International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Isolation, Identification, Detection of Bacteria from Meat, Fish, Poultry Samples and Detection of *Staphylococcus Aureus* Enterotoxin Gene

Ahmed Wahab Raisan

Master of Science in Microbiology, Department of Microbiology

University College of Science, Osmania University

INTRODUCTION

Meat putrefies , if the meat is untreated, in a matter of hours or days and results in the meat becoming unappetizing, poisonous or infectious. Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself or by the people handling the meat, or by their implements. Meat can be kept edible for a much longer time – though not indefinitely – if proper hygiene is observed during production and processing, and if appropriate food safety, food preservation and food storage procedures are applied (Brul, S. and Coote, P. 1999).

Meats are the most perishable foods due to its rich nutrient content. There are several intrinsic and extrinsic parameters affecting the growth of bacteria in meat and meat products.

Owing to its high water content and abundance of important nutrients available on the surface, meat is recognized as one of the most perishable foods. Spoilage can be defined as any change in a food product that makes it unacceptable to the consumer from a sensory point of view [Gram *et al* 2002]. Apart from physical damage, oxidation, and color change, the other spoilage symptoms are due to the undesired growth of microorganisms to unacceptable levels. In the case of meat, microbial spoilage leads to the development of off-odors and often slime formation, which make the product undesirable for human consumption (Jackson T. C. *et al* 1997). The organoleptic changes may vary according to the microbial association contaminating the meat and to the conditions under which the meat is stored. The development of organoleptic spoilage is related to consumption of meat nutrients such as sugars and free amino acids by the bacteria and the release of undesired volatile metabolites. Microbial loads from 10^7 CFU cm^{-2} are usually

associated to occurrence of bad odors such as "cheesy" or "buttery" odors; these may evolve into "fruity" odors when the loads increase and become putrid smells as the result of free amino acid consumption at loads as high as 10^9 CFU cm^{-2} (Dainty R. H. *et al* 1985).

In fact, once the glucose present in the aqueous phase has been utilized, other substrates are sequentially consumed until odorous nitrogenous compounds such as ammonia and dimethyl-sulfide are released (Stanbridge L. H., and A. R. Davies. 1998). Different spoilage-related species and strains can colonize the meat surface through different stages involving adsorption to the meat surface (Chung, K.-T.,et al. 1989) The development of these phases depends on the intrinsic and extrinsic ecological factors of a particular meat ecosystem such as pH, meat surface morphology, O_2 availability, temperature, and the presence and development of other bacteria (Ellis, D. I., and R. Goodacre. 2001).

Food spoilage and food poisoning are the main causes that led man to preserve food and prevent diseases due to food. The production of bread, alcoholic beverages and a variety of acid-fermented foods, the preservation of meat and fish products by drying or adding salts and the production of other indigenous food s were critical to the development of stable societies. These microbial processes originated in different parts of the world at various times. People began to understand that food s should be kept away from contact with air, light and moisture(Ganzle, 1999). Some foods were preserved in early times by coating them with clay and olive oil. Salt became an especially valuable commodity because it was essential to human and useful for food preservation, the availability of which influenced the course of history.

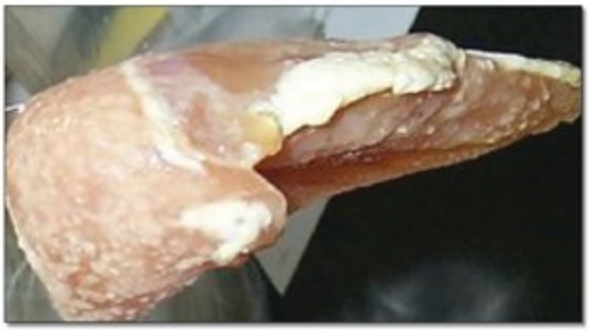


Figure 1: Spoiled breast fillet showing individual colonies that eventually become a slime layer

Approximately 5 billion chickens are processed in the India. each year, of which 80% are marketed as fresh product. It is estimated that 2% to 4% of this meat is lost as a result of poultry spoilage. Therefore,

spoilage is of great concern to the poultry industry (Lockhead, A. G. and G. B. Landerkin, 1935).

The primary causes of poultry products spoilage are as follows:

- Prolonged distribution or storage time
- Inappropriate storage temperature
- High initial bacterial counts
- High post-rigor meat pH

Dealing with spoilage factors

Companies are able to prevent prolonged storage times by properly rotating their stock. Product that is to be sold in locations far from the processing plant should be transported at temperatures that are below freezing (i.e. 26 F), but the temperature should be such that muscle tissue should not freeze(Kraft, A. A. and J. C. Ayres, 1952). Inappropriate storage temperatures or fluctuations in storage temperature are the most avoidable causes of spoilage. Temperature fluctuations can occur during distribution, storage, retail display or handling of the product by the consumer. Processors can determine whether product has been temperature abused by monitoring temperature or evaluating bacterial populations throughout the distribution system.

Bacteria responsible for spoilage

Research demonstrates that the populations of bacteria high in number on the carcass immediately after processing are not the ones that grow under refrigeration and spoil carcasses. Instead, the bacteria found after carcasses spoil are very difficult to find on carcasses at the time of processing. Just after processing, the spoilage bacteria are present in very low numbers, but they can multiply rapidly to cause spoilage odors and slime (Dainty, R. H., et al, 1985). These spoilage bacteria are called psychrotrophic bacteria (psychro=cold; trophic=able to grow) because they are able to multiply under cold conditions. Fresh poultry products held long enough at refrigerator temperatures will spoil as a result of the growth of psychrotrophic bacteria. In contrast, the bacteria that exist in higher numbers at the time of processing on the skin of chickens and in their intestinal tracts are primarily mesophiles (meso=middle; phile=love). These bacteria do not multiply to an appreciable degree at refrigerator temperatures. Examples of mesophiles are Salmonella, *E. coli* and other bacteria found on chickens (Kim, A.Y., and Thayer, D.W.

1996). When a company conducts an "Aerobic Plate Count" or "Total Plate Count" on a chicken carcass, it is measuring the mesophiles (Ingraham, J. L. and G. F. Bailey, 1959).

The figure below shows how these populations of bacteria behave on carcasses during refrigeration.

