Caning
- Chemical methods
  - Concentration
  - PH control,
  - Chemical preservatives, and packaging

Food preservation cont'd…

- Emerging methods:
  - Irradiation
  - Micro wave processing
  - Thermo-sonication
  - Modified atmosphere packaging (MAP) and active packaging

Food preservation cont'd…

- Preservation involves:
  - Preventing the growth of bacteria, fungi and other micro-organisms
  - retarding the oxidation of fats which cause rancidity.
  - Inhibiting natural aging and discolouration

Physical method of preservation

HEAT

- Thermal processes to preserve foods vary in intensity.

Sterility

- Ensures total destruction of the most heat-resistant bacterial spores in non acidic foods.

- Require a treatment of at least 250°F (121°C) of wet heat for at least 15 minute

- Less severe heating produce with pasteurization assure destruction of pathogens.

- Smoking uses a combination of heat to dry the meat without cooking it, and the use of the aromatic hydrocarbons to preserve the meat.

Refrigeration & freezing

- Refrigeration and freezing
  - Principle - slowing of biological and chemical activity with decreasing temperature.
  - When water is converted to ice, free water required for its solvent properties by all living systems is removed.

- Immersion freezing
  - Involves direct contact of the food or its container with refrigerants approved for food or a fast-freezing cryogenic liquid .

- The physical character of the tissue, and incidentally its taste, remained the same

Freezing and Refrigeration cont'd…

- Commercial freezing methods
  - Utilize refrigerated still air & high-velocity air,
  - Is faster and more efficient;
  - High-velocity air made to suspend particulate foods
- Indirect contact freezing
  - Utilizes hollow flat plates chilled with an internally circulated refrigerant
  - Freeze solid foods or with refrigerated tubular heat exchangers that rapidly slush-freeze liquids.

Clamping

- Many root vegetables are very resistant to spoilage and require no other preservation other than storage in cool dark conditions, usually in field clamps.

Dehydration

- When sufficient water is removed from foods, microorganisms will not grow

- Many enzymatic and nonenzymatic reactions will cease or be markedly slowed.

- Concentration preservation can be achieved by
  - Physically removing water,
  - Boiling or with lower-temperature vacuum evaporation,
  - Binding water through the addition of sugar, salt, or other solutes.

Dehydration cont'd…

- Foods preserved by dehydration contain considerably lower water activity and less total water than concentrated foods.

- Most dehydration methods utilize heat to vaporize and remove water.

- The heat and oxygen sensitivity of many foods necessitates vacuum dehydration for high quality.

Dehydration cont'd…

- Under vacuum, water can be removed at reduced temperature, and oxidative changes are minimized.
- In freeze-drying, foods are frozen quickly and placed in a chamber under high vacuum.
- A food's structure remains rigid as it goes directly from the frozen state to dryness.
- Drying is also the normal means of preservation for cereal grains.

Canning

- Is a method of preserving food by first heating it to a temperature that destroys contaminating microorganisms, and then sealing it in air-tight jars, cans or pouches.

- Because of the danger posed by *Clostridium botulinum* and other pathogens, the only safe method of canning most foods is both high heat and pressure, normally at temperatures of 240-250°F (116-121°C).

Caning cont'd…

- Foods that must be pressure canned include most vegetables, meats, seafood, poultry, and dairy products.

- The only foods that may be safely canned in a boiling water bath (without high pressure) are highly acidic foods with a pH below 4.6.

Chemical method of preservation

PH

- The natural acids of fruits and vegetables, acid added as a chemical, and acid produced by fermentation can inhibit or partially inhibit several pathogenic and spoilage organisms.

- The pH of acidic foods, however, is rarely sufficiently low to assure long-term preservation from acid alone.

Preservatives

- Preservatives function in two ways by:
  - Delaying the spoilage of the food,
  - Ensuring that the food retains, its original quality.
- The first method includes the use of
  - Sugar, vinegar for pickling meats and vegetables,
  - Salt, and alcohol.
- The second method includes the use of
  - ascorbic acid (which prevents color deterioration in canned fruits), benzoic acid, sulfur dioxide,
  - variety of neutralizers, firming agents, and bleaching agents

Salt

- Salting or curing draws moisture from the meat through a process of osmosis.

- Meat is cured with salt or sugar, or a combination of the two. Nitrates and nitrites are also often used to cure meat.

sugar

- Is used to preserve fruits

- either in syrup with fruit such as apples, pears, peaches, apricots, plums or

- In crystallised form where the preserved material is cooked in sugar to the point of crystralisation and the resultant product is then stored dry.

Sugar cont'd…

- The use of sugar is often combined with alcohol for preservation of luxury products such as fruit in brandy or other spirits.

- This method is used for the skins of citrus fruit (candied peel), angelica and ginger

Pickling

- Is a method of preserving food by placing it or cooking it in a substance that inhibits or kills bacteria and other micro-organisms.

- This material must also be fit for human consumption.

- Typical pickling agents include
  - Brine (high in salt),
  - Vinegar, ethanol,
  - Vegetable oil, especially olive oil but also many other oils.

Pickling cont'd…

- Most processes involve heating or boiling so that the food being preserved becomes saturated with the pickling agent.

- Frequently pickled items include vegetables such as cabbage, peppers, and some animal products such as corned beef and eggs.

- EDTA may also be added to chelate calcium.

- Calcium is essential for bacterial growth.

Lye

- Sodium hydroxide (lye) makes food too alkaline for bacterial growth.
- Lye will saponify fats in the food, which will change its flavor and texture.
- Lutefisk and hominy use lye in their preparation, as do some olive recipes.

Jellying

- Food may be preserved by cooking in a material that solidifies to form a gel.

- Such materials include gelatine, agar, maize flour and arrowroot flour.

- Some foods naturally form a protein gel when cooked

Modified atmosphere

- Is a way to preserve food operating on the atmosphere around it.

- Salad crops which are notoriously difficult to preserve are now being packaged in sealed bags with an atmosphere modified to reduce the oxygen (O_2) concentration and increase the carbon dioxide (CO_2) concentration.

- this method of preservation may not retain nutrients, especially vitamins.

Modified atmosphere cont'd…

- Nitrogen gas (N2) at concentrations of 98% or higher is also used effectively to kill insects in grain through hypoxia.

- However, carbon dioxide has an advantage in this respect as it kills organisms through both hypoxia and hypercarbia, requiring concentrations of only 80%, or so.

- This makes carbon dioxide preferable for fumigation in situations where an hermetic seal cannot be maintained.

Biological processes

- Some foods, such as many traditional cheeses, will keep for a long time without use of any special procedures.

- The preservation occurs because of the presence in very high numbers of beneficial bacteria or fungi which use their own biological defenses to prevent other organisms gaining a foot-hold.

Emerging techniques of food preservation

- **Ultra-high pressure hydrostatic processing**
- At pressures of 50 000 to 120 000 PSI, vegetative cells of spoilage organisms and pathogens can be destroyed with very little heating of the product.
- It is speculated that the mechanism of vegetative cell inactivation is through rupture of the cell wall during pressure release.
- With the addition of mild heating plus high pressure, some more fragile bacterial spores can also be inactivated.

- This process can also be extended to heat-sensitive fruits and vegetables.
- Future applications are likely to include liquid and semi-solid food products, for which rigid texture is a less important attribute and little or no heat for processing is desirable.

- Grains may be preserved using carbon dioxide. A block of dry ice is placed in the bottom and the can is filled with grain.
- The can is then "burped" of excess gas. The carbon dioxide from the sublimation of the dry ice prevents insects, mold, and oxidation from damaging the grain.
- Grain stored in this way can remain edible for five years.

