

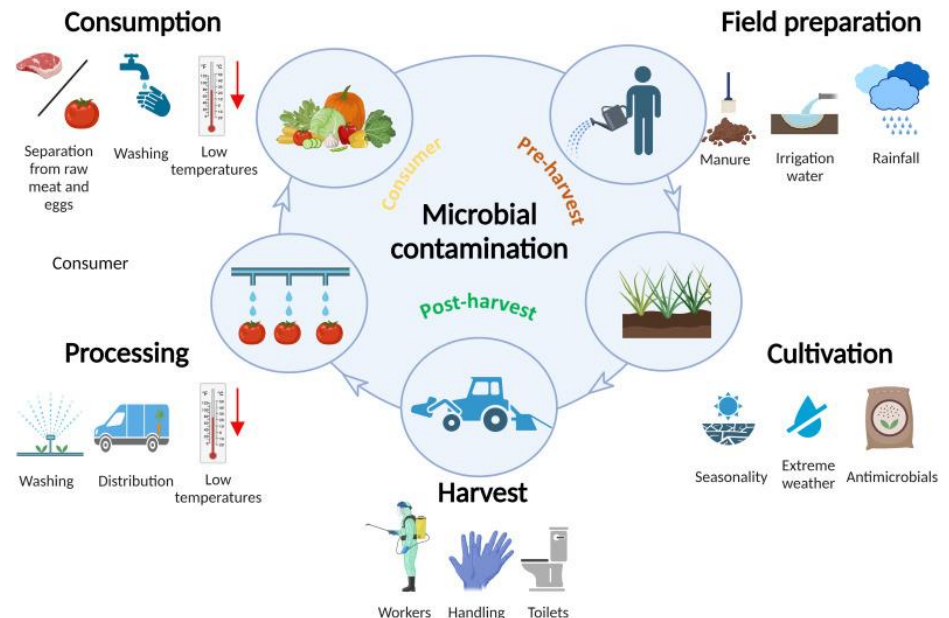
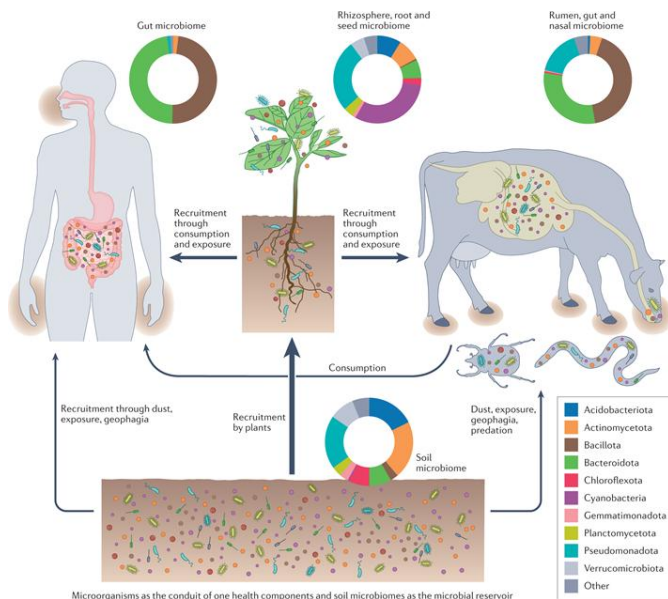
FE 305 FOOD MICROBIOLOGY

Source of Microbial Contamination of Foods

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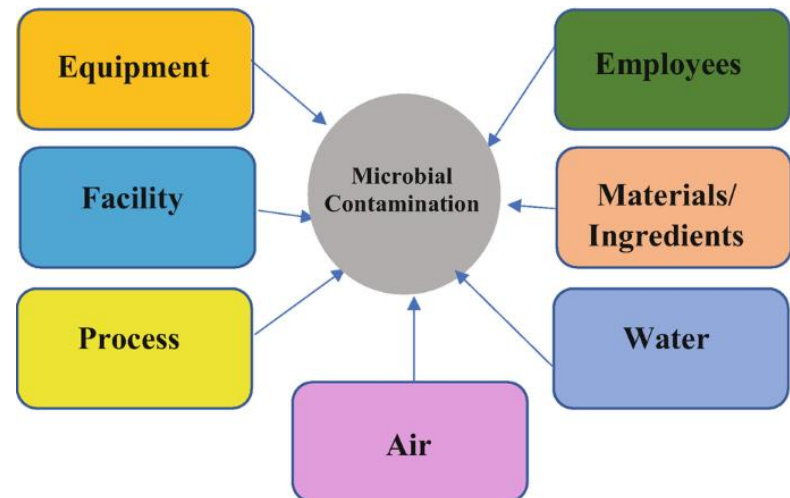
Introduction

- The microorganisms present in a food originate from two main sources:
 - the natural microflora (microbiota) of the food itself, and
 - contamination from soil, air, dust, water, animals, birds, insects, equipment and people
 - during harvesting, slaughtering, processing, storage, distribution and preparation.