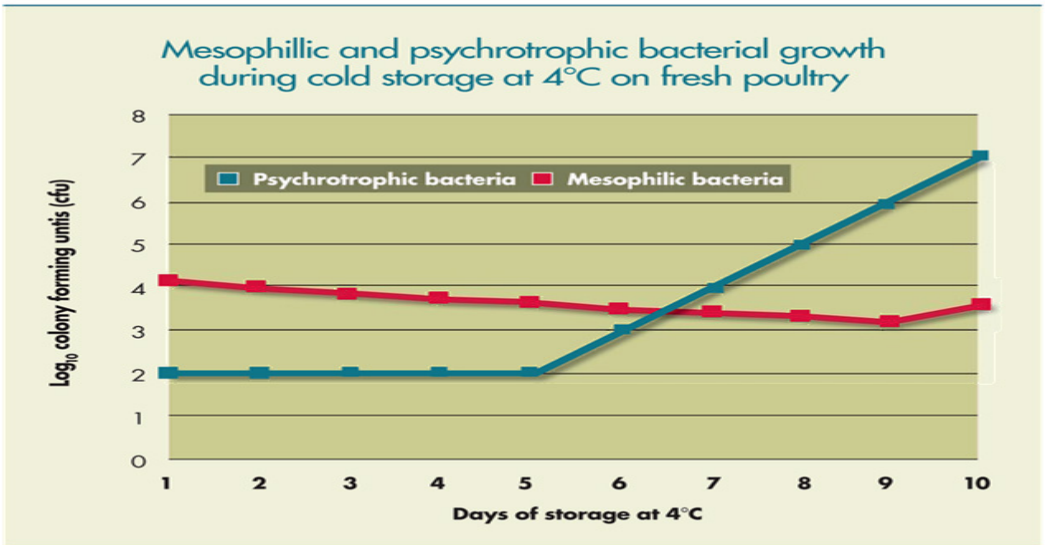


Figure 2: Mesophiles do not cause spoilage of poultry products Origin of spoilage bacteria

Spoilage bacteria on the carcass immediately after processing comes from 1. The feathers and feet of the live bird, 2. The water supply in the processing plant, 3. The chill tanks and 4. Processing equipment.

These spoilage bacteria are not usually found in the intestines of the live bird. High populations of *Acinetobacter* (10^8CFU/g) have been found on the feathers of the bird and may originate from the deep litter. Other spoilage bacteria, such as *Cytophaga* and *Flavobacterium,* are often found in chill tanks but are rarely found on carcasses. The psychrotrophic spoilage bacteria on chicken carcasses immediately after slaughter are generally Acinetobacter and pigmented pseudomonads. Although strains of non-pigmented Pseudomonas produce off-odors and off-flavors on spoiled

poultry, initially, they are difficult to find on carcasses and *Pseudomonas putrefaciens* (*Shewanella putrefaciens*) is rarely found (Brown, A. D., 1957).

Spoilage species

Rusell et al. (1995) conducted a study to identify the bacterial species responsible for spoilage of poultry from various locations around the U.S (Russell, S., 1992). The bacterial genera most isolated in high numbers on spoiled poultry was *Pseudomonas fluorescens*,*putida*, or *fragi* or *Shewanella* (formerly a Pseudomonas) *putrefaciens*. Identification of the genus and species most responsible for spoiling poultry is important because, once identified, it is easier to understand the mechanisms by which they produce spoilage (Alford, J. A. and L. E. Elliott, 1960). High numbers (10^5 CFU/cm^2) of psychrotrophic spoilage bacteria are required on poultry surfaces before off-flavors, off-odors and appearance defects are able to be detected organoleptically. Researchers have reported that higher numbers of bacteria ($3.2x10^7$ to $1x10^9$ CFU/cm^2) were required to produce slime than were needed for odor to become noticeable. Some of Pathogenic Microorganisms which are responsible for food borne illness are in the table below.

Table 1: Microorganisms and their infective doses

Microorganism	Infective dose (no. of microorganisms	Incubation period	Name of the disease
Clostridium botulinum	< nano grams	12-36 h	botulism
Clostridium perfringens	>10E8	8-22 h	Perfringens food poisoning
Shigella	<10	12–50 h	Shigellosis
Yersinia enterocolitica	unknown	1-3 days	Yersiniosis
Hepatitis A virus	10-100	Unknown	Hepatitis A
Norwalk virus / Norovirus	Unknown but presumed to be low	1-2 days	Viral gastroenteritis, stomach flu, Winter vomiting disease

Materials and methods:

Nutrient media, stains for morphological identification, Microscope, centrifuge, Laminar flow etc

Sample collection

Meat & Chicken samples were collected from butcher shops from areas like Moula Ali , A.S Rao Nagar, Malkajigiri, Tarnaka etc. The collected samples were aseptically processed in the microbiology laboratory.

Chemicals

Crystal violet, Grams iodine, ethanol, safranin, were used for morphological identification. The media and reagents used for the study such as Nutrient Broth, Nutrient agar, Eosin-Methylene Blue agar, Blood agar, Salmonella-Shigella agar, Thiosulfate-Citrate-BileSalt-Sucrose agar, Mannitol Salt agar, MacConkey agar, Methyl red and Voges Proskauer were procured from Himedia, India.

Total Bacterial Count:

The total bacterial count was performed on all the samples by Lazy Susan plating method in triplicate on solid Nutrient agar plates after serial dilution of samples at 1 in 10 concentrations. The seeded plates were sealed and incubated at 37 ^{0}C. The colony forming units were counted after 24 and expressed in CFU/ml. Meat, fish and poultry samples were procured from various retail outlets of Hyderabad , butchers ,slaughter houses etc and were subjected to microbial analysis. The procedures followed for sampling were as prescribed in the Standard procedures of Section 16(2)(c), of the FSS Act, 2006 which provides for the mechanism for accreditation of certification bodies for Food Safety Management Systems and Section 44 of FSS Act provides for recognition of organization or agency for food safety audit and checking compliance with Food Safety Management System required under the Act or the rules and regulations made there under.

Aerobic Mesophilic Plate count:

Indicates microbial counts for quality assessment of foods

Medium:

1.Plate count agar;

