# *MEAT MICROBIOLOGY*

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# Meat Microbiology

1- Food Borne Diseases.

2- Meat Spoilage

3- Meat Quality

4- Preservation

5- Flavor Alteration

# 1- Food Borne Diseases.

❖**Food Borne Diseases :**

– Diseases caused by the consumption and ingestion of infected meat contaminated by Pathogenic Bacteria and their toxins, Viruses and Parasites

– OR the diseases transmitted through the infected meat of Animals and Poultry.

# Bacterial Diseases

1. Salmonella
2. Escherichia coli
3. Campylobacter jejuni
4. Yersinia enterocolitica
5. Listeria monocytogenes
6. Clostridium perfringens
7. Clostridium botulinum
8. Others : Brucella , Corynebacterium , Mycobacterium Paratuberculosis & Pasturella spp .

# 2-Microbial Spoilage

A number of different types of microorganisms are responsible for the spoilages of meat. When they breaks different components of any food items, acids and other waste products are created. Sometimes the microbes itself may or may not be harmful but the metabolites or waste items produced may impart unpleasant taste and colour to our food which is harmful to human's health

# ➢ How it occurs?

- ✓ Normal slaughtering techniques
- ✓ External sources during bleeding, handling, skinning, cutting and processing.
- ✓ Intestinal tract of animals, exterior of animals (hide, hooves and hairs)
- ✓ Knives, cloths, air, and hands and clothing of workers.
- ✓ Depends on methods of slaughtering methods such as mechanical, chemical, electrical etc.

# Meat Spoilage

Raw meat starts deteriorating by its own enzymes and microorganism and chemically oxidize the fat.

- Autolysis changes include some proteolytic action on muscles and tissues resulted in hydrolysis of fats.
- Defects caused by autolysis called “souring”.

Invasion of tissue by micro organism -- Invasion of tissue by contaminating micro organism takes place after death of animals.

## Factors affecting the invasion includes :-

- Microbial load of the gut of slaughtered animal
- Health and Physical condition of the animal before slaughter
- Method used for slaughtering and bleeding
- Rate of cooling

# Growth of micro organism in Meat

Meat is an ideal food for a number of microorganism because its high moisture content, richness in nitrogenous components in meat with various degree of complexity, high amount of minerals and various growth factors. It also contains fermentable carbohydrate( glycogen) and favourable pH range which suits most of the contaminating micro-organism

# Factors influence growth of microorganism

1. The kind & amount of contamination with microorganism and the spread of these microorganism in the meat.
2. The physical properties of the meat Chemical properties of the meat
3. Availability of oxygen
4. Temperature

# Types of Spoilage of Meats

Spoilage under

1)Aerobic conditions

2)anaerobic conditions

Under aerobic conditions bacteria may cause the following- Surface slime – which may be caused by species of Pseudomonas, Acinetobacter, Moraxella, Alcaligenes, streptococcus, Leuconostoc, Bacillus, and Micrococcus, It is an indication of spoilage, often observed before expird date, Facultative and anaerobic bacteria can able to grow within the meat under anaerobic conditions and cause spoilage.

# Changes in colour of meat pigment

The red color of meat, called “bloom”, may be changed to shades of green, brown, gray as result of the production of oxidizing compound.

e.g:

hydrogen peroxides, hydrogen sulfide.
Lactobacillus and Leuconostoc are basically responsible.

# Changes in fats

The oxidation of unsaturated fats may takes place chemically in air and may be catalysed by light and copper. e.g. oxidative rancidity. Pseudomonas and Achromobacter are responsible for oxidative rancidity or by yeast.