Ohmic processing

- Electric current applied directly to a conductive food allows for rapid heating of the food product.
- The heat generated destroys microorganisms in a manner similar to classical thermal processing.

High-intensity light pulses

- Very intense white light (20 000 times the strength of sunlight on earth) can be pulsed with a duration of between 10-6 and 10-1 cycles per second, which results in the decontamination of food surfaces.

- Higher levels of energy have been shown to inactivate bacterial spores as well as vegetative cells.

- Pulsed light may destroy microbes through both rapid surface heating, with no real cooking of the product, and a photochemical mechanism.

- Future surface treatment of foods and package material decontamination applications are anticipated using pulsed light technology.

High electric field pulses

- Electrical pulses with a field strength of 10 to 20 kV per centimeter have been shown to disrupt and rupture cell membranes.

- The pulsing creates an uneven distribution of the electrical charge across the cell's membrane, which leads to microbial inactivation.

High electrical field cont’d…

- Although the process generates little heat, it is likely that it may find commercial applications in conjunction with mild heating

- Future applications may include pasteurizing fruit products and alcoholic beverage products.

Radio-frequency (RF) heating

- Food material is placed in an electrical field consisting of pulses of radio waves.
- This generates heat throughout by a rapid reversal of the polarity of molecules.
- RF has both current and future applications for bakery products as well as for comminuted meat products.
- Other potential applications include reduction of *Salmonella* in eggs and destroying harmful bacteria in fresh fruit juices

Irradiation

- Governmental interest in the process is emerging for many reasons, largely related to persistently high food losses from infestation,
  - contamination and spoilage;
  - mounting concerns over food-borne diseases;
  - and growing international trade in food products

- that must meet strict import standards of quality and quarantine - all areas in which food irradiation has demonstrated practical benefits when integrated within an established system for the safe handling and distribution of food.

- FAO has estimated that, worldwide, about 25 percent of all food production is lost after harvesting to insects, bacteria and rodents.
- The use of irradiation alone as a preservation technique will not solve all the problems of post-harvest food losses, but it can play an important role in cutting losses and reducing the dependence on chemical pesticides.
- Many countries lose huge amounts of grain because of insect infestation, moulds and premature germination. For roots and tubers, sprouting is the major cause of losses.
- Radiation processing offers an alternative to fumigation and some other treatments.

- Food irradiation remains highly controversial, partly because of fears that the safety of products and processes cannot be adequately regulated.

Microwave processing

- A well-accepted technology for heating and thawing for the past 20 years,
- Microwave processing has yet to have wide commercial application.
- The lack of uniformity of heating has been a significant technical hurdle.

Microwave cont;d…

- However, because of its properties, the process has significant potential as a technology and may be used in combination with other methods.

- Its use in many food processing steps, such as blanching, baking and pasteurization, is projected for the future.

Thermo-sonication

- The combination of ultrasound and heat at moderate temperatures can cause enhanced inactivation of microorganisms.
- This may be particularly useful for pasteurization of certain beverages where a reduced temperature is desirable.
- Ultrasound has potential application for emulsified foods, especially where a product's rheological qualities can be improved by ultrasound treatment.

Modified atmosphere packaging (MAP) and active packaging

- Controlled atmosphere storage and preservation of packaged food products is a widely utilized technology for fresh foods, prepared foods and baked products.
- The utilization of inert gases, reactive gases or vacuum can allow for unique applications that control microorganisms as well as maintaining product color and freshness.
- Packaging material can have functions other than its traditional barrier properties for oxygen control, moisture control and light restriction and against insect infestation

- For example, active packaging material not only acts as a barrier to oxygen, but can also function as an oxygen absorber and scavenger.

- This active role reduces destructive chemical reactions in oxygen-sensitive products and can also help restrict the growth of oxygen-requiring microbes.

- The shelf-life of many different product types could be increased under reduced-oxygen conditions.

Summary

- Food preservation process will maintain :
  - Safety, nutritive value
  - Appearance, texture
  - Flavor of raw and processed food by
    - Preventing the growth of different microorganisms
    - Retarding the oxidation of fat

- Three methods of food preservation
    - Physical
    - Chemical
    - Emerging techniques

Chapter Nine : Laboratory methods for detecting microorganism and their products in food

Outline of presentation

- Objective
- Culture, microscopic and sampling method
- Methods to detect and enumerate microorganisms
- Important characteristics of assay method
- Standard plate count
- Membrane filter
- Microscopic count
- Multiple Probable number analysis

Outline cont'd..

- Dye reduction
- Impedance
- Other enumeration methods
- Methods to detect specific components
- Immunologic method
- Other identification method
- Bioassay
- Cell culture system

Objective

- At the end of this chapter, the student should be able to:
  - Describe different methods for detection and enumeration of microorganisms in food.
  - List the important characteristics of assay techniques
  - Perform different methods for detection and enumeration of microorganisms in food.
  - Comply with the standard operational procedures for detection and enumeration of microorganisms in foods.

I. Culture, Microscopic and Sampling Methods

- There are many methodology but none of them are accurate and utilized for all kinds of foods/ beverages at all conditions.

Methods to Detect and Enumerate Microorganisms

- **Why organisms are enumerated?**
  - It gives history of product
  - One can predict safety and keeping quality

- **Specific organisms could be looked as :-**
  - Specific pathogens known to contaminate the product
  - It may indicate specific problems
  - A desired organism present at certain level

What are important characteristics of assays (assay methods)?

- Accuracy
- Sensitivity
- Specificity
- Reproducibility
- Fast (Turnaround time)
- Cheap

What are basic methods of enumeration?

- Viable counts (plate counts)
- Most probable number analysis (statistical estimate)
- Assays for metabolic products (dye reduction, impedance)
- Microscopic counts

Plate Counts

Rationale

- Viable cells given chance to form colonies
- Organisms are homogeneously distributed
- Sample taken is representative of the whole
- One cell or cluster forms one colony
- Growth on plate not restricted

Standard plate count

- Sample pour-plated in plate count agar (PCA) and incubated at 35 o C for 48 hr.
- All colonies counted.

What gets counted?

- Organism that can form a visible colony in the given incubation time, at the given temperature, on the growth medium provided

Standard plate count cont'd…

- **What doesn't get counted?**
  - Slow growers
  - Anaerobes
  - Obligate psychrophiles or thermophiles
  - Organisms with special nutrient requirements
  - Poor competitors (on crowded plates)

Basic steps

- Representative sample
- Mix well (blender, Stomacher)
- Dilute as required (phosphate, peptone)
- Pour plate or spread plate
- Proper incubation
- Count colonies (25-250 per plate)

- **One can make plate counts more specific by:-**
  - Use of selective media
  - Use of differential media
  - Incubating at different atmospheric conditions (air vs. anaerobic)
  - Use of different incubation temperature

- **Aids for plate counts**
    - Spiral plater
    - Colony counter
    - Petri film
- **Injured cells are cells that may be damaged by different factors.**
- Physical or physiological damage
    - membranes
    - ribosomes
    - enzymes

- Exposure to stress conditions
  - heat
  - freezing
  - chemicals
- Won't form colonies on selective medium
  - count on nonselective medium > count on selective medium

- Repair/recovery time required
  - complex, nonselective medium
  - proper pH
  - proper temperature
  - specific additives (pyruvate, catalase)

- Viable, nonculturable cells
  - viable microscopic count > viable plate count

Membrane Filters

Rationale

- Collect cells from a large filterable volume
- Examine microscopically or let colonies grow
- Useful for low concentrations of cells