2.Peptone water 0.1%,

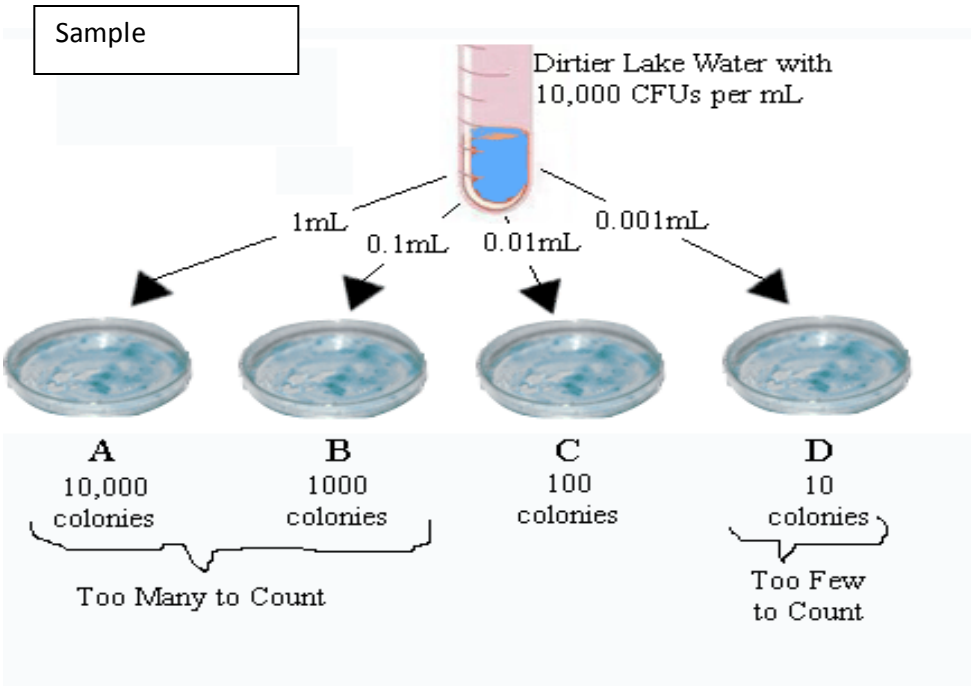


Figure 3: Sample dilution

Procedure:

Preparation of sample homogenate

Make a 1:10 dilution of the well mixed sample, by aseptically transferring sample to the desired volume of diluent. mix thoroughly with the appropriate volume of diluent (1ml into 9 ml, or 10 ml into 90 ml or 50ml into 450 ml).

In most of the meat samples particulate matter floats in the dilution water. In such cases allow the particles to settle for two to three minutes and then draw the diluent from that portion of dilution where particles are minimum and proceed.

Dilution:

If the count is expected to be more than 2.5 x10^3 per ml or g, prepare decimal dilutions as follows. Shake each dilution 25times. For each dilution use fresh sterile pipette. Alternately use auto pipette. Pipette 1 ml of homogenate into a tube containing 9 ml of the diluent. From the first dilution transfer 1ml to second dilution tube containing 9ml of the diluent.

Repeat using a third, fourth or more tubes until the desired dilution is obtained.

Isolation of bacteria from the meat samples:

About 5 grams of each meat sample was suspended in TSB broth and incubated overnight at 37^0c.Next day, the samples were diluted 10 fold and 5^{th} dilution and 6^{th}

dilution samples were plated on LB agar to get isolated colonies. Isolated colonies from each sample was picked and streaked on a fresh nutrient agar plate , the master plate.

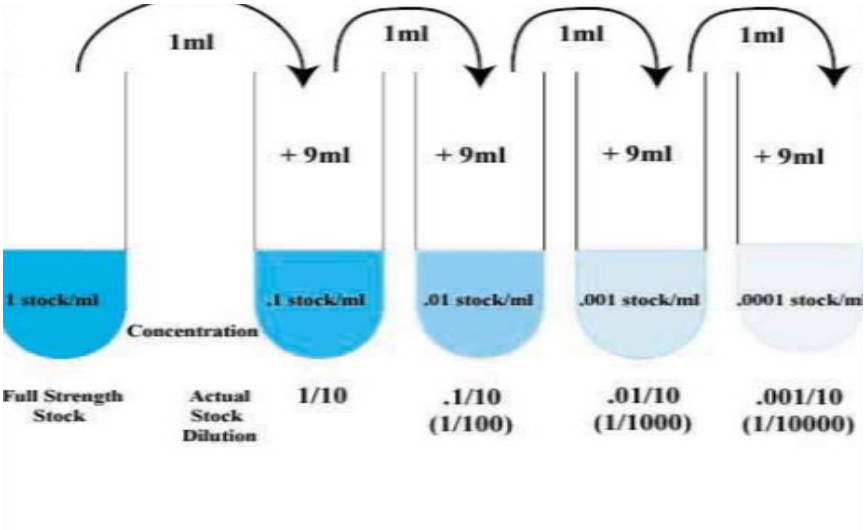


Figure 4: Serial Dilutions and plating onto agar plates for colony counts

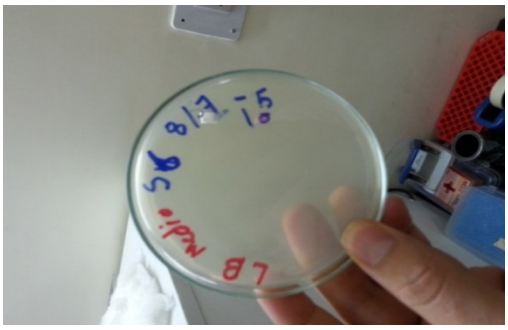
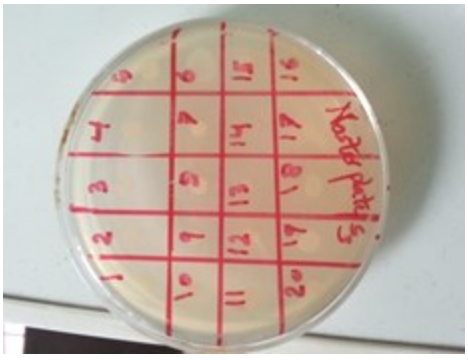


Figure 5.Diluted sample plated on nutrient agar



Preparation of master plate:

Master plates on LB Agar was made by streaking each pure colony on gridded plates and stored at 4 ^{0}c .Each time master plate was sub cultured for carrying out various tests.

Figure 6: Master Plate for Poultry

Figure 7: Master plate for Meat

RESULTS

Morphological identification

Each of the isolated colony on the master plate was identified by grams staining and motility experiment. The results are compiled in Table below.