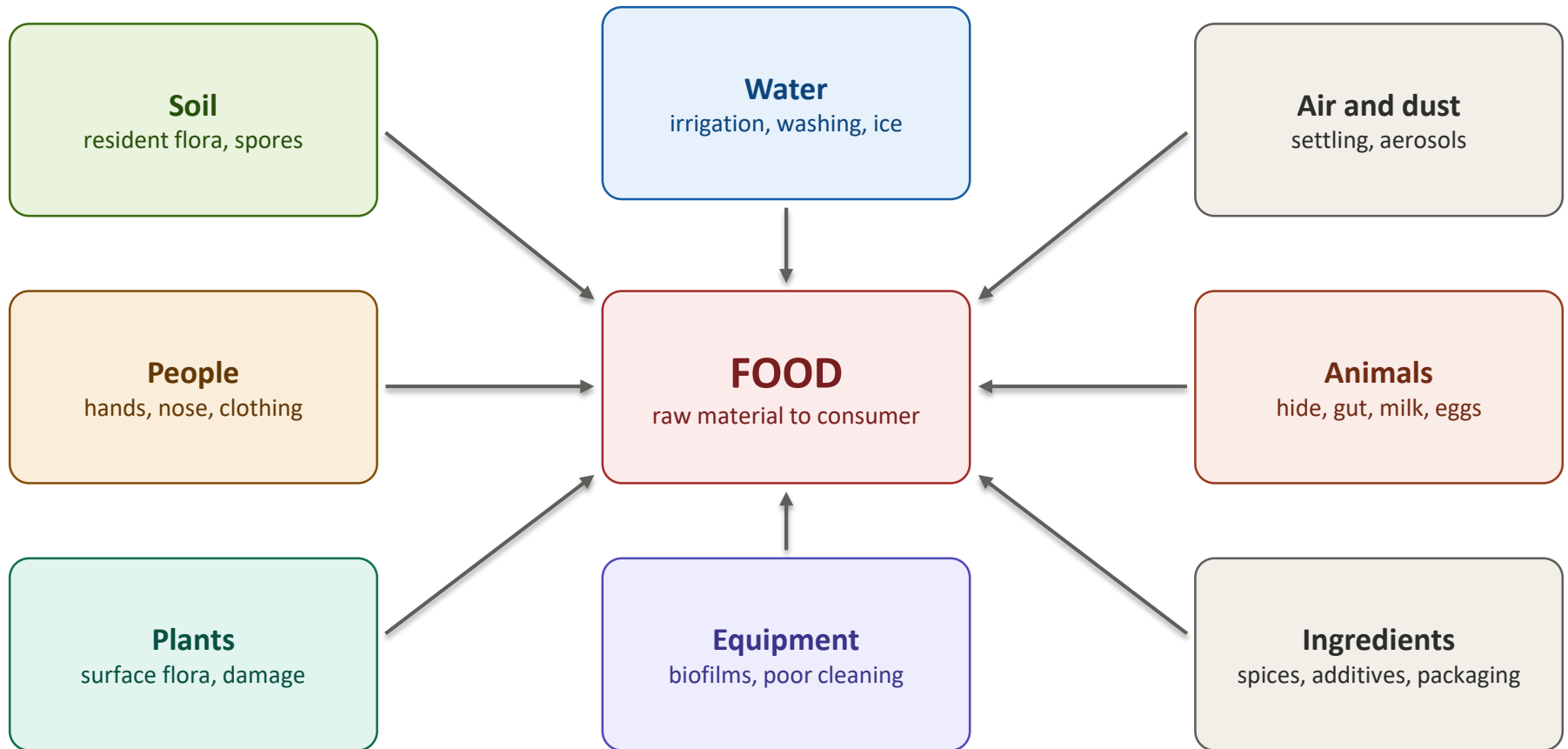


Sources of Microbial Contamination

- The microflora of a food consists of the microorganisms naturally present in it together with those that contaminate it from the environment during harvesting, storage, transport and processing.
- In order to prevent foodborne infections and intoxications and to extend the shelf life of foods, contamination must be prevented or kept to a minimum.
- The sources of microbial contamination of foods are:
 - Soil
 - Water- sewage
 - Air
 - People
 - Animals and plants
 - Equipment
 - Raw materials and ingredients



Sources of Microbial Contamination



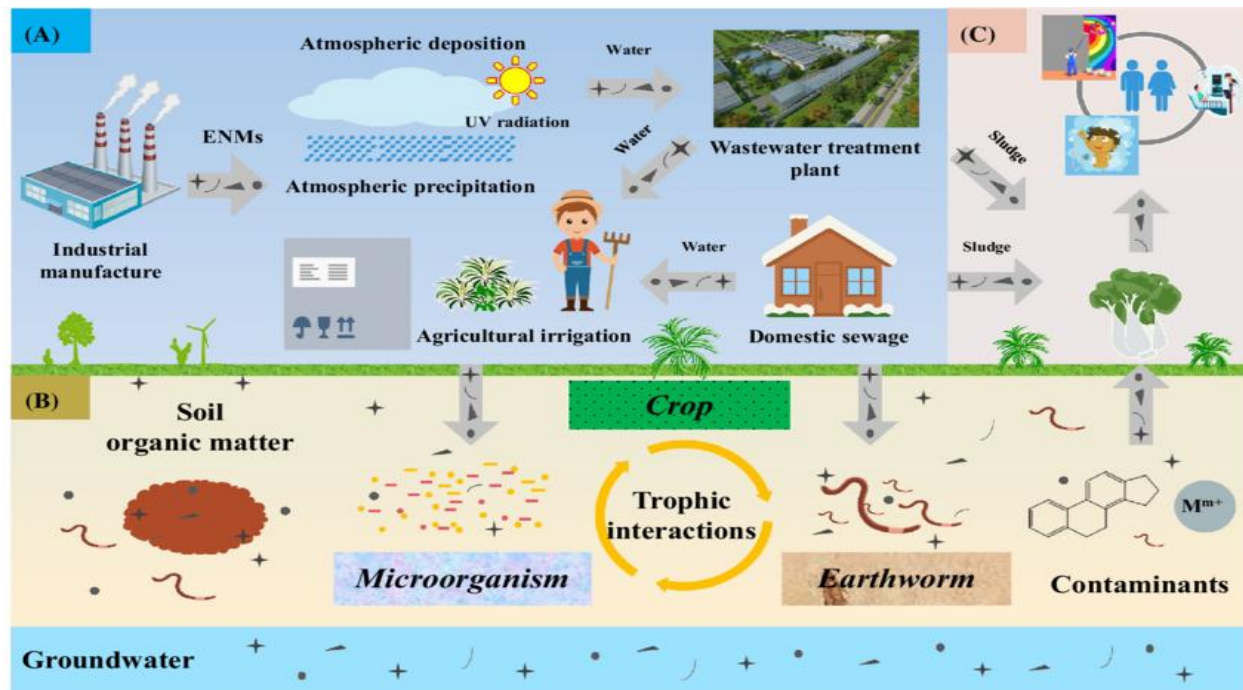
The natural flora of the food is joined at every step by organisms from these eight sources; preventing contamination is always cheaper than removing it later.

Soil

- Soil is the natural habitat of a very large number of microorganisms. The microbial density is higher at the surface than in the deeper layers; most microorganisms are found in the upper 50 cm of the soil profile.
- The number of microorganisms in soil varies with its moisture content, pH, temperature, organic matter and whether or not it has been fertilised.
- Soil microorganisms are transferred to fruits and vegetables by direct contact and through tubers and roots. Contamination also occurs when the wind carries dust from the soil surface, or when heavy rain splashes soil onto crops growing close to the ground.
 - Cereals are most susceptible to contamination during harvesting.

