Isolation and Characterization of Some Bacteria from Chicken Meat, Ovary and Intestine

Ohnmar Ye Win[1], Min Min Soe[2]

[1]*Associate Professor, Botany Department, Panglong University, Panglong, Southern Shan State, Myanmar*
[2] *Professor, Botany Department, Panglong University, Panglong, Southern Shan State, Myanmar*

***Abstract*— Foods are naturally nutritious and suitable substrates for the growth and metabolism of microorganisms. Consuming unhygienic products from meat and meat products make humans food-borne illness. This is the main cause by pathogenic microorganisms. In the present study, a total of ninety samples were purchased from Taunggyi market during June, 1999 to January, 2000. Fifty-four samples were from chicken meat, twelve samples from ovary and twenty-four from intestine. The aim of this study was to attempt the microbial contaminants of chicken meat, ovary and intestine sold at Taunggyi markets. The present study deals with some bacteria isolated from chicken meat, ovary and intestine using selective culture techniques. Mac Conkey agar was used to detect *Escherichia coli* and Salmonella-Shigella agar was used to detect *Salmonella* and *Shigella* species. Identification of bacteria was conducted by primary and secondary tests of biochemical activity. In this study, seven species were collected from chicken meat, three species were from chicken ovary and two species were from chicken intestine. *Shigella dysenteriae* was found as the highest percentage (18.5%) in chicken meat and another 18.5% bacteria were not identified. *Escherichia coli* were found as the highest percentage (58.3%) in chicken ovary. *Enterobacter aerogenes* was found as the highest percentage (75.0%) in chicken intestine. The present study revealed that raw chicken meat, ovary and intestine were heavily contaminated with bacterial pathogens. It can be concluded that the contamination of meat, ovary and intestine sold in market places must be minimized by proper sanitation and inspection practices.**

***Keywords*— identification, bacteria, chicken meat, ovary, intestine.**

I. INTRODUCTION

Bacteriology deals with bacteria and their related forms of life. The development of modern medicine, surgery, sanitation, etc. was made possible through the revelations of bacteriology (Clifton C.E., 1950). Thus, bacteriology performs the key role in solving problems of health, agriculture, and industry, and has served to pave the way, for some of the greatest of scientific advances. A number of bacteria cause diseases as diphtheria in human while many others produce mild to severe infections in plants and animals (Bryan A.H. *et al*., 1874).

Meats are among the most nutritious form of foods but they are very susceptible to microbial spoilage. Moreover, they are agents to transmission of several serious infections from animals to humans (Genta, M.L. *et al*., 2001). Many types of diseases like listeriosis, tuberculosis, anthrax and food poisoning can be transmitted through the consumption of diseased or contaminated meat (Gracey, J.F. *et al*., 1999).

In Myanmar, most of the meat on sale in open markets is prepared from freshly or recently slaughtered food animals and poultry. Meats on the counters are usually in cut forms. Depending on the weather, conditions of market activities, availability of transportation facilities and the degree and types of consumer demands, food animals and poultry are slaughtered and dressed at different times of the day. However, contamination of meat occurs even in relatively fresh cut and minced meat (Gracey, J.F. *et al*., 1999).

In the present study, bacteria were identified following the methods and procedures used in most diagnostic laboratories. The objective of this research work was to gain more understanding and knowledge about the techniques of isolation and identification of various bacteria from chicken meat, ovary and intestine. Moreover, the contamination rate of chicken meat, ovary and intestine was going to be calculated.

II. MATERIALS AND METHODS

This research was conducted at the Regional Veterinary Diagnostic Laboratory, Taunggyi, in the year 1999 – 2000. Different culture media for the bacterial isolation were prepared by the methods mentioned by Cowan and Steel (1993).

A total of 90 samples were collected from various shops in Taunggyi Market. Among them, 54 samples were chicken meat, 24 were chicken intestine and 12 were chicken ovary. Bacterial cultures were made from the raw samples on the day of sample collection.

Glassware: New glassware such as petridish, conical flask, etc. were immersed in 2% acetic acid in alcohol for 24 hours and washed in tap water and finally with distilled water thoroughly. Whenever used glassware were sterilized under steam pressure. After that, they were washed with detergent powder and soaked in distilled water in a few minutes. All glassware was dried in Hot Air Dryer. Test tubes and containers were plugged with non-absorbent cotton wool, packed with aluminium foil and sterilized in Hot Air Oven at 160˚C for one hour.