Table4 a: morphological ,cultural and microscopic characteristics of potential bacteria

Name of the test	S1C1	S1C2	S1C3	S1C4	S1C5	S1C 6	S1C7	S1C8	S1C9	S1C10
Gram-stain	Gram - positive	Gram-negative	Gram - positive	Gram-positive	Gram - positive	Gram-positive	Gram - positive	Gram - positive	Gram - positive	Gram-positive
Cell shape	Cocci	Straight -rod	Cocci	cocci	Cocci	cocci	cocci	cocci	cocci	cocci
Colony shape	Circular	circular	Circular	circular	Circular	circular	circular	circular	circular	circular
Margin	Entire	entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Appearance	Non-shiny	Dull-non swarming	Non-shin	Non-shiny	Non-shiny	Non-shiny	Non-shiny	Non-shiny	shiny	shiny
Elevation	Convex	flat	Convex	convex	Convex	convex	convex	convex	convex	convex
Surface texture	Smooth	smooth	Smooth	smooth	Smooth	smooth	smooth	smooth	rough	rough
Colour	White	colourless	White	white	White	white	white	white	yellow	yellow

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table4 b: MORPHOLOGICAL ,CULTURAL AND MICROSCOPIC CHARACTERISTICS OF POTENTIAL BACTERIA

Name of the test	S2C11	S2C12	S2C13	S2C14	S2C15	S2C16	S2C17	S2C18	S2C19	S2C20
Gram-stain	Gram-nagative	Gram-positive	Gram-nagative	Gram-positive	Gram-nagative	Gram-positive	Gram-positive	Gram-nagative	Gram-nagative	Gram-nagative
Cell shape	Rod	Cocci	Rod	cocci	Rod	Cocci	cocci	Rod	Rod	Rod
Colony shape	Pointed	Circular	Circular	circular	Pointed	Circular	circular	Pointed	Pointed	Large Flat
margin	Entire	Entire	Entire	Entire	entire	Entire	Entire	entire	entire	entire
Appearance	Mucoid	Non-shiny	translucent	Non-shiny	mucoid	Non-shiny	Non-shiny	mucoid	mucoid	puncti form
Elevation	Convex	convex	Convex	convex	convex	convex	convex	convex	convex	raised
Surface texture	Smooth	smooth	Smooth	smooth	Smooth	smooth	smooth	smooth	smooth	smooth
Colour	Yellow	White	Ceam	white	yellow	White	white	yellow	yellow	white

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table4 c: MORPHOLOGICAL ,CULTURAL AND MICROSCOPIC CHARACTERISTICS OF POTENTIAL BACTERIA

Name of the test	S3C21	S3C22	S3C23	S3C24	S3C25	S3C26	S3C27	S3C28	S3C29	S3C30
Gram-stain	Gram-positive	Gram-nagative	Gram-positive	Gram-nagative	Gram-nagative	Gram-positive	Gram-positive	Gram-positive	Gram-nagative	Gram-positive
Cell shape	Cocci	Rod	Cocci	Rod	Rod	cocci	cocci	cocci	Rod	Cocci
Colony shape	circular	Large flat	Circular	Pointed	Large flat	circular	circular	Circular	Pointed	Circular
Margin	Entire	entire	Entire	entire	entire	Entire	Entire	Entire	entire	Entire
Appearance	Non-shiny	punctiform	Non-shiny	mucoid	punctiform	Non-shiny	Non-shiny	Non-shiny	mucoid	Non-shiny
Elevation	convex	raised	convex	convex	raised	convex	convex	convex	convex	convex
Surface texture	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth	smooth
Colour	White	white	White	yellow	white	white	white	White	yellow	White

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table4 d: MORPHOLOGICAL ,CULTURAL AND MICROSCOPIC CHARACTERISTICS OF POTENTIAL BACTERIA

Name of the test	S4B1	S4B2	S4B3	S4B4	S4B5	S4B6	S4B7	S4B8	S4B9	S4B10
Gram-stain	Gram-nagative	Gram-positive	Gram-nagative	Gram-negative	Gram-positive	Gram-nagative	Gram-nagative	Gram-positive	Gram-positive	Gram-nagative
Cell shape	Rod	Cocci	Rod	cocci	Cocci	Rod	Rod	cocci	cocci	Rod
Colony shape	Pointed	Circular	Pointed	circular	Circular	Pointed	Pointed	circular	circular	Pointed
Margin	entire	Entire	Entire	Entire	Entire	Entire	entire	Entire	Entire	Entire
Appearance	mucoid	Non-shiny	Mucoid	Non-shiny	Non-shiny	Mucoid	mucoid	Non-shiny	Non-shiny	Mucoid
Elevation	convex	Convex	Convex	convex	convex	Convex	convex	convex	convex	Convex
Surface texture	smooth	Smooth	Smooth	smooth	smooth	Smooth	smooth	smooth	smooth	Smooth
Colour	yellow	Creany	Yellow	creany	White	Yellow	yellow	white	white	Yellow

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table4 e: MORPHOLOGICAL ,CULTURAL AND MICROSCOPIC CHARACTERISTICS OF POTENTIAL BACTERIA

Name of the test	S5L1	S5L2	S5L3	S5L4	S5L5	S5L6	S5L7	S5L8	S5L9	S5L10
Gram-stain	Gram-nagative	Gram-nagative	Gram-nagative	Gram-nagative	Gram-nagative	Gram-positive	Gram-nagative	Gram-nagative	Gram-positive	Gram-nagative
Cell shape	Rod	Rod	Rod	Rod	Rod	Cocci	Rod	Rod	Cocci	Rod
Colony shape	Pointed	Pointed	Pointed	large	Pointed	Circular	Pointed	Pointed	Circular	Pointed
Margin	Entire	entire	Entire	entire	entire	Entire	entire	entire	Entire	Entire
Appearance	mucoid	mucoid	Mucoid	mucoid	mucoid	Non-shiny	mucoid	mucoid	Non-shiny	mucoid
Elevation	convex	convex	Convex	umbonate	convex	Convex	convex	convex	convex	convex
Surface texture	smooth	smooth	Smooth	smooth	smooth	Smooth	smooth	smooth	smooth	smooth
Colour	Yellow	yellow	Yellow	white	yellow	White	yellow	yellow	White	Yellow

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table4 f : MORPHOLOGICAL ,CULTURAL AND MICROSCOPIC CHARACTERISTICS OF POTENTIAL BACTERIA

Name of the test	S6F1	S6F2	S6F3	S6F4	S6F5	S6F6	S6F7	S6F8	S6F9	S6F10
Gram-stain	Gram-negative	Gram-negative	Gram-negative	Gram-positive	Gram-posative	Gram-negative	Gram-negative	Gram-negative	Gram-negative	Gram-positivee
Cell shape	Rod	cocci	coccobacillus	coccobacillus	Coccobacillus	coccobacillus	Rod	Straight rod	curved	Coccobacillus
Colony Shape	Large	Pointed	flat	flat	Flat	flat	large	large	circular	Flat
Margin	Entire	entire	entire	entire	entire	entire	entire	entire	irregular	Entire
Appearance	Mucoid	mucoid	Cloudy	Cloudy	Cloudy	Cloudy	mucoid	mucoid	Non swarming	Cloudy
Elevation	Umbonate	convex	flat	flat	Flat	flat	umbonate	convex	convex	Flat
Surface Texture	Smooth	smooth	Smooth	smooth	smooth	smooth	smooth	smooth	smooth	Smooth
Colour	White	yellow	Opaque	opaque	opaque	opaque	white	gray	creamy	Opaque