# Surface colour discolouration due to pigment forming bacteria

red spot may be caused by Serratia marcescens or other bacteria with red pigment. Pseudomonas syncyanea can impart blue colour to the surface, Micrococcus or flavobacterium imparts yellow colour

# Off odours and off taste

“Taints” or undesirable odours and off taste that appear in a meat

. “ souring” that gives the sour odour that may be due to volatile acids. e.g. formic, butyric, propionic acids by the action of mos. Sometimes “Cold storage flavour” or taint observed in meat and its nothing but stale flavour. In case of musty or earthy flavour, meat contaminated with Actinomycetes are the causative agent for the same

# Genus Salmonella

Family Enterobacteriaceae

- Gram-negative, rod shaped,
- Non-spore forming,
- Facultative anaerobic;
- Endotoxins producing bacteria
- Incubation time: 10–16 h

- Growth Regulator

Growth temperatures: 5 - 45°C.
Destroyed by a temperature of 72 °C
Aw above 0.95 pH above 5.5 in order to produce toxins,
Aw at or above 0.92 and a pH above 4.4 for growth.

# Samples collection

A total of 150 samples of meat products represented by 70 minced meat, 40 sausage and 40 beef burger were randomly collected from different supermarkets and retailers of different sanitation levels at Gharbeia Governorate, Egypt. Each sample was separately packed, identified and immediately transferred in icebox under sanitary precaution to the laboratory where they were subjected to the bacteriological

examination within limited time.

2. Preparation of samples:

At the laboratory, frozen samples were thawed by overnight refrigeration. Each sample was aseptically and carefully freed from its casings and mixed thoroughly in sterile mixer. Twenty five grams of the examined samples were weighed aseptically into sterile blender container and thoroughly homogenized with 225 ml of sterile peptone broth (Oxoid) as pre-enrichment. The homogenate was incubated

at 37°C for 24 hrs.

One ml of the incubated pre-enrichment homogenate were transferred to Selenite cystine broth (SC) (Difco) as selective enrichment and incubated at 37°C for 24 h. At the end of the incubation period, a loopful from the selective enrichment broth was streaked onto XLD agar , MacConky's agar and Salmonella –Shigella agar (SS) (Oxoid) and incubated at 37°C for 24 h. The plates were examined for the presence of typical colonies of Salmonellae. Smears of suspected colonies were stained with Gram's stain and examined morphologically for staining characters. Presumptive Salmonella colonies were then subjected to initial screening tests using triple sugar iron agar (TSI), lysine iron agar (LIA) , urea broth(Merck) and lysine decarboxylase . All biochemical tests were performed at 37°C for 18–24 hours including citrate utilization, indol production test, methyl red,urea hydrolysis, and Voges- Proskauer

# RESULTS

. Prevelance of S. Enteritidis: After culturing onto XLD (Xylose Lysine Deoxycholate) medium ,Salmonella appeared as smooth colonies with black center while onto Salmonella –Shigella agar , it appeared pale colored colonies indicated non lactose fermenting with or without black centers and onto MacConkey's agar appeared as pale colorless smooth ,transparent and raised colonies.

- All isolates showed similar pattern of reaction despite of the source of isolation .Urea hydrolysis, Indole reaction and Voges Proskauer reaction showed negative results ,while TSI, Lysine Iron ,Simmon's Citrate and Methyl Red reactions showed positive results. Further, S. Entritidis was isolated from minced meat ,sausage and burger with a percentage of 1.4 % ,2.5 % and 0 % respectively( table 2). 3.2. Results of antibacterial sensitivity test for S. Entritidis The two isolates show sensitivity to the following antibiotics (chloramphinicole, levofloxacin, gentamycin, amoxicillin, enrofloxacin and ciprofloxacin) while they were resistant to oxytetracycline.

# Genus Escherichia

Family Enterobacteriaceae

E. coli. is Gram negative Facultative anaerobic Incubation: 1-10 days Enterotoxins destroyed over 100 °C for 35 mins

Growth Regulator:

- Temperature range: 7-45 °C
- pH value for growth is 4.5-8.8
- Aw ≤ 0.95 to inhibit growth

# Isolation and identification of E. coli

- Isolation of E.coli was adopted by using MacConkey broth and Eosin Methylene Blue plates.

RESULTS

- The metallic green colonies and picked identified were up biochemically and serologically .