A. Culture Method

An isolated growth of a single strain must be free from mixture and contamination with other bacteria. The pure culture was normally achieved by the method "Plating Out" in a solid culture medium. In this method, a wire loop was sterilized by heating with a spirit lamp. It was allowed to cool and was charged with the bacterial mixture and smeared thoroughly over area. "A" gave a well-inoculum. Two or three parallel lines (B, B, B) were drawn from the well-inoculum (A) onto the fresh surface of the medium. This procedure was repeated as shown in Figure 1. At each step, the inoculum comprised the most distal part of the immediately preceding strokes.

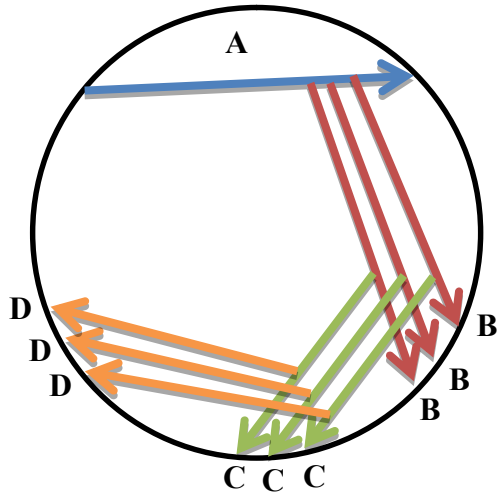

Fig. 1:- Culture Taking Method

B. Isolation of Bacteria

The Chicken meat samples were placed in different sterile petridish. The surface of meat was sterilized with a spatula heated by spirit burner. The sterilized surfaces of meat were cut with a sterilized scissor into a depth of half inch. The wire loop sterilized by red heat was dipped into the incision. The wire loop was then taken out from the incision and inoculated into the peptone broth and incubated at 37˚C overnight. On the next day, the growth of bacteria was checked by using Gram staining and streaked out onto the Nutrient and the Mac Conkey agar plates and incubated at 37˚C overnight. On the third day, the morphology, size and pigmentation of the bacterial colonies were examined and subcultures were carried out to get pure culture. In the case of chicken intestine and chicken ovary, the selenite broth and S-S (Salmonella-Shigella) agar were supplemented.

C. Identification of Bacteria

The Bacteria were identified by the methods described by Cowan and Steel (1993). Primary tests and secondary tests were undertaken in the identification process.

Primary Tests

Gram Reaction

The cells were first fixed to a glass slide by heat, and stained with a basic dye (crystal or methyl violet) that was washed off with an iodine-potassium iodide solution; then washed with water and cautiously decolorized with acetone or ethyl alcohol. The smear was counterstained with safranin. Gram-positive organisms retained the basic dye following decolorization with acetone or alcohol and appeared deep violet. Gram-negative organisms did not retain the violet stain but took up the counterstain (safranin) and stained red to pink.

Colony Morphology

Size and outline of the colonies and their elevation and colors were characterized on solid media (Nutrient agar, Mac Conkey agar and S-S (Salmonella-Shigella) agar plates.

Motility

Direct method was used to determine the motility of bacteria. One drop of distilled water was put on a clean glass slide. A small amount of colony was emulsified in distilled water. A cover slip was placed onto the suspension and examined under the microscope.

Spores

Spore shapes were oval, spherical or round. Locations of spores were central, subterminal or terminal.

O-F (Oxidation-Fermentation Test)

Duplicate tubes of solidified medium were inoculated by stabbing. One tube was promptly covered with a layer of sterile melted petroleum jelly (yellow soft petroleum) to depth of 5 – 10 mm and both were incubated for up to 30 days. Fermenting organisms produced in acid reaction throughout the medium in the sealed (anaerobic) and in the open (aerobic) tube. Oxidizing organisms produced in an acid reaction only in the open tube. This began at the surface and gradually extended downwards and might appear only after an alkaline reaction had been present for several days. Organisms that could not breakdown the carbohydrate aerobically or anaerobically produced an alkaline reaction in the open tube and no change in the sealed tube. This medium could be used for gas production and motility.