Practicalities

- Pore size: 0.45 micron (or 0.22 micron)
- Diameter: 13 mm to >150 mm
- Driving force: vacuum or positive pressure

Improvements

- Hydrophobic grid membrane filter
- Special filter composition (nylon, polycarbonate)
- Special filter color
- Fluorescent dyes

Microscopic Counts

Rationale

- Count cells in a known volume or area
- Translate to count per ml or count per gm of samples

Pros and cons

- Observe different cell types
- With proper stains, differentiate living from dead
- Rapid result, Tiring
- Insensitive, Large errors likely

Microscope counts in use

- Direct microscopic count (dairy industry)
- Howard mold count (vegetables)
- DEFT (direct epifluorescent filtre technique)

Most Probable Number Analysis

Rationale

- At low cell concentrations, the likelihood of finding a microbial cell in a particular place (test tube) can be defined statistically (Poisson distribution)

- Gives most likely number of organisms present, rather than precisely determined number

Method

- Make serial 10-fold dilutions (or volume changes) of sample
- Inoculate multiple tubes (usually 3 or 5) with each dilution
- Incubate, then count positive tubes at each dilution
- Look up pattern of positive tubes in table
- Read MPN from table

- **Merits and demerits**
  - Relatively simple
  - Can count a specific type in presence of others
  - Can use large sample volumes
  - Doesn't give the "real" value
  - Doesn't give isolated colonies
  - Lots of glassware generated

- **Uses**
  - Standard coliform analysis
  - Basis for HGMF reader
  - Low cell concentrations

Dye Reduction

Rationale

- As organisms metabolize, they use up oxygen
- As dissolved oxygen decreases and reduced metabolic products accumulate, redox potential (Eh) decreases
- Dyes that change color as they are reduced serve as indicators of Eh
  - Methylene blue (blue -> colorless)
  - Resazurin (blue -> pink -> colorless)

- More cells means quicker time to lose color
- Long used in dairy industry to grade milk

Merits and demerits

- Easy, rapid, and cheap
- Detects only viable cells
- Not very sensitive
- Not uniform across organisms or foods
- Reductive enzymes or products interfere

Impedance

Rationale

- Impedance measures resistance in a circuit to current flow
- In a food, charged molecules carry current; uncharged molecules don't
- As microorganisms metabolize, they convert uncharged substrates (sugars) to charged products (acids)

Impedance cont'd…

- Impedance decreases with increased growth
- Time to see impedance change or time to reach a certain value related to number of organisms

Applications

- As correlations to plate counts done for many products
- Requires standardized conditions, special medium and equipment (Bactomatic)
- Categorize samples: good -> bad
- Reduce time to determine microbial load

II. Other Enumeration Methods

Microcalorimetry

- Measure small amount of heat generated as organisms metabolize

- Use to enumerate organisms by measuring time to produce a certain response, or amount of heat generated

- Use to identify organisms by measuring heat generated in presence of various substrates

Radiometry

- 14-C-labelled substrate in growth medium
- Organisms release 14-CO2
- Amount released in a given time or length of time before given amount is released is proportional to cell numbers
- Isotope detection is very sensitive
- Isotopes not welcome in food industry

ATP Measurements

- ATP present in all living cells; quickly lost after cell dies
- Firefly luciferin-luciferase system uses ATP to emit light
- Light intensity proportional to cell numbers
- Interference from ATP in food

Methods to Detect Specific Components

Limulus Lysate for Endotoxin

- Protein from amoebocytes of horseshoe crab forms a gel in presence of Gram-negative endotoxin

- Release of colored substance from special substrate by activated limulus enzyme can also be detected

Limulus **lysate cont'd…**

- Very sensitive: small quantities of endotoxin and low cell concentrations detected
- Relate endotoxin to number of Gram-negative cells
- Used for quick analysis of quality of foods normally spoiled by Gram-negative organisms

Thermostable Nuclease

- Can food sample or extract cause DNA lysis after exposure to heat?
- High correlation of nuclease with enterotoxin-producing S. aureus
- Faster, easier to detect than enterotoxins
- Survive heat treatments as do enterotoxins

Fluoro- and Chromogenic Substrates

- Used to detect enzyme activity specific to particular organisms
- Substrate has an attached moiety that fluoresces or is colored upon release
- Add substrate to solid or liquid medium to detect presence of specific organism

Fluoro- and Chromogenic cont'd…

- Most common: MUG (4-methylumbelliferyl-ß-D-glucuronide) to detect ß-D-glucuronidase of E. coli
- ONPG (o-nitrophenyl-ß-D galactopyranoside converted to yellow moiety by ß-galactosidase
- Sensitive and rapid

Nucleic Acid Methods

DNA probes

- Single-stranded probe made from sequence unique to organism of interest

- Sample prepared so organisms are fixed to a solid support

- Cells lysed and DNA denatured

- Probe added; will stick to complementary sequence
- Probe detected by radioactive or enzyme label
- Applications:
  - Samples filtered and cells captured on membrane
  - Colonies transferred to membrane and blotted with probe

DNA Amplification

- Polymerase chain reaction (PCR) used to synthesize many copies of a particular DNA sequence, bounded by specific primer sequences
- Multiple cycles of replication can result in amplification to 107 molecules
- Detect amplified sequence by separating DNA fragments on agarose gel and labeling with probe
- Need specific sequence unique to particular organism

DNA application cont'd…

- Methods developed to detect specific pathogens: E. coli, Listeria monocytogenes, Shigella
- Increased sensitivity: can detect a few cells
- Time: Requires several hours to a few days
- It has complex procedures, cleanliness needed
- Value in detection of no cultivable forms

II. Immunological Methods

Basic Principle:

- Specific cell components (antigens) react with specific antibodies .

- Detection system can be:
  - Visible precipitation or agglutination
  - Label attached to antibody (fluorescent dye, radioisotope, enzyme)

The Case of Salmonella

- Various Salmonella "species" defined by cell surface antigens
-
- Zero tolerance for Salmonella in foods
- Extensive methods development to detect Salmonella, identify type, trace outbreaks

Fluorescent Antibodies

- Long-standing method (1942)
- Fluorescent dye attached to specific antibody
- Dye can be attached to second antibody (specific to first antibody)

Fluorescent Antibodies cont'd…

- Antigen-antibody (or antigen-antibody-antibody) complex emits fluorescence
- Detect with fluorescence microscope
- Observe single cells or microcolonies

Agglutination Tests

- Observe cells clump after reaction with antibodies
- Visibility enhanced if antibodies coated onto larger particles (beads, other cells, red blood cells = hemagglutination)
- Antibodies attached to other particles can detect cell products (e.g. toxins)

Agglutination Tests cont'd…

- Examples:
  - Agglutination = last step in Salmonella ID
  - Salmonella 1-2 Test
  - Gel diffusion assays for toxins
    - Oudin tubes (only antigen diffuses)
    - Ouchterlony plates/slides (both antigen and antibody diffuse)

RIA and ELISA

- Easily detected label (radioisotope or enzyme) attached to antigen or antibody (usually antibody, if detection of antigen in samples desired)
- Antigen-antibody complex must be retained in system for detection (usually attached to a surface)
- Detection by:
  - Radioactivity measurement (X-ray film, scintillation counting, etc) - not popular in food indus.
  - Addition of substrate for enzyme, with release of colored product

- Popular systems:
  - Antibody-coated microtitre plates
  - Horseradish peroxidase, alkaline phosphatase as enzyme labels
- Systems on market for Salmonella, other organisms, Staph toxins, mycotoxins, pesticides
- Both detection and quantization possible
- Time required = a few hours (after enrichment)