# Control

- Cleaning and sanitizing practices for premises and equipment
- Food handler hygiene
- Keep food ≤ 4°C
- Cook to temperature more than 72°C
- Primary production controls
- Keep kitchens and food-serving areas clean and sanitized

# Genus Campylobacter

- Gram negative
- Thermophilic
- Facultative anaerobic / microaerophilic
- Incubation: 2-5 days

Growth Regulator

- Temperature range: 30-45 °C
- pH value for growth is 6.5–7.5.
- Aw 0.97 is required to growth
- Growth inhibited below pH of 4.9 and above pH 9

# Detection of Campylobacter

Materials and methods In this study 40 raw meat samples including 22 from poultry and 18 from bovine source, were investigated for the presence of Campylobacter jejuni. These samples were collected randomly from butcheries and poultry shops in Tehran. At the beginning, 10 g of each sample was added to 90 ml of Preston Broth containing defibrinated sheep blood and antibiotics. For this purpose, 10 ml defibrinated sheep blood plus 2 ml antibiotic solution (vancomycin, polymixin, trimethoprim) were added to 200 ml Preston Broth. After that, the Preston Broth was placed in a jar containing Anearocult C and was incubated at 37°C for 4 to 6 hrs and then at 41.5°C for 44 to 48 hrs

Then the Preston Broth was surface plated on Campylobacter Agar containing defibrinated sheep blood and antibiotics as mentioned before. The inoculated plates were incubated at 41.5°C for 5 days in a jar containing Anearocult C and a piece of moist cotton to save the environmental humidity. When suspected colonies were detected, confirming tests including Gram stain, growth at 25°C, oxidase and catalase tests, sensitivity to nalidixic acid and cephalothin and hippurate hydrolysis were performed .results among 40 raw meat samples or out of 22 poultry samples, 3 chicken meats were contaminated with C. jejuni. Therefore, 7.5% of all raw meat samples and 13.6% of raw poultry contained C. jejuni.

The results of confirming tests on suspected colonies were as follows: we observed curved Gram negative bacilli, unable to grow at 25°C under microaerophilic conditions, oxidase and catalase positive, sensitive to nalidixic acid, resistant to cephalothin and able to hydrolyze sodium hippurate. These results completely match with Campylobacter jejuni biochemical characteristics.

# Control

- Keep food ≤ 4°C
- Core temperature more than 80°C
- Control raw material
- Control water treatment plant

# Genus Clostridium

Family Bacillaceae

- Gram positive
- Non- proteolytic
- Obligate anaerobic
- Incubation: 8-14 hours
- Create H2S- off flavor

Growth Regulator

- Temperature range: 20-45 °C
- pH value for growth is 4.9-8.3
- Aw more than 0.95 required to growth

# Detection of clostridium

- Cooked meat media . Sheep blood agar media. Egg yolk agar media. Nutrient gelatin media.

- Biochemical reactions Nitrate reduction test was done according to (Willis, 1977)

Detection and typing of Clostridium perfringens in some retail chicken meat products Zinc Test was done according to (Willis, 1977) Indole production test it was done according to (MacFaddine, 1980) Hydrogen sulphid test it was done according to (MacFaddine, 1980) Sugar fermentation test it was done according to (Willis, 1977) Detection of Clostridium perfringens toxins by using Multiplex PCR was done according to Kalender et al. (2005), Moller and Ahrens (1996), and Meer and Songer (1997).

# RESULTS

16% from examined raw breast, raw thigh, nuggets, panée and frankfurter samples, respectively. eight (29.6%) isolates were confirmed as C. perfringens type A after detection of alpha toxin gene that gave a characteristic ampilicon band at 402bp; and only one isolate (3.7%) gave the characteristic fragment of epsilon toxin encoded by etx at base pairs 541 indicated C. perfringens type D using multiplex PCR; In addition, it was determined that none of the isolates carried C. perfringens enterotoxin (cpe) genes.