Results	Open tube	Sealed tube
Oxidation	Yellow	Green
Fermentation	Yellow	Yellow
No reaction	Blue or Green	Green

Ability to grow in air (Aerobic)

All bacteria except the strict anaerobes can grow in air at 37˚C.

Catalase Test

The presence of catalase reduces H_2O_2. The reagent (3% H_2O_2) was placed by the sterilized wire loop to the slide. A single colony on the Nutrient agar was picked out and emulsified in the reagent. The production of gas bubbles from the surface of the reagent indicated a positive reaction. It occurred almost immediately.

Secondary Tests

Indole Test

Certain bacteria decompose the amino acid tryptophan to indole in the medium. Indole is tested by a colorimetric reaction with ρ-dimethyl aminobenzaldehyde. The medium was inoculated and incubated at 37˚C for 24 hours or sometimes, for 96 hours. Kovac's reagent (0.5 ml) was added and shaken gently. A red color indicated a positive reaction.

M-R Test (Methyl Red Test)

Certain bacteria produce sufficient acid during the fermentation of glucose and the maintenance of conditions such that an old culture is sustained below pH about 4.5. A change in the color of methyl red indicator is observed at the end of incubation period.

The fluid medium was inoculated slightly from a young agar slope culture and incubated at 37˚C for 48 hours. Five drops of methyl red reagent were added, mixed and read immediately. Positive tests were bright red and negative were yellow. If the results were equivocal after 48-hour incubation, the test was repeated with the cultures that had been incubated for 5 days.

V-P Test (Voges-Proskauer Test)

The plates were incubated at 37˚C for 48 hours and added 1 ml of 40% KOH and 3 ml of 5% solution of α-naphthol in absolute ethanol. A pink color indicated the positive reaction in 2 – 5 minutes. It became crimson in 30 minutes. The tube was shaken at intervals, color changes occurred.

Hydrogen Sulphide Production Test

Triple sugar iron (TSI) agar was used for the detection of H_2S production. The butt and slope of TSI agar were inoculated by stabbing and streaking with a straight wire and incubated at 37˚C for at least 7 days. Daily observation was made for blackening due to the production of H_2S.

Citrate Utilization Test

The culture was inoculated into Simmon's citrate medium. The inoculum was dipped into the butt of the medium and incubated at 37˚C for 96 hours. Blue color and the growth indicated the positive result and original green color and no growth the negative result.

Urease Test

The urea medium was heavily inoculated and incubated at 37˚C. This was examined after the overnight-incubation and no tube was ascribed as negative until 4-day incubation. A purple pink color indicated the positive result due to a change the color of the indicator.

Nitrate Reduction Test

Nitrate reduction test reagent (0.1 ml) was added to the test medium. A red color developed within a few minutes indicated the presence of nitrite.

Gelatin Liquefication Test

The culture was inoculated and incubated overnight. The medium was put into the fridge (0 – 4˚C) for 15 – 20 minutes. Liquefied indicated the positive result and solidified the negative result.

Phenylalanine Deaminase Test

The medium was inoculated with a fairly heavy inoculum and incubated at 37˚C for up to 24 hours. A few drops of 10% ferric chloride solution were allowed to run down over the growth on the slant. In the positive tests, a green color developed on the slant.

Arginine Hydrolysis Test

The bacterium was inoculated into the arginine broth (5 ml) and incubated at 37˚C for up to 24 hours. Nessler's reagent (0.25 ml) was used. A brown color indicated the positive results due to the presence of NH_3.

Lysine Decarboxylation Test

The bacterium was inoculated into the lysine broth (5 ml) with a straight wire through the paraffin layer and incubated and examined daily up to 14 days. The medium first became yellow due to acid production from glucose, later when decarboxylation occurred, the medium became violet.

Sugar Tests

These tests were used for acid production and gas formation. Fermentation reactions occurred within 24-hour incubation. The sugar tests were incubated for the maximum of 40 days and checked 24 hourly. Once fermentation occurred, the actual date at which it first appeared was recorded.

III. RESULTS AND DISCUSSION

In this research paper, 9 species belonging to 8 genera of mesophilic bacteria have been identified, described and recorded. Primary tests of biochemical activity were made as shown in Table 1.