Polyclonal vs. Monoclonal Antibodies

- Made in whole animal vs. hybridoma clone
- Multiple vs. single epitope specificity
- Cost of production (both time and trouble)
- Advantages depend on assay system

Other Identification Methods

Diagnostic Kits

- Purpose: automate or miniaturize conventional biochemical tests for organism identification

- Save reagents, preparation time

- May save incubation time

- Series of reactions compared to standards

Diagnostic Kits cont'd…

- Reliability depends on data base:
  - Adapted from clinical microbiology
  - Gram-negative identification is generally better
- Examples:
  - API systems
  - Micro-ID
  - Minitek
  - Enterotube
  - Automicrobic system

Other Methods

- Electrophoresis
  - get "fingerprint" of microbial proteins
- Gas chromatography
  - identify cellular fatty acids

III. Bioassays

Rationale

- Determine biological activity of organism or toxin
- Use whole animal, cultured cells, or enzymes

Whole Animal Assays

- Mouse lethality
  - nonspecific: many toxins can cause death
  - Increase specificity: can antiserum protect?
  - standard assay for botulinum toxin

- Monkey feeding, injection of kittens
  - observe animals for emesis (vomiting)
  - standard assay for staph enterotoxin
- Diarrhea in rabbits, mice (various pathogens)

Other Assays for Diarrheal Activity

- Suckling mouse (E. coli stable toxin)
  - Inject sample into stomach
  - Intestine weighs more as fluid accumulates

- Ligated intestinal loops (cholera toxin, E. coli LT)
  - Surgical procedure to tie loops and inject
  - Look for fluid accumulation in loop

- Skin assays for altered vascular permeability
  - Inject sample into skin (rabbit, guinea pig)
  - Look for reddened, swollen area (erythema)
  - Inject Evans blue dye, look for blue area
  - C. perfringens, B. cereus

- **Invasiveness**
  - Sereny test (Shigella)
    - Inject live cells into guinea pig eye
    - Observe for conjunctivitis (infection)
  - Anton test (Listeria)

Cell Culture Systems

Rationale

- Spare animals
- Easier to maintain
- More specific activity detected

Sources of cells

- Human and animal
- Normal cells vs. cancer cell lines
- Various organs

- **Activities detected**
  - Adhesion of bacteria to cells (esp. intestinal epithelium)
  - Ability of bacteria to invade
  - Cytotoxic activity
  - Altered metabolic activity (morphological change)

Enzyme Activity

Rationale

- As more becomes known about the specific function(s) of various toxins, in vitro assays of enzyme activity may replace cell culture as well as whole animal assays .

Summary

- By enumerating microorganisms one can predict safety and quality of food.
- Basic methods of enumeration
- Viable count
- Multiple probable number analysis
- Assay for metabolic product

Summary cont'd…

- Microscopic cell count
- In standard plate count, organisms which can form visible colony will be counted.
- Membrane filters is useful for low concentration of cells
- Microscopic counts are rapid but insensitive
- Multiple probable number gives most likely number of organisms present rather than precisely determine number.

Summary cont’d…

- Dye reduction is long used diary industry to grade milk.
- Impedance measures resistance in a circuit.
- There are different methods of used to enumerate microorganism in food.
    - Microcalorimetry
    - Radiometry
    - ATP mesurement
- Immunologic ,electrophoresis, gas chromatography and bioassay are used to enumerate/detect microorganism in foods.

Chapter ten : Bacteriological Analysis of Water , Beverage and Milk

Outline of presentation

- Objective
- Types ,safety quality of water, source of contamination and type of microorganisms
- Microbiology of water
- Source of contamination of water
- Types of organisms in water

Outline cont'd…

- Sampling methods
- Methods of microbial detection
- Analysis of milk
- Useful laboratory tests for milk analysis

Objective

- After completion of this chapter, the student should be able to:
  - Explain types of water safety and quality
  - Identify the sources of contamination of water
  - List the type of organisms which are found in water
  - Describe different sampling method for water analysis
  - Explain types of water pollution

Objective cont’d…

- Recognize water analysis methods
- Perform microbiological analysis of milk and water
- Conform with the standard operational procedure of microbiological analysis of milk and water

Types , safety , quality of water , source of contamination and types of microorganisms

Types of water

Atmospheric water :

- Rain and snow are included
- May contain considerable number of bacteria owing chiefly to the high content of dust in the air
- After snow or heavy rain the atmosphere is washes to nearly free of organisms so that many sterile plates each innoculated with one ml of water may be obtained

Surface water

- As soon as rain drops and snowflakes touch the earth, they may become contaminated by microorganisms in the soil .
- The extent of contamination of this water is dependent upon the number of bacteria in the soil and also upon the kinds and quantities of food materials dissolved out of the soil by the water .
- The first result of mild rain is greatly increased the bacterial contamination of a body of water

Surface water cont'd…

- However , a prolonged rain exerts an opposites effect owing to the fact that after the main impurities have been removed from the upper layers of the soil , the subsequent rain fall acts merely as diluents of the body of water ,

- Rivers show their highest count during the rainy season .

Stored water

- The effect of storage is to greatly decrease the number of bacteria in water.

- This is due to factors that bring bacterial self purification such as sedimentation,

- Sedimentation : bacteria have a specific gravity slightly less gravity than that of distilled water, this means that they will slightly settle in still body of water.

Stored water cont'd…

- However , the greatest factor responsible for the sedimentation of bacteria is their attachment to the suspended particle .

- The suspended particles mechanically remove the bacteria from the upper layers of the water.

Stored water cont'd…

- Activities of other organisms
- Predatory protozoa present in water play an important role in decreasing the number of bacteria
- Bacteria require living or dead for food and easily engulf large number of bacteria , provided the water content sufficient oxygen ,
- In the absence of bacteria and oxygen , the protozoa gradually decrease

Direct sun light

- Is toxic to vegetative bacterial cell and even to spores

- If the action is sufficiently prolonged

- In water supply the toxicity of UV light is inversely proportional to turbidity

Temperature

- Increasing temperature exert a harmful effect up on the survival of pathogenic organisms particularly that cause disease.

Food supply

- As an increase in food supply usually results in an in increase in bacterial numbers however certain toxic substances such as acids and bases produce marked reductions in the number of variable bacteria and various dissolved gases such as carbon dioxide and hydrogen also show a toxic effect.

Ground water

- Relatively free from bacteria because of the filtering action of the earth through which the water has penetrated.

- This filtering action remove not only most of the bacteria but also any suspended organic food particles.

Ground water cont'd…

- Deep wells usually contain fewer organisms than water from shallow wells owing to the deeper layers of filtering materials.

- The distance that bacteria travels through soil depends up on a number of factors such as permeability of the soil , the hydraulic gradient of ground water and climatic condition.

Microbiology of Water

Water could be classified in to two based on sources.

- **Ground water**
  - This kind of water found in deep wells and in subterranean springs. It is free from microbe due to filtration action of soils.

- **Surface Water**
  - This form of water found in lakes, Streams, and Shallow wells. It has certain number of microbes.

Contaminated and polluted water

Contaminated water

- Water that is contaminated with chemicals and microbes.

Polluted water

- Contaminated water and the condition is known exactly .
- It is characterized by unpleasant test, smell and appearance.