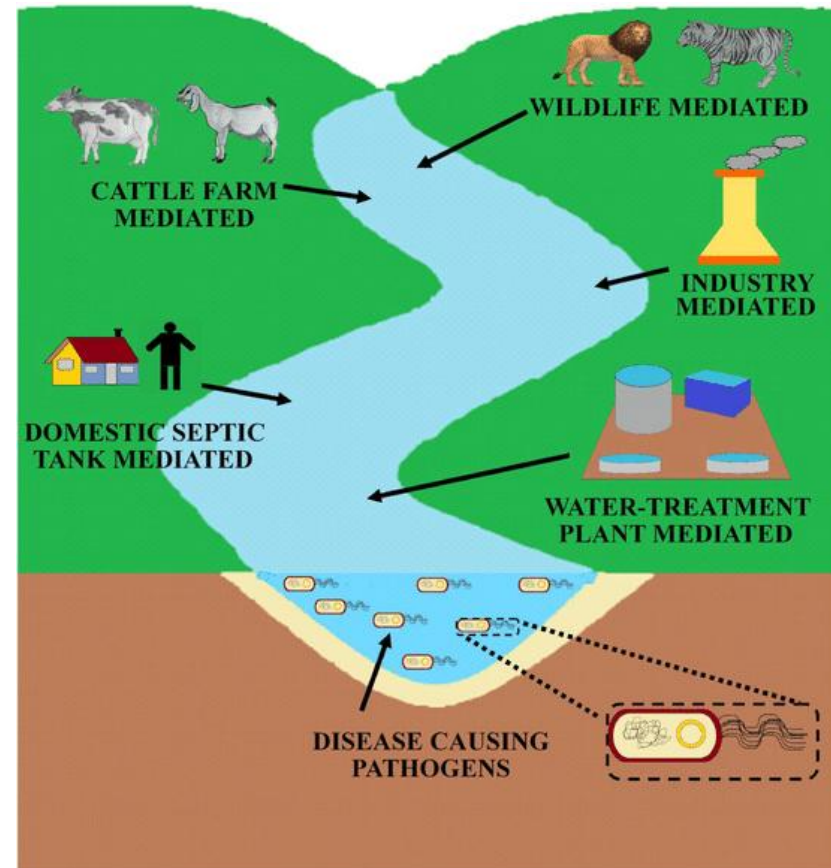
Soil

- When the soil surface dries, the microorganisms in the resulting dust are carried by the wind to other soils, rivers and oceans.
 - Bacteria of the genera *Alcaligenes*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Kocuria* (formerly *Micrococcus*), *Listeria*, *Pseudomonas* and *Serratia*, together with actinomycetes, moulds and many yeasts, are typical soil microorganisms.
 - Yeasts are especially common in the soils of orchards and vineyards.
 - Moulds occur in all soil types, usually in the form of spores.



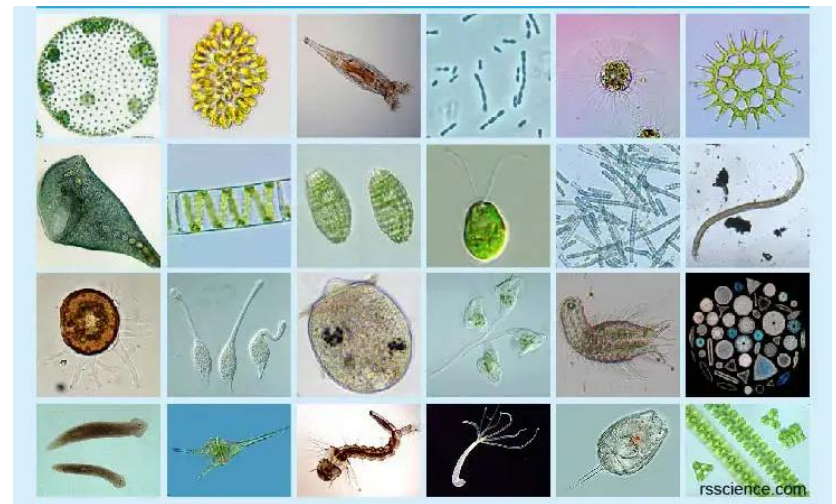
Water

- Water comes into contact with food at every stage, from crop production to the cleaning of food-contact surfaces. Water also carries its own natural microflora.
- Water is used for:
 - irrigation of crops;
 - drinking water for animals;
 - fisheries and the production of marine foods;
 - washing of foods;
 - processing and storage of foods (for example, icing of fish);
 - cleaning of processing plants and transport vehicles; and
 - as an ingredient in many processed foods.
- The quality of the water therefore has a major influence on the microbiological quality of the food.



Water

- Microorganisms are also dispersed when clouds formed over large bodies of water are carried by the wind and return to the soil as rain; the microorganisms of water and soil are therefore largely the same.
- The following foodborne bacteria are transmitted mainly from water and soil:
 - *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Citrobacter*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Kocuria*, *Proteus*, *Pseudomonas* and *Serratia*.
 - Almost all mould genera are found in water and soil.
 - Moulds degrade plant and animal material, cause plant diseases and are very widespread in nature.
 - For example: *Aspergillus*, *Rhizopus*, *Penicillium*, *Trichothecium*, *Botrytis* and *Fusarium*.
 - Because most yeasts are associated with plants, their numbers in water are relatively low.



Water

The microorganisms found in water can be examined in three groups:

1. Microorganisms naturally present in water

- Certain bacteria with an optimum growth temperature of 25 °C or below, such as *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Kocuria* and *Aeromonas*.

2. Microorganisms originating from soil

- This group includes saprophytic members of the genera *Bacillus* and *Streptomyces* and of the family Enterobacteriaceae, which reach the water when soil is washed away and which also have an optimum growth temperature of 25 °C or below.

3. Microorganisms from the intestinal flora of humans and animals

- *Escherichia coli*, *Enterococcus faecalis* (formerly *Streptococcus faecalis*), *Clostridium perfringens*, *Klebsiella*, etc., which indicate faecal pollution of the water. They reach the water from human and animal faeces and urine. Such water must not be used for drinking or for food production.