Table 1:- Primary Tests of Biochemical Activity of Bacteria

No.	Isolated Bacteria	Gram	Shape	Motility	Catalase	Oxidase	Growth on Mac	O-F
1.	*Staphylococcus epidermidis*	+	S	−	+	−	−	F(g)
2.	*Shigella dysenteriae*	−	R	−	+	−	+	F(g)
3.	*Citrobacter koseri*	−	R	+	+	−	+	F(g)
4.	*Escherichia coli*	−	R	−	+	−	+	F(g)
5.	*Klebsiella ozaenae*	−	R	−	+	−	+	F(g)
6.	*Enterobacter aerogenes*	−	R	+	+	−	+	F(g)
7.	*Streptobacillus moniliformis*	−	R	−	−	−	−	F
8.	*Salmonella pullorum*	−	R	−	+	−	+	F
9.	*Salmonella choleraesuis*	−	R	+	+	−	+	F

S: Spherical; R: Rod; +: (Positive); −: (Negative); Mac: Mac Conkey; O-F: Oxidation-Fermentation; F: Fermentative; F(g): Fermentative with gas.

After primary tests had been conducted, secondary tests of biochemical activity were continued to confirm the identification of isolated bacterial species as shown in Table 2.

Table 2:- Secondary Tests of Biochemical Activity of Bacteria

No.	Isolated Bacteria	Indole	P. alanine	T.S.I	Urea	KNO_3	Gelatin	M-R	V-P	Citrate	Arginine	Lysine	Dextrose	Lactose	Maltose	Mannitol	Sucrose	Xylose
1.	*Staphylococcus epidermidis*	−	−	−	−	+	−	( )	( )	−	+	( )	+	−	+	−	+	−
2.	*Shigella dysenteriae*	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−
3.	*Citrobacter koseri*	+	−	−	−	+	−	+	−	+	+	−	+	+	+	+	+	+
4.	*Escherichia coli*	+	−	−	−	+	−	+	−	−	+	+	+	+	+	+	+	+
5.	*Klebsiella ozaenae*	−	−	−	−	+	−	+	−	+	−	+	+	+	+	+	+	+
6.	*Enterobacter aerogenes*	−	−	−	−	+	−	−	+	+	−	+	+	+	+	+	+	+
7.	*Streptobacillus moniliformis*	−	−	−	−	−	−	+	−	−	+	−	+	−	( )	+	−	−
8.	*Salmonella pullorum*	−	−	−	−	+	−	+	−	+	+	+	+	−	+	+	−	+
9.	*Salmonella choleraesuis*	−	−	+	−	+	−	+	−	+	+	+	+	−	+	+	−	+

P.alanine: Phenylalanine; T.S.I: Triple Sugar Iron; ( ) : untested; M-R: Methyl-Red; V-P: Voges-Proskauer

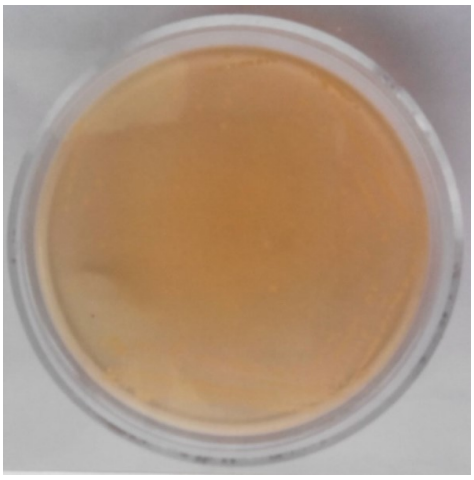

Fig. 2:- *Staphylococcus epidermidis* on Nutrient agar;