Sources of contamination of water

- Water receives its bacterial from
  - Air , Soil
  - Sewage
  - Organic wastes
  - Dead plants and animals
- Streams, rivers ,wells and ground water can be contaminated with feces of humans and animals
- Contamination of water polluted with animal or human feces is an important source bacterial disease

Types of microorganisms found in water

- Most important pathogens that man acquires through water
- Bacteria :
  - V.cholera
  - Salmonella species
  - Shigella , E.coli
  - Campylobacter, Leptospira

Type of microorganism cont'd…

- Parasites
  - Entameoba species
  - Cryptosporidium
  - Giardia
  - Balantidium
- Virus
  - Rota virus
  - Polio virus
  - Hepatitis A

Sampling methods

- Samples of water for bacteriological testing must be collected in sterile bottles and care *must* be taken to prevent accidental contamination of the water during its collection.

Sampling bottles

- Glass bottles used for water sampling should have a capacity of at least 200 ml.

- They should be fitted with ground glass stoppers or screw caps.

Sampling bottles cont'd…

- The stopper or cap and neck of the bottle should be protected from contamination by a suitable cover either of paper or thin aluminium foil.

- Silicon rubber liners, that will withstand repeated sterilization at 160 ºC, should be used inside screw caps.

- After being sterilized the bottle should not be opened before the sample is collected.

Neutralizing chlorine in water samples

- When the water to be examined is likely to contain, sufficient sodium thiosulphate (Na2S2O3.5H2O) should be added to neutralize chlorine or chloramine to each bottle as follows:

- Add 100–200 µl (0.1–0.2 ml) sodium thiosulphate 30 g/l (3% w/v) to each bottle of 200 ml capacity before it is sterilized.

- This will give a concentration of approximately 18 mg/liter of water.

- **Note**:
  - Sodium thiosulphate at a concentration of approx. 18 mg/litre has no significant effect on the coliform or *E. coli.*

Information to be supplied with water samples

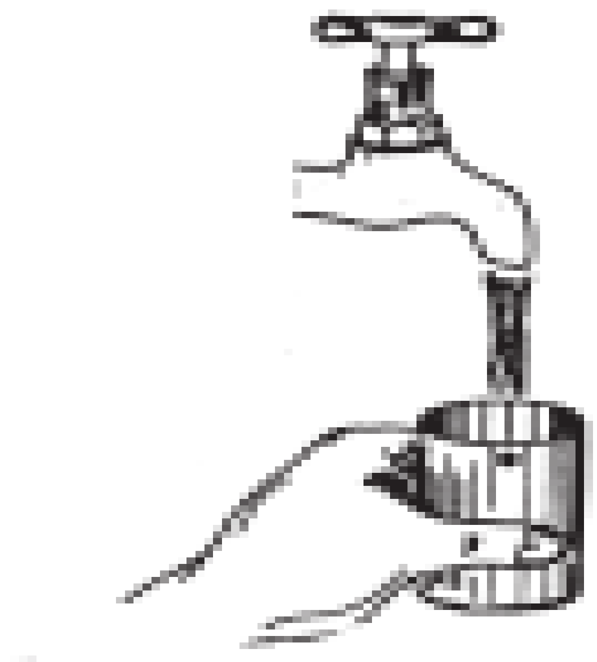
- Each water sample should be given a code number and the following information should accompany the sample (preferably using a standardized form):
    - Reasons for examination, e.g. whether a routine sample or otherwise.
    - Source and the exact place from where the water has been collected
    - State whether the water has been filtered, chlorinated, or treated in some other way.

Sample from tap

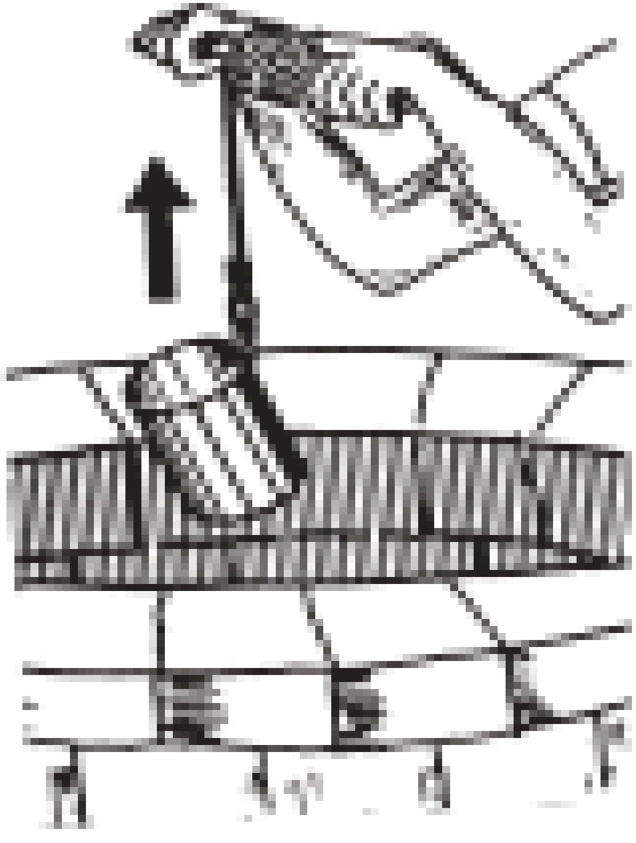
- *Sample from a house tap*:

Mention

- Whether the water was drawn from a cistern or direct from the main.



Sample from well



Sample from a well:

Give details of :

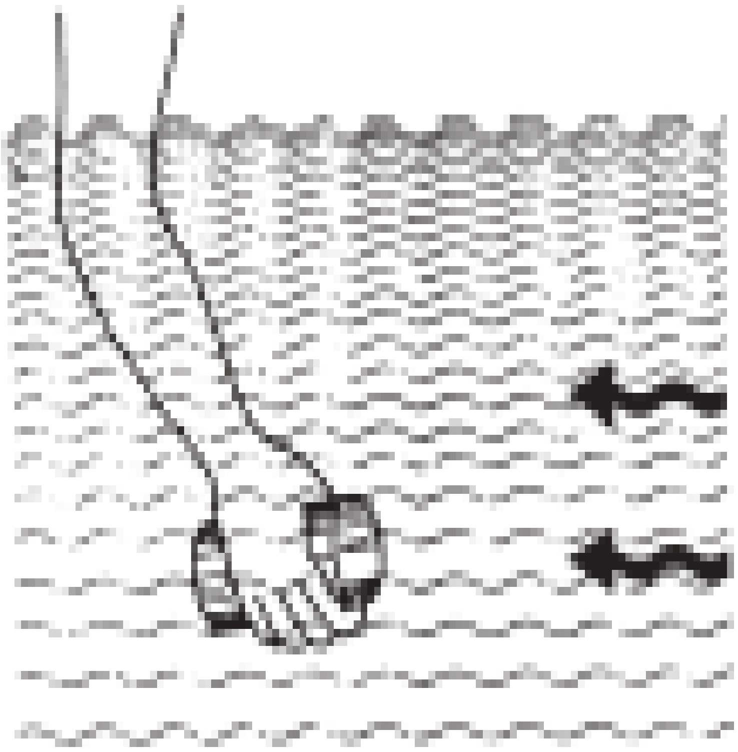
- The well’s depth
- Covered or uncovered
- Recently constructed or altered

***Sample from a spring*:**

- Describe the stratum from which it issued and whether the sample was taken directly from spring or from a collecting chamber.