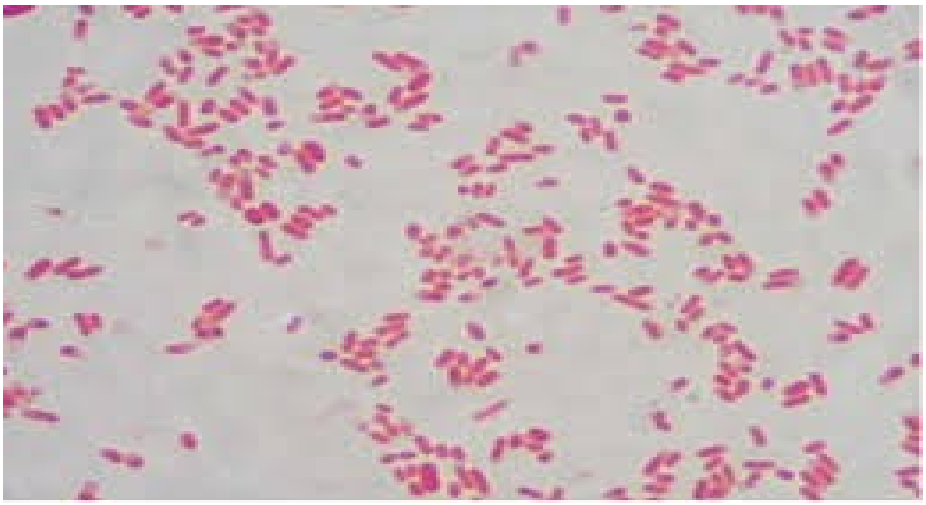


Figure 8*: E.coli*

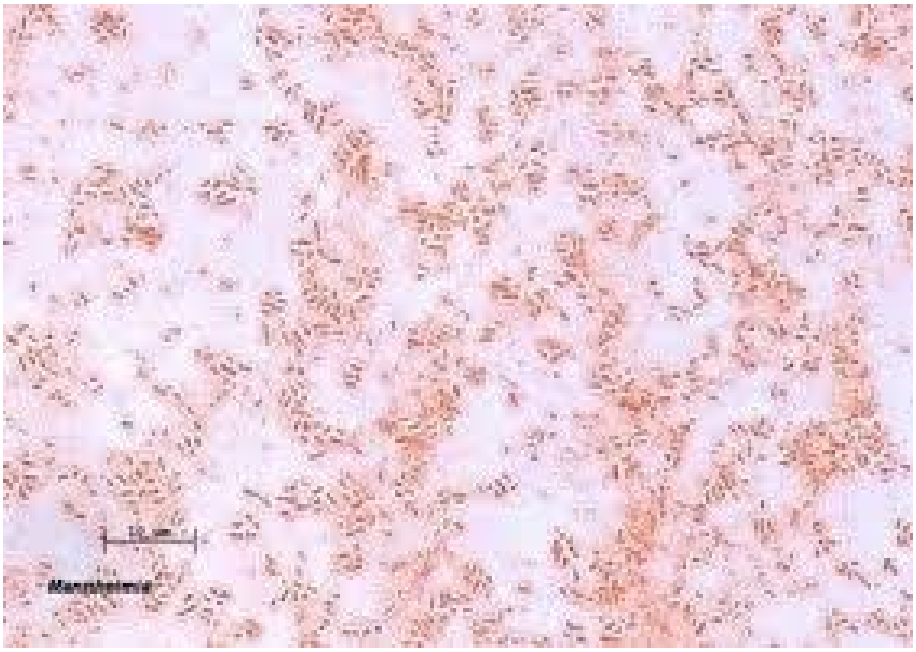


Figure 9*: Klebsiella oxytoca*

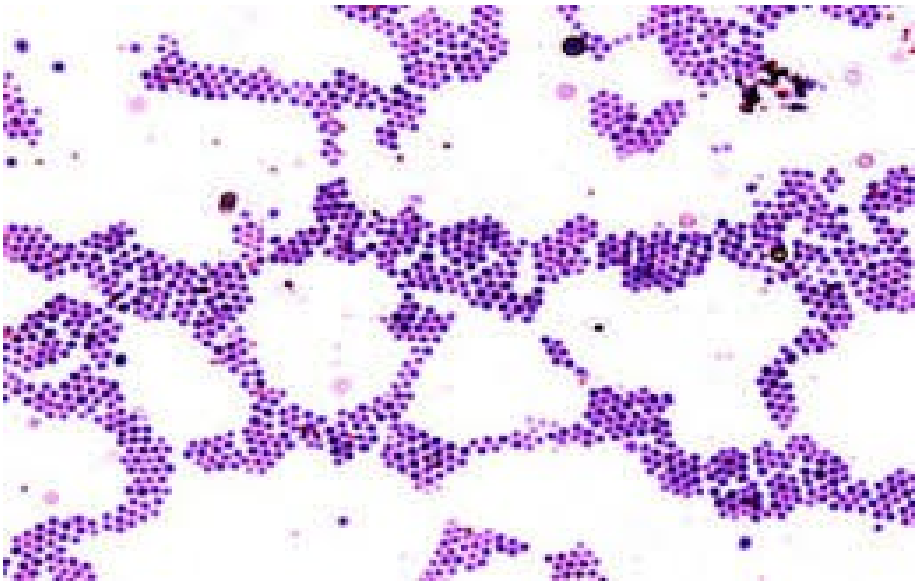


Figure 10: *Enterocoocus faecalis*

Biochemical identification result:

IMVIC tests were done as mentioned in methods and the results are compiled in table.

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table 5a-. Results of the Microbial loads in Chicken Samples (Gizzard)

Isolate	N0.OF colony	Biochemical Tests						Gram Reaction & Morphology	Identifecation of bacteria
		Indole	VP	MR	Citrate	Catalase	TSI		
S1A1	1	(+)	(-)	(-)	(+)	(+)	(-)	Gram + Cocci	*Enterococcus faecalis*
S1A2	2	(+)	(-)	(+)	(+)	(-)	(-)	Gram – Rod	*Providencia.sp*
S1A3	3	(+)	(-)	(-)	(+)	(-)	(-)	Gram + Cocci	*Enterococcus faecalis*
S1A4	4	(+)	(-)	(-)	(+)	(+)	(-)	Gram + Cocci	*Enterococcus faecalis*
S1A5	5	(+)	(-)	(-)	(+)	(+)	(-)	Gram + Cocci	*Enterococcus* ***faecalis***
S1A6	6	(+)	(-)	(-)	(+)	(+)	(-)	Gram + Cocci	*Enterococcus faecalis*
S1A7	7	(+)	(-)	(-)	(+)	(+)	(-)	Gram + Cocci	*Enterococcus faecalis*
S1A8	8	(+)	(-)	(-)	(+)	(+)	(-)	Gram + Cocci	*Enterococcus faecalis*
S1A9	9	(-)	(+)	(+)	(+)	(+)	(-)	Gram + Cocci	*Staphylococcus aureus*
S1A10		(-)	(+)	(+)	(+)	(+)	(-)	Gram + Cocci	*Staphylococcus aureus*