Microorganisms Found in Water

Natural water flora

Optimum growth 25 °C or below

Vibrio
Pseudomonas
Acinetobacter
Aeromonas
Kocuria

Washed in from soil

Saprophytes carried by run-off

Bacillus
Streptomyces
Enterobacteriaceae
Moulds
Actinomycetes

From human and animal gut

Indicate faecal pollution

Escherichia coli
Enterococcus faecalis
Clostridium perfringens
Klebsiella
Norovirus, hepatitis A

Cause spoilage of fish and chilled foods

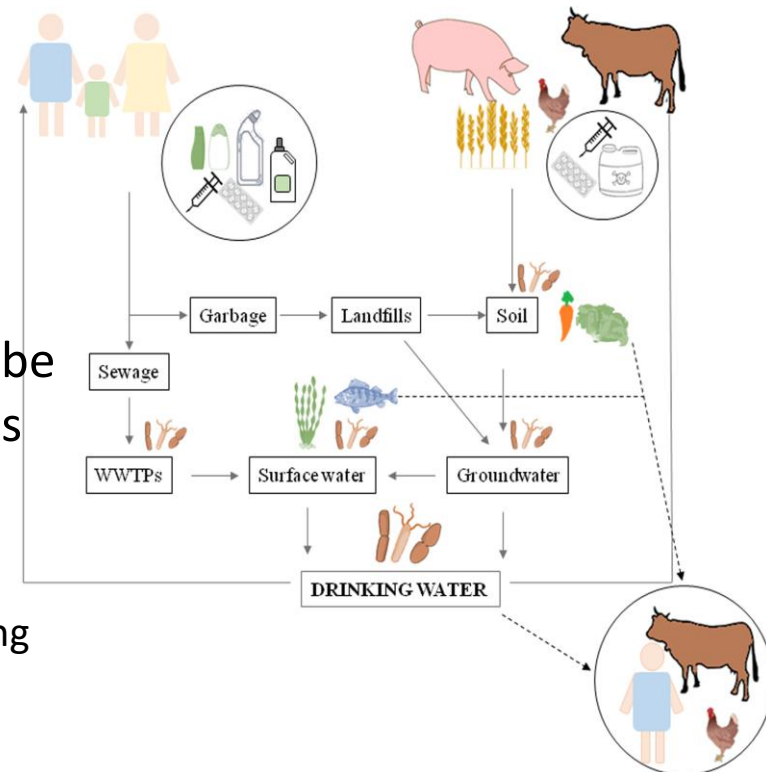
Bring spores into the process water

Such water must never be used for food

Water used in food production must meet drinking water standards. Chlorination, UV or ozone treatment is applied before the water contacts the food or the food-contact surfaces.

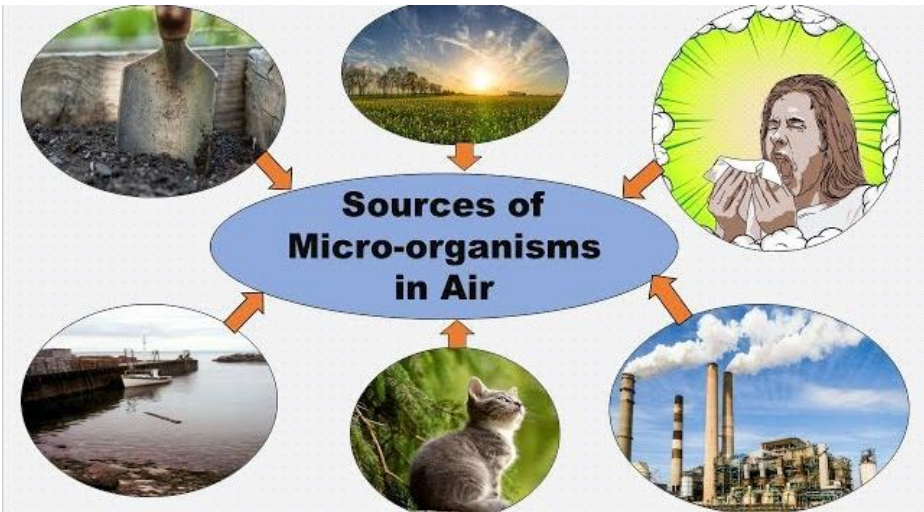
Water

- Water transfers microorganisms to foods
 - directly, or indirectly through hands, equipment and utensils.
- Water may contain spoilage microorganisms such as
 - *Alcaligenes*, *Bacillus*, *Chromobacterium*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Flavobacterium*, *Kocuria*, *Proteus* and *Pseudomonas*.
- Inadequately treated water may also contain pathogens such as
 - *Salmonella*, STEC, *Campylobacter*, *Vibrio cholerae*, norovirus and hepatitis A virus.
- Treated (for example chlorinated) water must be used for processing, washing, sanitation and as an ingredient.
- Water used in food production must
 - comply with the microbiological standards for drinking water and
 - be acceptable in sanitary and economic terms.



Air

- Air has no microflora of its own. The microorganisms in air generally originate from dust, soil and plants; they are raised by the wind and, once suspended, form the transient microflora of the air. People also affect it: millions of microorganisms are released into the air by coughing, sneezing and talking. In enclosed areas in particular,
 - the rate of air flow,
 - the number of people present and
 - the movement of those people determine the microbial load of the air.
- The microorganisms commonly found in air are *Streptococcus* and *Lactococcus* species in dairy plants and yeasts in breweries.
- In food establishments, the microbial load in the areas where raw materials or live animals are handled is much higher than in clean (high-care) areas.



Air

- Airborne contamination is prevented by filtering the air taken in from outside, by keeping the plant under slight positive pressure so that air moves from the inside outwards, and by directing the air flow from clean areas towards less clean areas.
- Air and dust may contain almost all types of microorganisms. Although they are not the main source of contamination, *Staphylococcus* and *Salmonella* species may also be present in air and dust.
- Among the microorganisms in air and dust, *Bacillus* and *Kocuria/Micrococcus* species, which are resistant to drying to various degrees, are particularly noteworthy. Various moulds and yeasts are also encountered from time to time.