Sample from a river

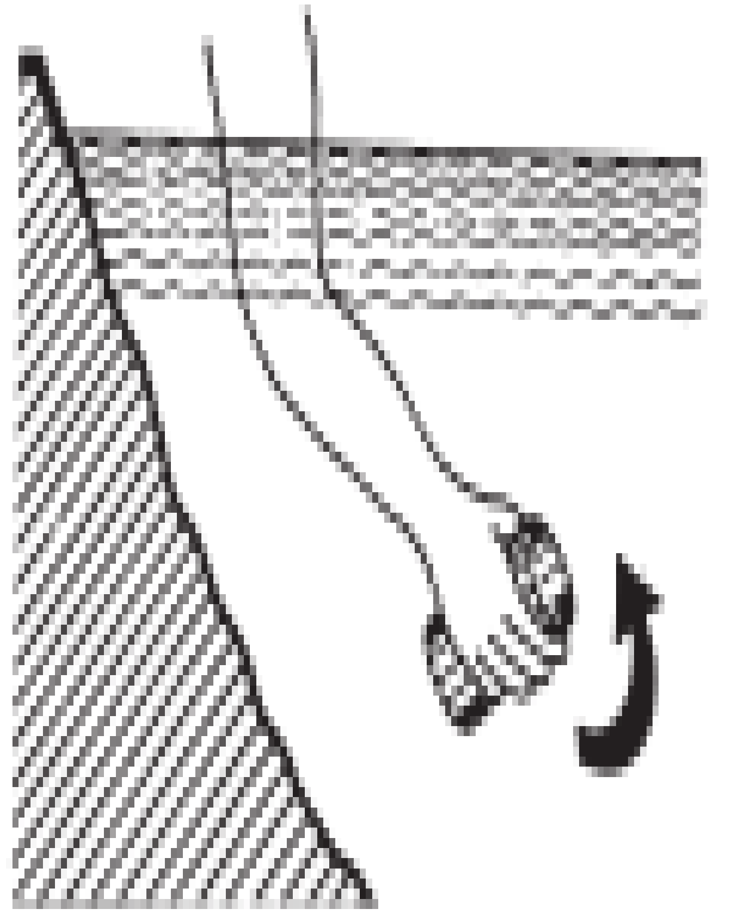
- *Sample from a river or stream*:
- Mention the depth at which the sample was collected
- Whether from the side or the middle of the stream
- Whether the water level was above or below average
- Whether there had been heavy rainfall or flooding.



Sample from a lake or reservoir :

Give the detail of :

- Exact position and the depth
- Temperature of the source
- Possible sources of pollution in the area
- Their approximate distance from the sampling point.
- Date and time when the sample was taken (and dispatched if sent to a testing laboratory).



Sampling

- Hold the sterile bottle by the base in one hand. Use the other hand to remove the stopper and cover together.
- The stopper and cover should be retained in the hand while the bottle is filled and then they should be replaced together.

- To prevent contamination, the person collecting the water must not touch, or allow any surface to touch, the screw thread of the bottle neck or the inside of the cap.
- If the bottle becomes contaminated, it must not be used.

Collecting a sample from a tap

- Remove any external fittings from the tap, such as an antisplash nozzle or rubber tube.
- Clean carefully the outside nozzle of the tap, especially any grease which has collected.
- Turn the tap on full, and allow the water to run to waste for 1 minute.
- This allows time for the nozzle of the tap to be flushed and any stagnant water in the service pipe to be discharged.

Sample from the tap cont'd…

- Sterilize the tap using the flame of a blowlamp or gas torch, or by igniting a piece of cotton-wool soaked in methylated spirit and holding it with a pair of tongs close to the nozzle until the whole tap is unbearably hot to the touch.

- Allow the tap to cool by running the water to waste for a few seconds.

- Fill the sample bottle from a gentle flow of water, and replace the cap of the bottle.

Sample from the tap cont'd…

- Using a water-proof marker or grease pencil, number the bottle with the sample code number.

Note:

- Leaking taps may cause contamination of the sample from sources outside the water pipe and therefore leaks should be reported when sampling.

- A bacteriological sample should not be taken until the leak is repaired.

Collecting a sample from a street hydrant

- Collect a sample from a suitable tap supplying water direct from the main.
- Where this is not possible, obtain a sample from a street hydrant by the following method:

1. Pour hypochlorite solution (5–10% v/v) into the duckfoot or hydrant bowl. If the hydrant box is full of water it must be baled out until 1 inch below the top of the duckfoot or bowl and enough water must be displaced to allow room for the hypochlorite solution.

2. Immediately screw on the standpost with the bibcock shut.

3. Open the hydrant valve and bibcock until water runs, then shut the bibcock

- Using a water-proof marker or grease pencil, number the bottle with the sample code number.

Note:

- Leaking taps may cause contamination of the sample from sources outside the water pipe and therefore leaks should be reported when sampling.

- A bacteriological sample should not be taken until the leak is repaired.

4 Allow at least one minute to elapse.

5 Open the bibcock fully and allow water to run for at least 2 minutes.

CAUTION: Avoid being splashed by hypochlorite-charged water.

6 Make a residual chlorine test (see the end of this subunit) to make sure all the hypochlorite-charged water has been flushed to waste.

7 Screw down the bibcock to a small stream, and aseptically collect the sample.

8 Number the bottle with the sample code number.

Collecting a sample from a river, stream, or other surface water

1 Aseptically remove the cap and cover of the sterile sample bottle, and face the mouth of the bottle upstream (i.e. towards the flow of water).

Note: To avoid entering the water, the bottle should be clamped to the end of a stick.

One way of doing this is to fix the bottle neck in a retort stand clamp and mount this on a stick.

2 Plunge the neck downwards about 30 cm below the water surface, and then tilt the neck slightly upwards to let it fill completely before carefully replacing the cap and cover.

- Where there is no current, push the bottle forward horizontally until it is filled.

3 Label the bottle with the sample code number.

Collecting a sample from a tubewell

1 Continuously operates the handpump for 5 minutes.

2 Heat the mouth of the pump, preferably by means of a blow lamp or gas torch, and pump several gallons of water to waste.

3 Aseptically collect a sample of water by allowing the water from the pump to flow directly into the sterile bottle.

Collecting a sample from an open well

- If the well is one from which water can be raised only by means of a bucket and rope, use a weighted bottle to collect the sample as follows:

1 Tie a sterile sample bottle on to a weighted length of rope or strong string.

- Use a stone or heavy piece of metal as a weight, and attach the bottle just above the weight.

From an open well cont'd…

2. Aseptically remove the cap from the bottle, and lower the bottle into the well to a depth of about 1 meter.

3. When no more air bubbles rise to the surface, raise the bottle out of the well and carefully replace the cap.

4. Label the bottle with the sample code number.

Transporting water samples to a water testing laboratory

- Immediately after collection, samples should be placed in an insulated cold box for transport to a water testing laboratory.

- Water samples should be examined as soon as possible on arrival and always within 6 hours of collection.

- Whenever possible, process water samples in the field.

Frequency of sampling

- In large treatment plants, it should be a routine to sample water daily at each stage of treatment.
- In many tropical rural areas, however, untreated sources of water are used.
- In these situations, periodic sanitary surveys of the raw water should be carried out to establish the level of risk of epidemic waterborne disease to which the population is exposed.

Frequency cont'd …

- The survey should include an on-site inspection and evaluation of the water supply system, and a bacteriological analysis of the water.
- From such a survey, sources of pollution can often be identified and measures taken to prevent future contamination.
- The frequency of sampling water in distribution pipes, unchlorinated water supplies before distribution, and chlorinated water before distribution is as follows:

Water in distribution pipes

- Water quality deteriorates as a result of corrosion in pipes allowing leaks and infiltration.
- The larger the population served, the greater the risk of contamination.
- At least one sample /5000 population / month should be examined
- Random routine sampling procedure should be established .