Table 5b. Results of Microbial loads in Chicken(breast)

Isolate	NO. OF colony	Biochemical Tests						Gram Reaction & Morphology	Identifecation of bacteria
		Indole	VP	MR	Citrate	Catalase	TSI		
S1A11	1	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S1A12	2	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S1A13	3	(-)	(-)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Proteus maribilis*
S1A14	4	(+)	(-)	(-)	(+)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S1A15	5	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S1A16	6	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S1A17	7	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S1A18	8	(+)		(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S1A19	9	(+)		(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S2A20	10	(+)	(-)	(+)	(-)	(+)	(-)	Gram Negative Rods	*Escherichia coli*

Table 5c:. Results of Microbial loads in Chicken (Thigh)

Isolate	No of colony	Biochemical Tests						Gram Reaction & Morphology	Identifecation of bacteria
		Indole	VP	MR	Citrate	Catalase	TSI		
S3A21	1	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S3A22	2	(+)	(-)	(+)	(-)	(+)	(-)	Gram Negative Rods	*Escherichia coli*
S3A23	3	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S3A24	4	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S3A25	5	(+)	(-)	(+)	(-)	(+)	(-)	Gram Negative Rods	*Escherichia coli*
S3A26	6	(+)	(-)	(-)	(+)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S3A27	7	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S3A28	8	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S3A29	9	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S3A30	10	(+)	(-)	(-)	(-)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table-5d. Results of Microbial loads in Beef.

Iolate	No. of colony	Biochemical Tests						Gram Reaction & Morphology	Identification of bacteria
		Indole	VP	MR	Citrate	Catalase	TSI		
S4B1	1	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S4B2	2	(+)	(-)	(+)	(+)	(+)	(-)	Gram Negative Rods	U.I
S4B3	3	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S4B4	4	(+)	(-)	(+)	(+)	(+)	(-)	Gram Positive Cocci	U.I
S4B5	5	(+)	(-)	(-)	(+)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S4B6	6	(+)		(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S4B7	7	(+)		(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S4B8	8	(+)	(-)	(-)	(+)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S4B9	9	(+)	(-)	(-)	(+)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S4B10	10	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 10
June 2016

Table-5e. Results of Microbial loads in Meat(Lamb).

Isolate	No of colony	Biochemical Tests						Gram Reaction & Morphology	Identifecation of bacteria
		Indole	VP	MR	Citrate	Catalase	TSI		
S5L1	1	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S5L2	2	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S5L3	3	(+)		(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S5L4	4	(+)	(-)	(+)	(+)	(-)	(-)	Gram Positive Cocci	*Serratia marcesncs*
S5L5	5	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S5L6	6	(+)	(-)	(-)	(+)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S5L7	7	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S5L8	8	(+)		(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*
S5L9	9	(+)	(-)	(-)	(+)	(+)	(-)	Gram Positive Cocci	*Enterococcus faecalis*
S5L10	10	(+)	(+)	(-)	(+)	(+)	(-)	Gram Negative Rods	*Klebsiella oxytoca*

Table 5f: Microbial Loads in Fish.

Isolate	No of colony	Biochemical Tests						Gram Reaction & Morphology	Identifecation of bacteria
		Indole	VP	MR	Citrate	Catalase	TSI		
S6F1	1	(+)	(-)	(+)	(+)	(-)	(-)	Gram Negative Rods	*Serratia marcesncs*
S6F2	2	(+)	(+)	(+)	(-)	(-)	(-)	Gram Negative Rods	U.I
S6F3	3	(+)	(-)	(+)	(-)	(+)	(-)	Gram Negative Rods	*Morgenella*
S6F4	4	(+)	(-)	(+)	(+)	(-)	(-)	Gram Positive Cocci	U.I
S6F5	5	(+)	(-)	(-)	(-)	(-)	(-)	Gram Negative Rods	U.I
S6F6	6	(+)	(-)	(+)	(-)	(-)	(-)	Gram Positive Cocci	*Morgenella*
S6F78	7	(+)	(-)	(+)	(+)	(-)	(-)	Gram Negative Rods	*Serratia marcesans*
S6F8	8	(-)	(-)	(+)	(+)	(-)	(-)	Gram Negative Rods	*Citrobacter*
S6F9	9	(-)	(-)	(-)	(-)	(-)	(-)	Gram Negative Rods	*Clostridium perfringens*
S6F10	10	(+)	(-)	(-)	(-)	(+)	(-)	Gram Negative Rods	U.I

Table 6:. VII Key for the Biochemical identification of microorganisms adopted from(FDA – Bacteriological Analytical Manual, 7th Ed.AOAC Intnl.)

Indole	Methyl red	Voges Proskauer	Citrate	Suspected Pathogenic organism
+	_	+	+	*Klebsiella oxytoca*
+	+	_	_	*E.coli*
+	_	_	+	*E.faecalis*
-	_	_	+	*P.Mirabilis*
+	+	_	_	*Morganella morganii*
-	+	_	_	*Yersinia*
-	+	_	+	*Citrobacter*
-	+	_	+	*Salmonella*

***Out of the examined 60 samples 30 of chicken ,10 of beef ,10 of lamb,and 10 of fish were shown a percentage as following in the table**