People

- The microflora of the human body begins to develop at birth. People become contaminated with microorganisms from every environment with which they come into contact.
- They can also transfer these microorganisms to their clothes, to objects, to other people and to foods.
- The microflora of the human body varies from person to person and with external factors. Washing and personal cleaning agents particularly affect the skin microflora, while medicines taken and foods consumed affect the microflora of the digestive system.
- Food comes into contact with people at many stages between harvest and the final consumer (preparation, processing, packaging, storage and transport).



People

- This makes people the single most important source of contamination. Hands, clothes, hair and beard, jewellery, breath and saliva are the main points affecting the microbial load of the food.
- The principal microorganisms involved are *Staphylococcus aureus* in the nasal and throat mucosa and *Escherichia coli* in the intestine.
- For this reason, personnel hygiene and training are essential for the prevention of contamination.
- The microbiota found on the hands of food workers and on the outer surface of their clothing gives important clues about the worker's habits and environment.
 - This microbiota may consist of organisms from any object the person has touched, or it may originate from dust, water, soil and similar sources.
 - Many different microorganisms are transferred from the hands, the nasal cavity and the mouth.
 - The most notable of these genera are *Staphylococcus* and *Kocuria/Micrococcus*; staphylococci are commonly found on the hands, arms, nasal cavity, mouth and other parts of the body.
 - *Salmonella* and *Shigella* are mainly of intestinal origin and are transferred to foods when sanitary rules are not observed.

Hand Washing: Breaking the Human Route

1. Wet hands

with clean running water

2. Apply soap

enough to cover all surfaces

3. Palm to palm

rub hands together

4. Backs and between

right over left, fingers interlaced

5. Backs of fingers

interlocked, rub against palms

6. Thumbs

rotational rubbing of each thumb

7. Fingertips and nails

rub against the opposite palm

8. Rinse and dry

single-use towel; turn the tap off with it

When

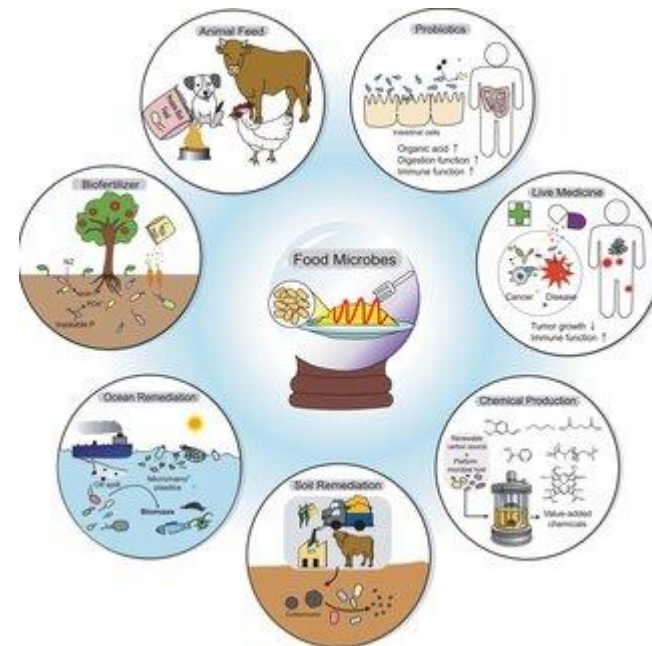
before handling food and after breaks
after the toilet and after raw meat or eggs
after touching face, hair or phone
after handling waste or cleaning agents

Remember

40 - 60 seconds in total
most often missed: thumbs, fingertips,
between the fingers
gloves and alcohol gel do not replace washing

Animals

- The muscle tissue of a healthy animal is considered to be essentially sterile.
- After slaughter it begins to be contaminated with dust, soil, hide and intestinal microorganisms.
 - These include pathogenic species such as *Brucella*, *Coxiella*, *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, *Salmonella* and *Campylobacter*, as well as non-pathogenic soil bacteria and some parasites.
- Farm animals become infected through contaminated feed or through contact with the droppings of other animals and birds.
- Milk in the udder of a healthy animal is practically sterile; contamination occurs during and after milking from the udder surface, the hands of the milker and the milking equipment and containers.
 - The raw milk flora may contain *Staphylococcus* and *Kocuria/Micrococcus* species.
 - *Staphylococcus aureus* is found in the milk of animals suffering from mastitis.
- Eggs from healthy hens are regarded as sterile immediately after laying, but they may be contaminated with *Salmonella* from the faeces, or infected via the ovary (transovarian transmission of *S. Enteritidis*).
- Insects, flies, pests and rodents play an important role in carrying microorganisms and transferring them to foods.



Animals

Animal Feeds

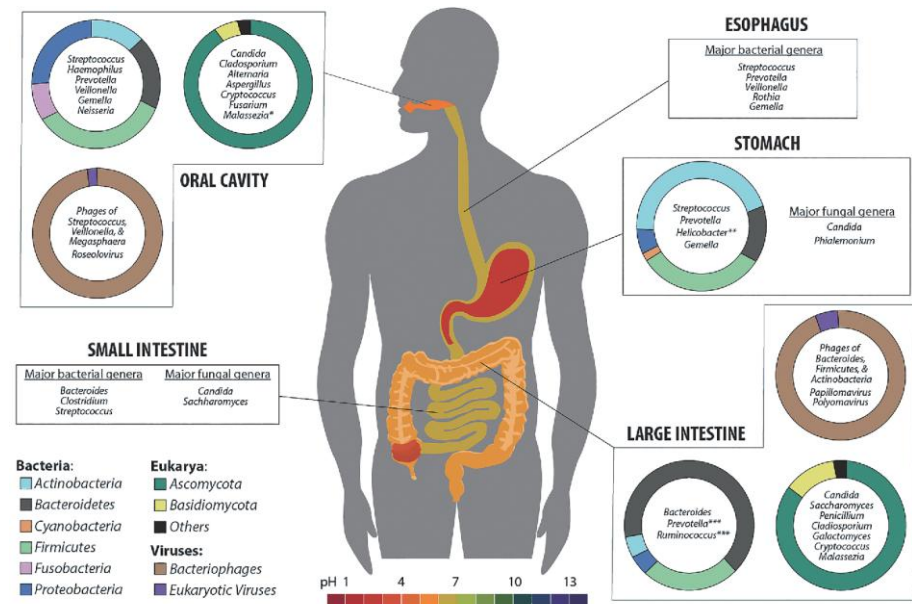
- Any or all of the bacteria, moulds and yeasts mentioned so far may be present in animal feed.
- The types of microorganism found in a feed depend primarily on the source and type of the feed, on whether a microbicidal process has been applied, on the packaging material in which it is stored and on similar factors.
- Feeds play an important role in the spread of *Salmonella*, which causes foodborne infection. Once these organisms appear in one part of an establishment, they spread very rapidly.