Unchlorinated water supplies before distribution

- According to WHO - maximum interval between successive samples for bacteriological analysis :
  - **Population served Maximum interval**
    - Less than 20 000 . . . . 1 month
    - 20 000–50 000 . . . . 2 weeks
    - 50 000–100 000 . . . . 4 days
    - >100 000 ………………… 1day with continuous chlorine residual recording

Potability

- The term that refers to the drink ability of water (Potable or unpoatble/ non potable).

- In unpolluted water (Mountains, lakes or streams) the number and types of bacteria is low and mainly Actinomyces, Bacillus, Sterptomyces, Clostridium species are commonly found.

- In polluted water, on the other hand you may have Coli forms, Enterobacter, Sterptocoous, Proteious and Pseudomonas species may found in high number

Types of Water Pollution

A. ***Physical Pollutions***:

- When a particulate matters, like sand or soil make water as cloudy.
- Some living materials also present.

B. ***Chemical Pollution***:

- This is due to introduction of organic and inorganic wastes.

C. ***Biological Pollution***:

- When microorganisms contaminate water bodies.

The Biochemical Oxygen Demand

- It is a critical measurement in polluted water .
- It refers to the amount of oxygen that microbes require to decompose water organic matter.
- The dissolved oxygen is measured at different intervals usually immediately after collection and after five days intervals.
- The BOD is reported in PPM.

Bacteriological Analysis Of Water

- Water samples could be contaminated with different bacteria.

- All bacteria may not be detected for quality measure. Hence indicator organism like Coliforms could be detected in routine public health laboratory.

Water Sample Collection

- Raw or tap water could be taken us a sample under standard conditions with appropriate containers.

Methods of Microbial Detection

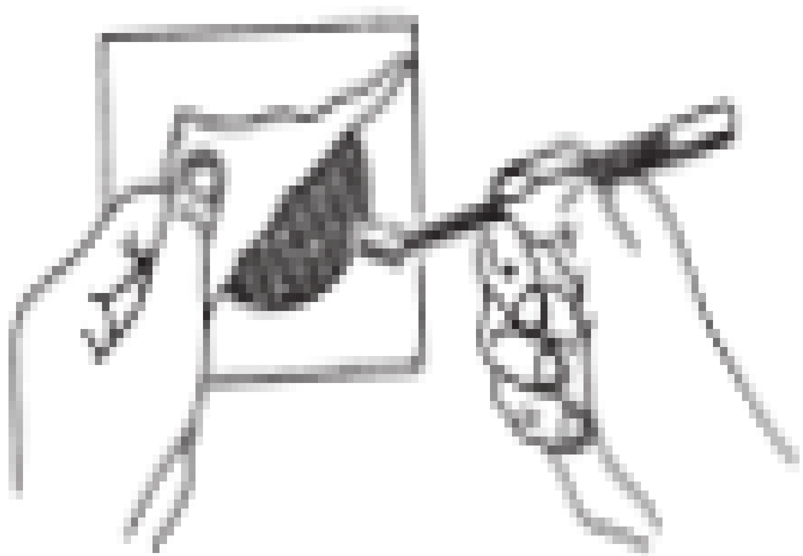
I. MEMBERANE FILTER TECHNIQES

- This method is a popular method, were sample of water (100 ml) is passed in a filter by negative pressure and the filter is plated on different medium.

- The bacterial colony is counted and reported per 100 ml of sample.

Membrane filter technique procedure

1. Adding sterile broth in
cellulose pad in a petridish



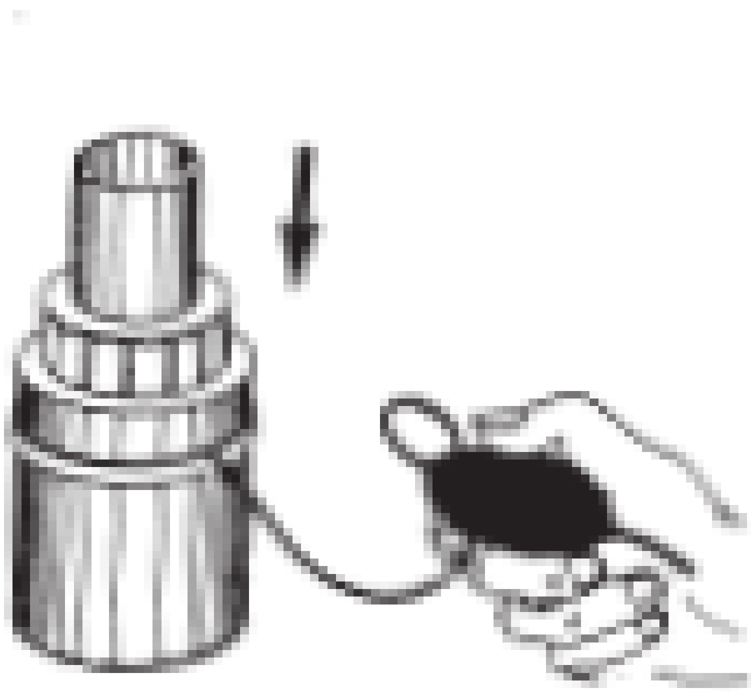
2. Aseptically removing the
sterile membrane

Procedure cont'd…

3. Placing the membrane on the filter base

4. Pouring the water sample In the filter unit

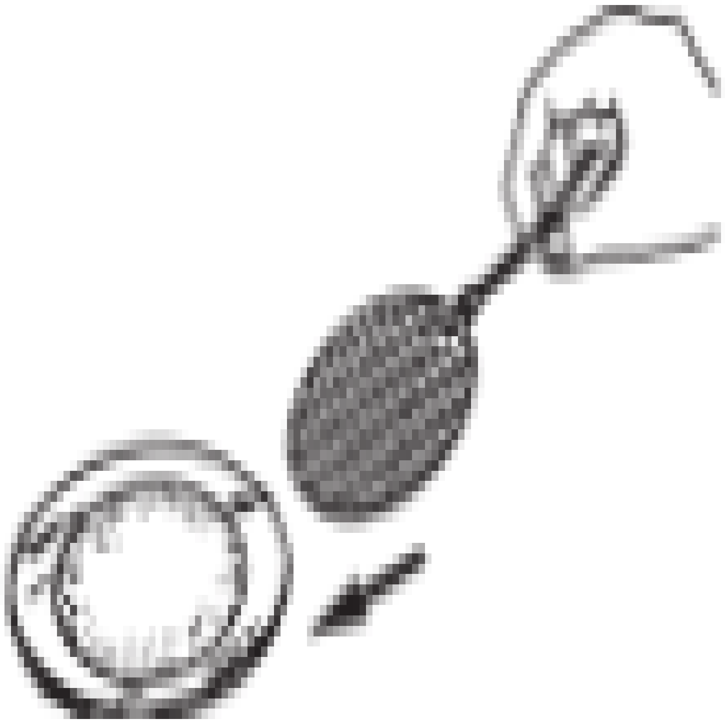
Procedure cont'd…



5. Drawing the water through the Membrane by suction

6. Removing the membrane

Procedure cont'd…



7.Placing the membrane on
broth imprignated pad

8. Labeling the Petridish
before incubation

II. STANDARD PLATE COUNT METHOD

- A sample of water is diluted in a sterile buffer solution and specified amount will be inoculated in agar medium and incubated.

- The total colony will be enumerated and multiplied by the dilution factor for final report.