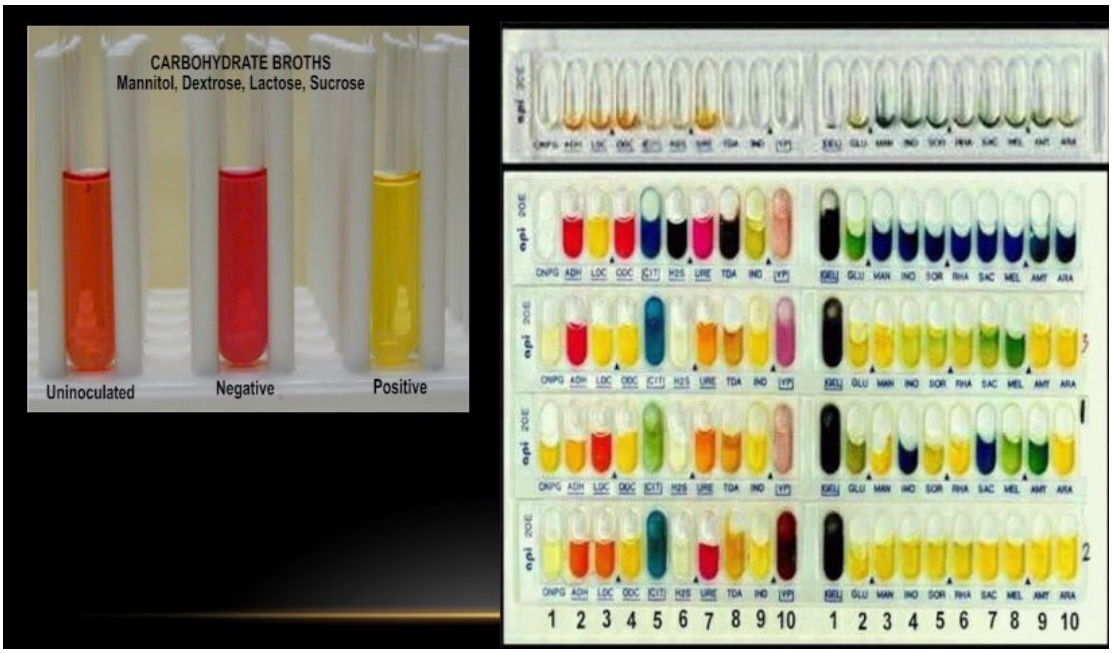


Figure 11: Biochemical Tests for characterization –Utilization of carbohydrates, sugars,urea etc

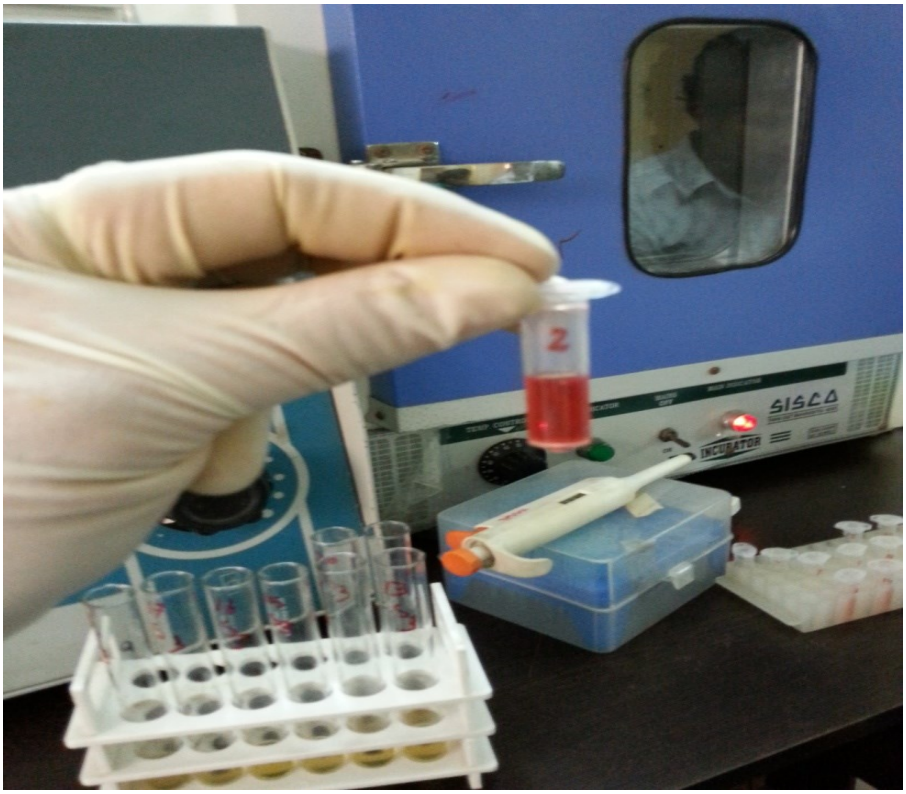


Figure 12: Methyl Red test positive.