Animal Skins and Hides

- All the microorganisms found in soil, water, feed, dust and faeces may also be found on animal skins and hides.
- These microorganisms can be transferred from the hides to the hands of workers or directly to the food.
- Some organisms from the hide microbiota can settle in the lymphatic system of slaughter animals and pass into the muscle tissue after slaughter.

Intestinal Tract of Animals and Humans

- The intestinal tract harbours bacteria that occur there far more frequently than in soil, water or other environments.
 - The most common of these are *Bacteroides*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Lactobacillus*, *Proteus*, *Salmonella*, *Shigella*, *Staphylococcus* and *Streptococcus*.
 - The most notable of these is the genus *Escherichia*, whose natural habitat is the intestinal tract of humans and other mammals.
- Other genera frequently found in the intestinal tract are *Clostridium*, *Citrobacter*, *Enterobacter*, *Klebsiella* and *Pseudomonas*.
- Intestinal bacteria pass directly from the gut of animals into soil and water, and from there they contaminate plants, dust and food containers.
- Although moulds are not generally considered to be part of the intestinal flora, yeasts of the genus *Candida* are frequently encountered, particularly in the human intestinal tract.



Intestinal Tract of Animals and Humans

- When foods are handled with contaminated hands,
 - pathogens of intestinal origin have a good chance of contaminating the food.
- Bacterial and viral pathogens from the intestinal tract can cause cholera, bacillary dysentery, typhoid fever and hepatitis A.
- The bacterial genera most commonly present in the intestinal tract of humans and animals are:

Bacteroides

Bifidobacterium

Citrobacter

Clostridium

Enterobacter

Escherichia

Fusobacterium

Klebsiella

Lactobacillus

Enterococcus

Pseudomonas

Salmonella

Shigella

Staphylococcus

Streptococcus

- Sanitation of foods and drinking water and the hygienic disposal of sewage are essential to prevent the spread of these microorganisms to the environment.
- People suffering from an infectious disease, and carriers, must not work in food production.

Plants and Plant Products

- Plants are contaminated by microorganisms from many different sources: soil, water, fertiliser, animals and so on. The natural flora also differs from one fruit or vegetable to another.
 - Yeasts such as *Saccharomyces*, *Wickerhamomyces* (formerly *Hansenula*), *Candida* and *Rhodotorula* occur in the natural flora of fruits and vegetables;
 - bacteria such as *Pseudomonas*, *Alcaligenes*, *Kocuria*, *Flavobacterium*, coliforms and lactic acid bacteria are frequently found in the plant flora.
- In vegetables irrigated with sewage-contaminated water, bacteria such as *Salmonella*, *Shigella* and *Vibrio cholerae* and protozoa such as *Entamoeba histolytica* may be encountered.
- Ground maize, wheat and rice contain spore-forming bacteria such as *Bacillus cereus* and *Clostridium perfringens*.
- Some bacteria are more closely associated with plants than with soil.
 - Among these, the genera *Acetobacter*, *Pectobacterium* and *Dickeya* (both formerly *Erwinia*), *Flavobacterium*, *Kurthia*, *Lactiplantibacillus*, *Leuconostoc*, *Pediococcus* and *Xanthomonas* are important. Genera other than those listed here may also occur on plants and plant products.
 - Among the moulds, the most important plant-associated species are those that cause spoilage during the marketing of fruits and vegetables.
 - The yeast genus *Saccharomyces* is found on many plant products, especially fruits; *Rhodotorula* is also a common plant yeast.

Plants and Plant Products

- Plants may also carry human pathogens,
 - originating from contaminated soil, water, hands, animals, air, harvesting equipment, untreated sewage and other sources.
- Fruits and vegetables carry a natural surface flora.
- Microorganisms are present mainly on the surface;
 - their type and number vary with the condition of the soil, the type of fertiliser and water used and the quality of the air.
- The natural surface flora of plants usually includes species of the following genera:

Acetobacter
Alcaligenes
Bacillus
Clostridium
Enterobacter
Pectobacterium

Flavobacterium
Lactobacillus
Leuconostoc
Listeria
Micrococcus

Pediococcus
Pseudomonas
Streptococcus
Xanthomonas

Plants and Plant Products

- Important plant-borne yeasts include species of *Saccharomyces*, *Rhodotorula* and *Candida* (formerly *Torula/Torulopsis*); they cause spoilage of fruits and vegetables (so-called market diseases).
- The mould genera commonly present on plant foods are *Aspergillus*, *Alternaria*, *Botrytis*, *Fusarium*, *Penicillium* and *Cladosporium*.
- Enteric pathogens may also be associated with plant foods,
 - especially if the soil has been fertilised with untreated sewage.
- The following factors increase the number and variety of microorganisms on plant foods:
 - plant diseases;
 - damage to the surface before, during and after harvesting;
 - long delays between harvesting and washing; and
 - unfavourable storage and transport conditions after harvesting.

Plants and Plant Products

- The microbial load of plant foods can be reduced by:
 - using proper handling practices (for example, using treated sewage or other types of fertiliser);
 - reducing mechanical damage during harvesting;
 - washing rapidly with good-quality water to remove soil and dirt; and
 - storing at low temperature until processing.