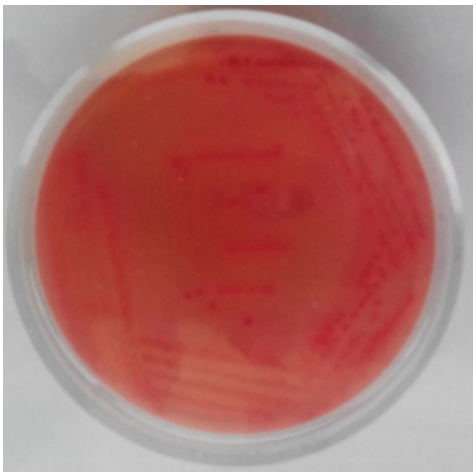

Fig. 3:- *Escherichia coli* on Mac Conkey agar

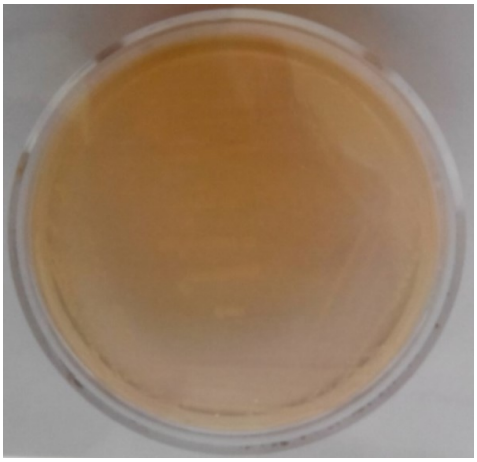

Fig. 4:- *Escherichia coli* on Nutrient agar;

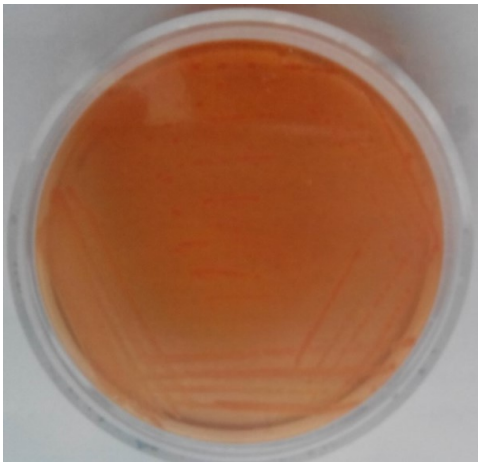

Fig. 5:- *Salmonella* on S-S (Salmonella- Shigella) agar

In Table 3, the percentage of the isolated bacteria has been calculated.

Table 3:- Percentage of Bacteria isolated from Chicken meat, Ovary and Intestine

No.	Isolated Bacteria	Source	Percentage
1.	*Staphylococcus epidermidis*	Chicken meat	14.3%
2.	*Shigella dysenteriae*	Chicken meat	18.5%
3.	*Citrobacter koseri*	Chicken meat	3.7%
4.	*Escherichia coli*	Chicken meat	14.8%
5.	*Klebsiella ozaenae*	Chicken meat	11.1%
6.	*Enterobacter aerogenes*	Chicken meat	14.8%
7.	*Streptobacillus moniliformis*	Chicken meat	3.7%
8.	Unidentified	Chicken meat	18.5%
9.	*Salmonella pullorum*	Chicken ovary	16.7%
10.	*Salmonella choleraesuis*	Chicken ovary	25.0%
11.	*Escherichia coli*	Chicken ovary	58.3%
12.	*Escherichia coli*	Chicken intestine	25.0%
13.	*Enterobacter aerogenes*	Chicken intestine	75.0%

During 7 months, a total of ninety samples were used in the isolation of bacteria. In the present research, some isolates could not be identified with the techniques employed. Chicken meat, ovaries and intestines were used because they serve as good sources of media in which bacteria could be readily isolated and studied. Cowan and Steel, (1993) stated that staining methods were used the morphology of bacteria and their affinity for certain dyes (Table 1). Genta, M.L., *et al*., (2001) discussed that the microbial flora invaded to food, two major problems arose. First was the pathogenicity of several microbes, and second was the change on the food characteristics. In the present investigation, all the isolated bacterial species were identified by using secondary tests of most diagnostic laboratory (Table 2).

Cruickshank R., (1965) stated that *Staphylococcus epidermidis* was commonly present on the skin and hair of many animals and birds. It was an occasional opportunist and slightly pathogenic. In the present study, *S. epidermidis* was found as 14.3% in chicken meat (Fig. 2); (Table 3). *Shigella dysenteriae* was the most frequent organism responsible for dysentery in man. In the present study, *S. dysenteriae* was isolated the highest amount (18.5%) from chicken meat (Table 3). *Citrobacter koseri* caused urinary tract infections and were found in wound, respiratory, meningitis and sepsis.