III. Most probable number (MPN)

- Water sample of 0.1 ml, 1ml, and 10 ml will be inoculated in a medium (lactose broth tube and coli forms will be detected.
- By comparing the MPN table with the dilution on different tubes an estimate will be done.
- Specific tests are also available for individual indicator organisms.
- MPN analysis is based on certain assumption and formula

- According to Thomas Formula
- MPN/ ml or gm of sample = (∑ gi)/ (∑ti mi ∑(ti.gi)mi) (1/2)

- Where; The summation is over selected dilutions;

- ∑ gi …..No. of positive tubes

- ∑ timi…….The volume or gm of sample in all tubes in the selected dilution

- ∑ (ti.gi)mi …… The volume of sample (gm) in all negative tubes in the selected dilution

Eg.1. Assume the dilutions are 1.0; 0.1, 0.01, 0.001, and 0.0001 ml or gm

- For outcome (5/5, 10/10, 4/10, 2/10, 0/5) use only tubes (--4/10, 2/10,..);
- $\sum$ timi= 10 x 0.01+ 10 X 0.001 = 0.11

- If the No. of negative tubes at 0.01 and 0.001 dilutions are 6 and 8 respectively,====
- 6 x 0.01 + 8 x 0.001= 0.068;   and the number of positive tubes are 6;
- Then MPN/ ml of samples = 6 / (0.068 x 0.11) (1/2) = 6 / 0.086 = 70 coli forms/ ml of water sample.
- For Outcomes (5/5,10/10, 10/10, 0/10, 0/5…); Here use only)--,10/10,..) by Thomas Formula
- MPN= 10/ (0.01 X 0.11) (1/2) = 10 / 0.322 = 300/ ml of samples.

Analysis of milk

- Milk is extremely nutrious food. It is an aqueous solution of proteins, fats, and carbohydrates.
- The PH of milk is about 7.0 and this PH is an excellent medium for growth of microorganism. 87 % of milk is water and 2.5 % is Caseins.
- In addition Lacto albumin is present.
- Lactose the commonest carbohydrates in milk and called Milk sugar.

Spoilage of Milk

- Lactobacillus and Streptocoous can ferment lactose in milk that will result in sour curd.

- Sweat Curdling: This happens when Bacillus, Proteious, Micrococous attacks caseins enzymatically.

- It can be also contaminated by gram negative rods, E.coli, E.aerogens

- **Ropiness**: Formed from Enterobacter, Klebsella and Alcalegens.

Milk Born infection

- Theses are mainly caused by M. bovis, Q-Fever, Brucelosis, Coxiella burneti, Campylobacter, Salmonella, Listeria.

Pasteurization

- It is the process of eliminating the least microorganism to make sterile milk (Relative sterility or commercial sterility.

Methods

- LTLT: Low temperature, long Time: This is pasteurization of milk at 62 0c for 30 minutes .

Methods of pasteurization cont'd…

- HTST: High temperature Short Time pasteurization: This is pasteurization of milk at 71 0c for 15 minutes.

- Ultrapasturization: Pasteurization at 82 degree centigrade for 3 minutes. The shelf life of this type of milk is long.

Milk Standards

- The quality of milk may vary from place to place (state to state) in terms of temperature, bacterial load and other chemical tests.

Grade A milk: Pasteurized grade A milk

- This type of milk is pasteurized and the total bacterial limit is 20,000/ml.

- Coli forms should not be more than 10 /ml and Phosphatase activity must be less than 1 ug/ml.

Milk grade cont'd…

Grade B milk/ Pre pasteurized Milk

- This type of milk may not be pasteurized and the total plate count should not exceed 1000000/ml.

- The coli form count should not exceed 10/ml and there should be no inhibitory substances.

Some Useful Laboratory Tests For Milk Analysis

I. Somatic cell count (SCC):

- **SCC** is a measure of the white blood cell count in milk.

- The SCC in milk of an individual ewe indicates her udder health status, and bulk tank milk SCC can indicate the general state of udder health in a cow/ sheep .

- Somatic cells are always present in milk, but the SCC will rise when an infectious agent enters the udder or when the udder has been injured.

SCC cont'd …

- A major consequence of rising SCC is a decrease in raw milk quality, which has implications for milk processing.

- Most studies have found SCC levels for a healthy udder could range 250,000 to 1,600,000 cells/ml.

- There are negative economic consequences for the producer if somatic cell counts are elevated in the animals.
- A high SCC is a strong signal of an udder infection, referred to as mastitis.
- Common clinical signs of mastitis are a swollen udder half, abnormal color of milk, or clots present in the milk.

- High SCC also has a negative effect on milk processing through decreased yields and off-flavor development in finished products.

- Milk with a SCC>1,000,000 decreased the cheese yield and increased the development of rancid flavors in the cheese

II. The phosphatase test

- Phophatase normally present in milk.
- The enzyme is heat tolerant similar with the phosphatase of M.bovis and Coxelella burntii.
- The test is done by, milk sample is incubated with substrate like Sodium diphenyl phosptae and the mixture is incubated.
- The phosptae released by the enzymes and the colour is changed and detected with chromogen.
- In properly pasteurized milk the test is negative and the colour change is absent and vice versa.

III. The Standard Plate Count

- It is based on the total bacterial colonies grow on a culture medium in 1 ml of sample.
- A serial dilution of milk sample is done as 1;10, 1;100, 1;1000, 1;100000 and incubated.
- The colony falls between 30-300 is selected and the total bacterial count is estimated by multiplying the dilution factor.

- Disadvantages
  - Do not detect Psychrophilic and Thermophilic bacteria
  - Do not detect anaerobic bacteria, viruses and molds
  - The coli form bacteria is used as an important indicator of the sanitary quality of milk that is isolated on a violet red bile agar.

IV. The Dye Reduction Test

- It tell us the relative amount of bacteria in the sample.

- Test principle:
  - A sample of milk usually I1 ml is mixed with dyes (methyline blue or Resazurin) and incubated at 37 0c.

  - Up on growth of bacteria the blue color is reduced to colorless product in case of methyline blue or pink to colorless in case of resazurin.

- Poor quality milk may have complete reduction of methyline blue after 2 hrs.

- While good quality milk has minimal colour changes.

- The test has to be done on lactose fermenter organism and most useful if the CFU is more than 100,000/ml of milk sample.

V. THE BREAD COUNT METHODS

- In this test, 0.01 ml of milk sample is taken using a breed pipitaes and spreaded on microscopic slide.
- One square cm area is taken and after staining with Newmann-Lampart stains (Methylne blue), the average number of bacteria per field is reported.
- One of the disadvantage of breed counting method is it does not differentate live and dead cells , debris and the procedure is tiresome.

VI. Antibiotic detection test

- The test is useful for animals that are treated for M.bovis and Q.fever, Brucellosis.
- The milk may have the antibiotic and inhibit a certain indicator organism (B. subtils) (zone of inhibition).
- Penicillin antibiotic can be detected using Penicilinase test

Summary

- Milk is contaminated by gram positive rods , E.coli and E. aerogens
- There are three methods for pasteurization of milk
- Based on coliform count and some chemical activity , milk is graded as grade A and B

Summary cont'd…

- Different laboratory tests for milk analysis
  - Somatic cell count
  - Phosphatase test
  - Standard plate count
  - Dye reduction test
  - bread count methods
  - Antibiotic detection test

Summary cont'd….

- water can be polluted/unpolluted based on the number and type of bacteria present
- There are three types of water pollution
- Different methods of microbiological analysis of water
  - Membrane filter method
  - Standard plate count
  - Most probable number

END

THANK YOU