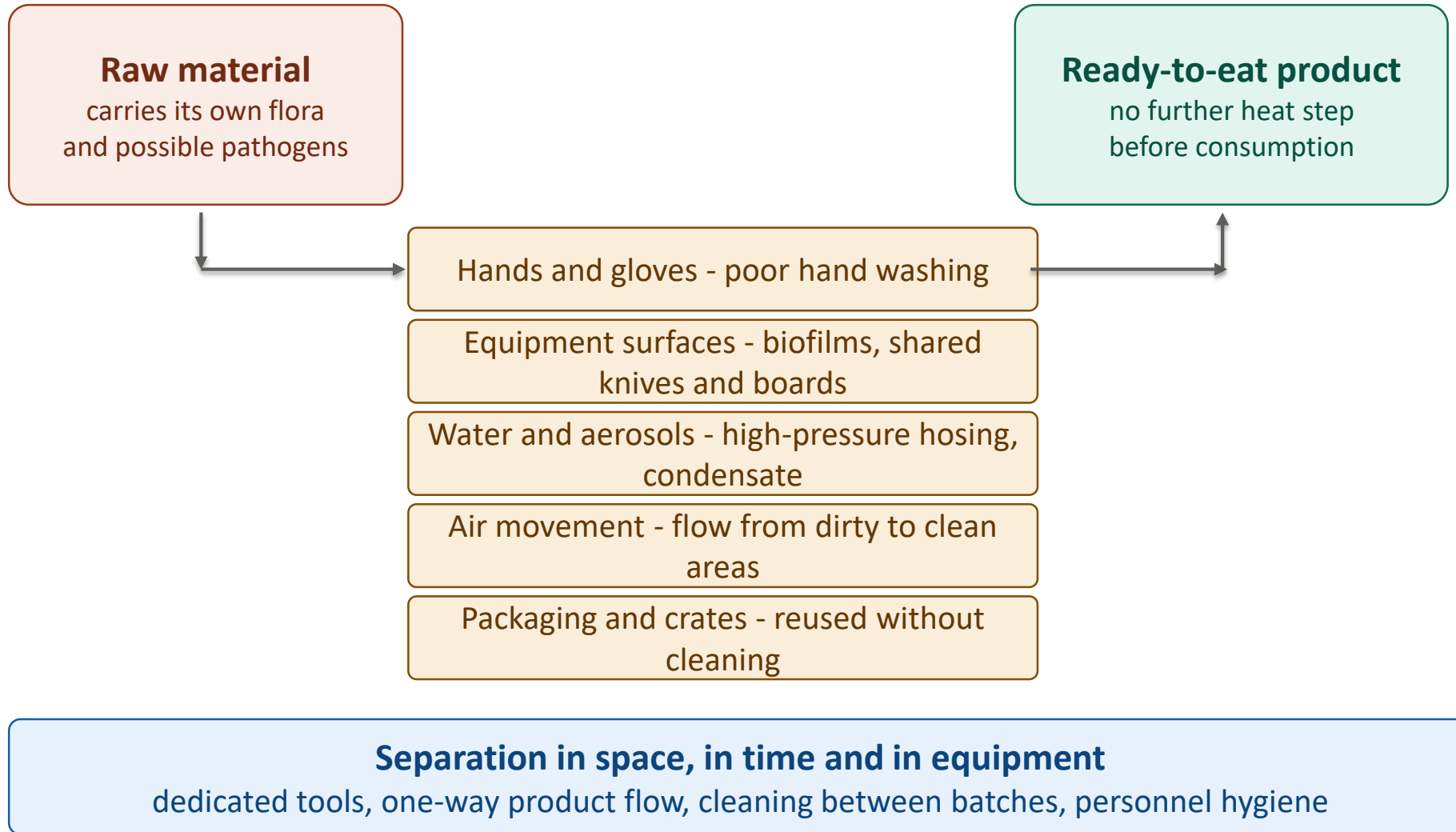
Equipment

- To allow effective cleaning in food establishments, sufficient space must be left between items of equipment and between the equipment and the walls and ceiling.
- All tools, equipment and containers that come into contact with food and are used repeatedly must be made of materials that are safe for health, smooth, easy to clean and non-contaminating. These materials must also resist the alkaline, acidic and saline agents used in cleaning and be resistant to heat and steam.
- Premises should be designed so as to prevent the entry of pests and environmental pollutants that may be sources of contamination.
- Wooden materials, which support microbial growth and are difficult to clean and disinfect, should not be used except where unavoidable.
- Separate equipment should be used for raw and cooked foods in order to avoid cross-contamination.
- The microorganisms present on food containers depend on the type of food processed, on how the containers are treated, on their storage conditions and on other factors.