# Control

- Packaging- Absent of oxygen and presence of nitrite
- Cool quickly after cooking ≤ 4°C
- Cook to temperature more than 121°C for 4 mins or 111°C for 30 mins
- Keep kitchens and food-serving areas clean and sanitized
- Wash properly before processing

# Genus Listeria

Family Listeriaceae

- Gram-positive, Non-spore-forming,
- Facultative anaerobic
- Rod-shaped bacterium
- Incubation time: 3-70 days

Growth Regulator

- Temperature range: 0-38 °C
- pH value for growth is 5.0-5.9
- Aw ≤ 0.92 and pH < 4.1 inhibit growth
- Temperature below –2°C inhibits growth

# Habitat

- Listeria species can replicate in the environment.
- They are widely distributed and can be recovered from herbage, faeces of healthy animals, sewage effluent and bodies of fresh water.
- They have been isolated from soil, organic matter, residual waters, animals feed, fresh and frozen chicken, processed foods, cheese, raw milk, water and gastrointestinal tract of asymptomatic humans and animals
- L. monocytogenes has been isolated from several mammal, birds, fishes and insect species.
- Nevertheless its principal habitat is the soil and decomposing organic matter

# Media used for isolation

FDA, USDA, ISO methods

Enrichment: Cold enrichment,
UVM I & II (University of Vermont Modification Medium (UVM) )
Fraser's broth
Tryptose phosphate broth with polymixin

Selective media : McBride
PALCAM
Oxford agar,
DRIA,
LiCl-ceftazidime agar, modified
Trypaflavine Nalidixic acid-serum agar

- ✓ L. monocytogenes hydrolyzes esculin to esculetin and dextrose. Esculetin reacts with ferric ions and produces black zones around the colonies.
- ✓ Polymyxin- Acriflavin- Lithium chloride-Ceftazidime - Aesculin-Mannitol (PALCAM) Agar was formulated by Van Netten et al (1) and is recommended for the isolation of L. monocytogenes from foods.

# CULTURE OF *LISTERIA*

- On BA: small translucent drop-like colonies (moist) with small zone of slight beta-hemaolysis

# Control

- Appropriate cleaning and sanitizing of premises and equipment before/after handling and preparing uncooked foods.
- Rinse raw produce thoroughly under running tap water before eating.
- Keep cooked and uncooked meats separate
- Store the product below –2°C
- Internal temperature.( >72°C)I
- Bacteriocins- sacacin inhibit the growth
- Using listericidal processes
- Control Good Hygiene Practices (GHP)

# Genus Staphylococcus

Family: Micrococcaceae

- Gram-positive
- Facultative Anaerobic
- Spherical bacterium
- Produces enterotoxins.
- Incubation time is 2–6 h

## Growth Regulator

- Temperature range: 7-45 °C
- pH value for growth is 7.2–7.6,
- Production of toxins stops at pH ≤5.2 or an Aw ≤ 0.90.

# Samples: Meat products

- A grand total of 75 raw meat products samples represented by minced meat, sausage and beef burger (25 of each) collected randomly from different supermarkets and butcher shops at Alexandria Province during the period extended from January to June 2017. Samples were kept in a separate plastic bag and transferred with the minimum delay to the laboratory under possible aseptic conditions to be examined for detection of S. aureus.

# Isolation and identification of S. aureus

- Isolation and identification of S. aureus were carried out according to per Bergey's manual of determinative bacteriology (Holt et al., 1994). From each of previously prepared dilution, 0.1 ml was evenly spread over a dry surface of Baird parker agar plate medium with egg yolk Tellurite with a sterile bent glass rod using surface plating Technique. The inoculated plates were incubated at 37° C for 24 hours in an inverted position. The black shiny colonies with narrow white margins surrounded by a clear zone were S. aureus.

# Control

- Hygiene of food handlers, Control GMP
- Keep hot foods ≥60°C and cold foods cold ≤4°C
- Avoid preparation of food with nose or eye infection, wounds or skin infections
- Preventing unnecessary contact with food
- Using gloves, tongs or other implements to handle food
- Avoiding sneezing, coughing or blowing over food or food contact surfaces.
- Avoid eating perishable food prepared more than two hours earlier


ThankYou!