Escherichia coli inhabits in the intestines of humans, animals and poultry. Some species were commensals and others were responsible for enterotoxemia in young animals. It frequently caused diarrhea in humans and animals. It was widely distributed in water and its presence indicates contamination. It produced serious infections if introduced into the tissues or other parts of the body other than the gastrointestinal tract. *Klebsiella ozaenae* generally was non-pathogenic and some strains were pathogenic for mice.

Enterobacter aerogenes caused bovine mastitis. It could confuse with *E. coli*. Differentiation could be done with citrate test. *E. aerogenes* was a common inhabitant of the intestines. *Streptobacillus moniliformis* occurred as a normal inhabitant in nasopharynx of rats; caused arthritis in rodents and rat bite fever in man. Genta, M.L. *et al*., (2001) found that eggs, milk, chicken and hamburger were the most frequent vehicles for *Salmonella* sp. In the present study, *E. coli* was found as the highest percentage (58.3%) in chicken ovary (Fig. 3 & 4). *E. aerogenes* was found as the highest percentage (75.0%) in chicken intestine (Table 3). *Salmonella pullonum* (16.7%) and *S. choleraesuis* (58.3%) were isolated from chicken ovary (Fig. 5); (Table 3).

IV. Conclusion

In conclusion, the present study showed that chicken meat, ovary and intestine were highly contaminated with fecal materials. These results were in agreement with those described by the authors: Bryan, A.H. *et al*., (1874); Cruickshank R. (1965); Saif, Y.M. (1997); and Gracey, J.F. *et al.*, (1999); Genta, M.L. *et al*., (2001). Moreover, Win, O.Y. (2019) stated that duck meat sold in retail markets in Taunggyi was highly contaminated with fecal materials. Based on these findings, it is necessary to implement sanitary step to minimize the growth of pathogenic and spoilage microorganisms. The consumption of contaminated meat poses the risk of foodborne diseases and it had little attention in epidemiological studies. Thus, hygienic practices should be improved at the retail markets. Moreover, all the meat sellers should be adopted hygienic practices.

Acknowledgment

We would like to acknowledge Dr Nwe Nwe Yin, the Acting Rector of Panglong University for her permission to submit this article. We would like to thank Dr Min Min Soe, Professor and Head, Department of Botany, Panglong University for her review of this paper. We are greatly indebted to U Maung Maung Lwin, Lecturer (Retired), Institute of Animal Husbandry & Veterinary Science, Yezin for his invaluable guidance, advice and constructive criticisms upon reading the manuscript. Our sincere thanks are due to Dr Aye Aye Than, Researcher and all the staff at the Regional Veterinary Diagnostic Laboratory, Taunggyi, for their invaluable guidance, advice, suggestion and comments. comments.

REFERENCES

[1.] Bryan, A.H. and Bryan, C.G. 1874. *Bacteriology Principles and Practice*, 5th. ed., Barnes & Noble, Inc. New York.
[2.] Clifton C.E. 1950. *Introduction to the Bacteria*, 1st. ed., M.C. Graw-Hill Book (Company), Inc. New York.
[3.] Cowan and Steel, 1993. *Handbook of Microbiological Media*, 3rd. ed., Cambridge University Press, pp. 1 – 173.
[4.] Cruickshank R. 1965. *Medical Microbiology*, 11th. ed., The English Language Book Society and E. & S. Livingstone Ltd.
[5.] Genta, M.L. and Heluane, H. 2001. "Biochemical Identification of Most Frequently Encountered Bacteria that cause Spoilage", *Food Microbiology Protocols*, Humana Press Inc., Totowa, NJ. Vol. 14, pp. 494.
[6.] Gracey, J.F., Collins, D.S., Huey, R.J. 1999. *Meat Hygiene*, 10th. ed., John Wiley & Sons Ltd., pp. 14, 89 – 112.
[7.] Saif, Y.M. 1997. Diseases of Poultry. 10th. ed., Iowa State University Press, USA, pp. 619 – 988.
[8.] Win, O.Y. 2019. "Quantitative Analysis of Bacteria in Duck Meat as Hygiene Indicator". *Panglong University Research Journal*, Vol. 10, No. 1, pp. 188 – 195.