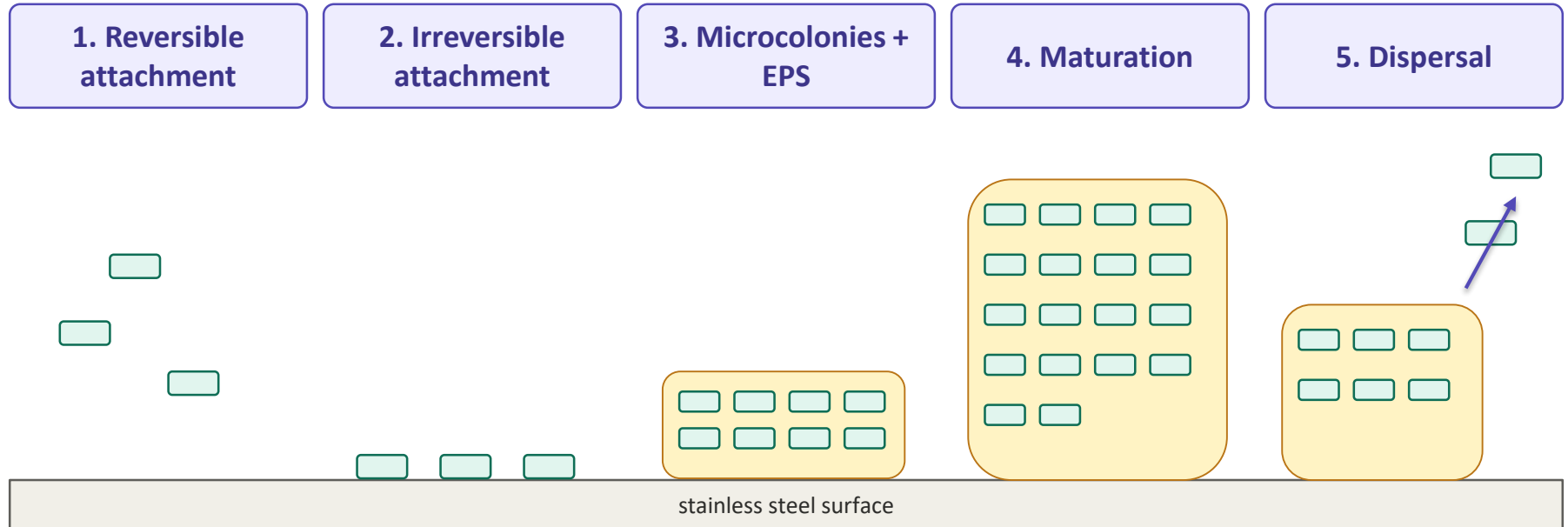
Equipment

- If the same containers are used continuously for the same vegetable, they will naturally carry the microorganisms associated with that vegetable.
- If the containers are washed with hot or boiling water, the remaining microbiota consists of the organisms that survive this treatment.
- If, on the other hand, the containers are stored in an open, dusty place, they will also carry airborne bacteria, yeasts and moulds.
- When processing equipment is used continuously for long periods, microorganisms multiply on it and form biofilms.
 - The equipment can then act as a continuous source of microorganisms for the product.
- Inadequate sanitation of utensils, cutting boards, knives, spoons and similar items can be a source of cross-contamination.
- *Bacillus*, *Clostridium*, *Enterobacter*, *Escherichia*, *Lactobacillus*, *Listeria*, *Leuconostoc*, *Kocuria*, *Pseudomonas* and *Salmonella*, as well as yeasts and moulds, can contaminate food from equipment.
- Packaging materials used in food production must also comply with the appropriate microbiological standards.
- Contamination from equipment can be reduced by:
 - adequate equipment,
 - hygienic design,
 - sanitation and
 - effective cleaning and disinfection.

Cross-Contamination in the Plant



Biofilm Formation on Food-Contact Surfaces



EPS = extracellular polymeric substances: a slimy matrix of polysaccharides, proteins and DNA

Why it matters

cells in biofilms are 10 - 1 000 times more resistant to sanitisers
Listeria, Pseudomonas, Salmonella
persist in drains, seals, conveyors

Control

clean first, then disinfect
mechanical action (brushing) is essential
hygienic design: no dead ends or crevices
verify with swabs and ATP tests

Environmental Monitoring in the Plant

Zone 1	Food-contact surfaces	conveyors, slicers, filling heads, moulds, hands and gloves
Zone 2	Non-contact, next to the product	equipment frames, control panels, covers, nearby walls
Zone 3	Remote areas in the production room	floors, drains, wheels, forklifts, cleaning tools
Zone 4	Outside the processing area	corridors, locker rooms, canteen, loading bay

What to look for

Listeria spp. as an indicator of the genus -
not only *L. monocytogenes*
Enterobacteriaceae as a hygiene indicator
ATP tests verify cleaning, not microbiology

How to sample

sample DURING production, not after cleaning
sponge a large area; target seams, gaskets,
hollow rollers, drains and standing water
record trends, not single results

Seek and destroy

A positive Zone 3 result is not a failure - it is information. Follow it with vector sampling towards Zone 1, dismantle the equipment, find the harbourage site, clean, re-test three times. EU Regulation 2073/2005 requires producers of ready-to-eat foods to sample the processing environment for *L. monocytogenes*; a persistent house strain can survive in a drain for years.

Raw Materials and Additives

- In addition to its natural flora, contamination of a food with microorganisms from air, water, soil, tools, equipment and personnel makes it a health risk. The consumption of contaminated foods can cause a variety of diseases.
- Additives, although used in small amounts, may carry very high numbers of microorganisms into the food. They are therefore an important source of contamination, especially in processed foods.
 - Since spices are of plant origin, they carry many microorganisms of soil, water and animal origin.
 - Heat-resistant *Bacillus* and *Clostridium* spores may survive in foods preserved by commercial sterilisation.
 - Fish salted with sun-dried (solar) salt containing halophilic bacteria may spoil (pink discoloration).
 - Osmophilic yeasts and xerophilic moulds present in concentrated syrups added to reduce water activity can cause spoilage.
 - Starch, sugar and flour may contain the spores of thermophilic bacteria.
 - Pathogens such as *Salmonella* may be present in dried egg and in chocolate.

Primary Sources of Foodborne Disease Agents

- The most important foodborne pathogens and their usual sources are:
- *Staphylococcus aureus* – the nasal cavity, skin and infected wounds of people and animals.
- *Salmonella enterica* serovars Typhimurium and Enteritidis – the intestinal tract of humans and animals, poultry meat and eggs.
- *Streptococcus pyogenes* – the nasal cavity and throat of people.
- *Bacillus cereus* – starchy foods such as rice and pasta dishes, spices and dried foods.
- *Campylobacter jejuni* – water, poultry meat and raw milk.
- *Listeria monocytogenes* – vegetables, raw milk, soft cheeses and ready-to-eat products; it persists in wet processing environments.
- *Brucella* – meat from infected animals, raw milk, cream and cheeses made from raw milk.
- *Clostridium perfringens* and *Clostridium botulinum* – soil, dust and the intestinal tract.
- Pathogenic *Escherichia coli* (including STEC O157:H7) – the intestinal tract of humans and, especially, of cattle.

Where the Main Pathogens Come From

Pathogen	Primary reservoir	Typical foods
<i>Salmonella</i> Enteritidis	Poultry gut, ovary	Eggs, poultry meat
<i>Campylobacter jejuni</i>	Poultry gut, water	Poultry meat, raw milk
<i>Listeria monocytogenes</i>	Soil, wet plant surfaces	Soft cheese, RTE products
STEC O157:H7	Cattle gut	Minced beef, raw milk, sprouts
<i>Staphylococcus aureus</i>	Human nose, skin, wounds	Handled cooked foods, cream
<i>Clostridium perfringens</i>	Soil, dust, gut	Large meat dishes cooled slowly
<i>Clostridium botulinum</i>	Soil, sediments	Home-canned low-acid foods
<i>Bacillus cereus</i>	Soil, spices, cereals	Cooked rice and pasta
Norovirus, hepatitis A	Human gut	Shellfish, salads, handled foods

Knowing the reservoir tells you where in the chain to place the control measure.