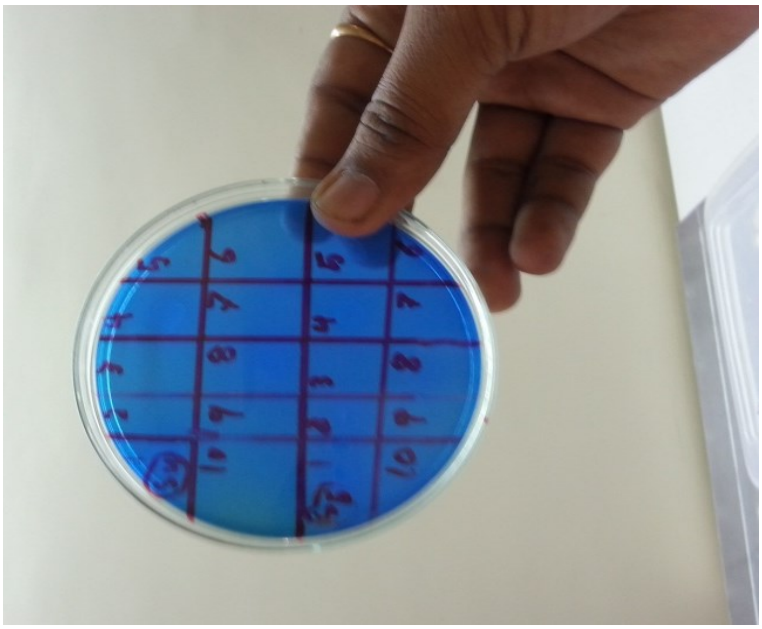


Figure 13:Citrate Test Positiv

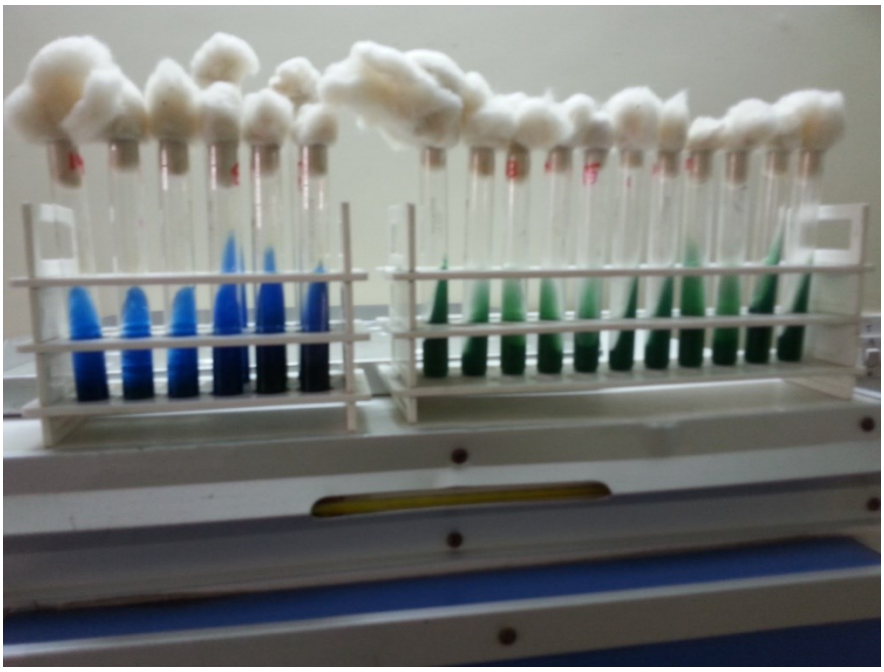


Figure 14: Simon citrate test in tube slants

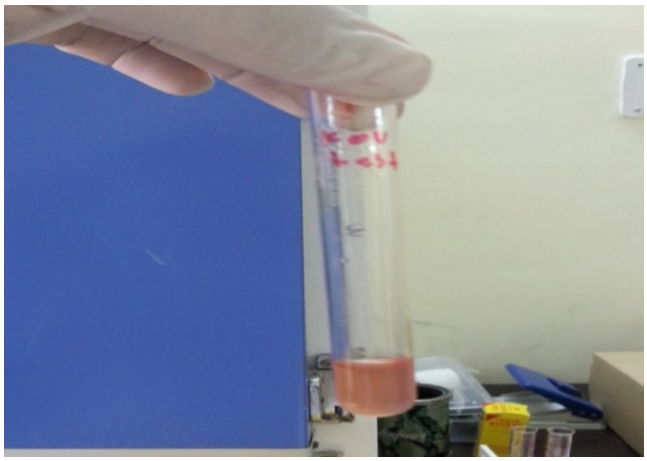
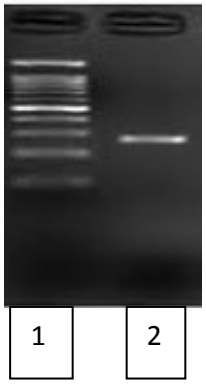


Figure 15: Indole test positive

Table .7 . frequency distribution of the isolated bacteria

Bacteria isolates	Chicken meat	Beef Meat	Lamb meat	Fish meat	Percentage of occurrence
Enterococcus faecalis	**17**	3	2	_____	**36.6%**
Klebsiella oxytoca	6	5	7	_____	**30%**
Serratia marcesans	_____	_____	1	2	**5%**
Escherichia coli	3	_____	_____	_____	**5%**
Morgenella	_____	_____	_____	2	**3.3%**
Staphylococcus aureus	2	_____	_____	_____	**3.3%**
Citrobacter	_____	_____	_____	1	**1.6%**
Clostridium perfringens	_____	_____	_____	1	**1.6%**
Proteus maribilis	1	_____	_____	_____	**1.6%**
Providencia.sps	1	_____	_____	_____	**1.6%**
non-identified	_____	2	_____	4	**10%**

PCR Results:



1-100 bp ladder
2. *Staphylococcus aureus enterotoxin* A amplicon

Figure 34: PCR amplification of *Staphylococcus aureus enterotoxin* A

Using 100 bp ladder the size of enterotoxin A gene was found to be 270 bp
PCR amplification of enterotoxin sea is useful for detection of *S. aureus* carrying *sea* in food samples in the food industry and outbreak investigation.We detected the presence of staphylococcus aureus in chicken by PCR method.

CONCLUSION:

Microbial content present in different samples (meat, poultry, fish) obtained from Buchner house, slaughter houses, cold freezer in super market etc was enumerated. A systematic morphological,and bicochemical identification was carried out once isolated colonies were isolated from each sample. From the results obtained it is observed that enterococci faecalis ranked as number one organism contaminating the meat products followed by *Klebsiella oxytoca.*A Few counts of *Serratia marcesens*, *Morganella* ,*Staphylococcus aureus*, providentia were also observed. Since *Staphylococcus* is a known enteropathogen,PCR was used to detect the presence of staphylococcus. The presence of these microorganisms indicate the importance of high pressure cooking at consumer end and a regular auditing by health safety inspectors at these slaughter houses to monitor hygiene and cleanliness at these sites.

REFRENCES

1. Alford, J. A. and L. E. Elliott, 1960. Lipolytic activity of microorganisms at low and intermediate temperatures. I. Action of Pseudomonas fluorescens on lard. Food Res. 25:296-303.
2. Ayres, J. C., W. S. Ogilvy, and G. F. Stewart, 1950. Post mortem changes in stored meats. I. Micro-organisms associated with development of slime on eviscerated cut-up poultry. Food Technol. 4:199-205.
3. Ayres, J. C., 1960. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Res. 25(6):1-18.
4. A'lvarez, I,. Man as, P., Condo N.S. and Raso, J. (2003a). Resistance variation of salmonella enterica serovars to pulsed electric field treatments. J. Food Sci. 68: 2316-2320.
5. Aba bouch, L.H., Grimit, L. Eddafry, R., and Busta, F.F. (1995) Thermal inactivation kinetics of Bacillus subtilis spores suspended in buffer and in oils. L.Appl. Bacteriol. 78: 669-676.
6. Abee, T. and Wouters, J.A. (9199). Microbial stress response in minimal processing. Int.J. Food microbial. 50:65-91.
7. Acott, K., Sloan, A.E. and Labuza, T.P. (1976). Evaluation of antimicrobial agents in a microbial challenge study for an intermediate moisture dog food. J. Food Sci. 41: 541-546.
8. Allison,D.G., D'Emmanuele, A. Egington, P. and Williams, A.R.(1996). The effect of ultrasound on Escherichia coli viability. J. Basic Microbiol. 36: 3-11.
9. Alpas, H., Kalchayanand, N., Bozughu, F. and Ray, B (1998). Interaction of pressure , time and temperature of pressurization on viability loss of Listeria innocua. World J.Microbiol. Biotech. 14: 251-253.
10. Barnes, E. M., 1960. Bacteriological problems in broiler preparations and storage. R. Soc. Health J. 80:145-148.
11. Barnes, E. M. and C. S. Impey, 1968. Psychrophilic spoilage bacteria of poultry. J. Appl. Bacteriol. 31:97-107.
12. Barnes, E. M. and M. J. Thornley, 1966. The spoilage flora of eviscerated chickens stored at different temperatures. J. Food Technol. 1:113-119.