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# Isolation and biochemical characterization of bacteria in processed chicken meat balls and determination of antibiotic susceptibility

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## Abstract

In emerging countries like Sri Lanka, urbanization, religious beliefs, and health influence meat consumption. Meat contains essential amino acids and high protein content. Meat has a short shelf life and spoils quickly if preservation methods are not followed properly. Pre-slaughter handling and post-slaughter handling of meat affect the quality of the meat product. Therefore, meat processing is important to increase the shelf life. Temperature, storage atmosphere, water activity and pH level affect how quickly meat spoils. Proper hygiene procedures should be followed to prevent the emergence of antibiotic-resistant bacteria and microbial contamination of food. The objective of this research study was to isolate and characterize bacteria in selected five different chicken meatball brands in local market and determine antibiotic susceptibility. This sampling consisted of five different chicken meatball brands in local market. After the enrichment process, several subculture procedures were used to obtain single colonies from the culture plates. Motility test, gram's staining and endospore staining was done to observe the bacteria morphology. Indole, methyl-red, Voges-Proskauer (VP), citrate, catalase, and Triple Sugar Iron (TSI) biochemical tests were performed for biochemical characterization. Finally, the antibiotic resistance of the isolated organisms to ampicillin, erythromycin, vancomycin, tetracycline, and chloramphenicol was evaluated by Kirby Bauer's disc diffusion method. Evidence of microbial growth was detected in all five samples. The organisms from each sample were predicted as *Vibrio nigripulchritudo*, *Bacillus cereus*, *Aeromonas salmonicida*, *Paenibacillus alvei* by MATLAB®. However, 16S sequencing needs to be performed to accurately determine the organisms present in the samples. Regardless of standards, poor hygiene controls, poor heat treatment, contaminated minor ingredients, can lead to bacterial contamination. For this, the responsible institutions should check and update the quality assurance processes.

**Keywords:** Food microbiology, Food quality, Biochemical tests, Antibiotic susceptibility

## 1. Introduction

Processed meat is defined as meat that has been salted, cured, fermented, smoked, or given another flavoring.[1] Meat and animal products, particularly well-known meat products like sausages, ham, salami, meatballs, etc., are highly valued in many cultures all over the world.[2]

Processed poultry meat is made by treating and processing fresh poultry meat to extend its shelf life and enhance flavor. Some treatments employ chemical preservatives like sodium nitrite.[2]

Processing refers to the mechanical recovery of chicken flesh from a chicken carcass or the mixture of chicken flesh and skin. There are numerous unique forms of preparations that can be done on different meat products. Any meat that has gone through a procedure like drying, curing, maturing, or precooking is referred to as transformed meat.[3]

In Asian countries, chicken meatballs are a common chicken meal. As with red meatballs, the chicken can simply be substituted for the red meat. The most crucial concept is that they are an exquisite food product.[3]

The poultry industry continued to develop and industrialize throughout the world, it was primarily responsible for the increase in global meat production for the last five years. The Food and Agriculture Organization (FAO) reports that 119 million pounds of poultry meat were produced worldwide.[4]

The fastest-growing agricultural sub-sector in emerging nations, particularly, is the chicken industry, which plays a crucial role in nutrition. Future expansion of the sector is anticipated to be influenced by variables like population increase, income level growth, and urbanization. The poultry industry, with a market size of $ 310.7 billion in 2020, is anticipated to increase by 3.8% annually to reach $ 322.55 billion in 2021.At a 7% Compound Annual Growth Rate, the market is anticipated to reach $ 422.97 billion in 2025.[5]

Poultry was the most widely consumed form of meat in the world in 2021 with an estimated 132.3 million tons consumed. By 2031, 153.85 metric kilotons of poultry meat are anticipated to be consumed globally.[6] In 2020, Sri Lanka's population consumed 10.4 kg of poultry meat annually. This is an increase of 19.2% from the prior year. In the past, Sri Lanka's consumption of poultry meat per person reached an all-time high of 10.4 kg in 2020 and a record-low of 0.630 kg in 1961.Regarding the interest rate on poultry meat intake per capita, Sri Lanka is rated 107$^{th}$ out of the 161 nations.[7]

The contamination of processed chicken products with pathogenic microorganisms, particularly bacteria, is one of the most challenging problems facing the worldwide food industry.[8] The carcasses, the cuts made from them, and processed meat products are all contaminated during and after slaughter by the atmosphere of the slaughterhouse, the instruments used, and the microbes from the animal microbiome.[9] While food is being prepared and kept, some of these bacterial contaminations might emerge or continue to exist. Contamination of equipment surfaces by bacteria can occur early in the process. This includes rubber fingers that are used to remove feathers or conveyor belts as sources of bacterial contamination. Even new rubber fingers can harbor bacteria and be a source of contamination for carcasses. Cross-contamination can occur between cuts or carcasses through contact with a contaminated surface or direct contact. Air, manipulators, and equipment surface are the main sources of contamination during subsequent processing processes such as cutting, deboning, mixing, and mincing, for the production of meat-related food items. Transformation processes expand the surface area of meat in contact with air and work surfaces. As a result, there is a higher level of bacteria in the transformed products than in the primary cuttings.[9] *Escherichia coli*, *Bacillus subtilis*, *Campylobacter*, and *Salmonella* sp. are typical bacteria found in chicken products.[10]

These bacteria are resistant to some antibiotics. Antibiotic resistance occurs when bacteria acquire resistance to antibiotics meant to kill, stop their growth. When exposed to antibiotics, antibiotic-resistant bacteria are capable of proliferating, expanding, and infecting the host. Antibiotic resistance becomes an obstacle in the treatment of bacterial infections. The end result is that some antibiotics can no longer be used to treat some bacterial infectious diseases.[11] Antibiotic resistance mechanisms can be divided into four categories. The first involves beta-lactamases to modify the antibiotic's enzymes, thereby breaking the beta-lactams ring of penicillin and cephalosporins. Other enzymes (e.g., AmpC), hydrolyze most beta-lactams. Alteration of the target site of the bacterial cell wall is the second mechanism of antibiotic resistance. This involves the production of modified penicillin-binding proteins that reduce the target affinity for the antibiotic. Third, bacteria can use efflux pumps to prevent the accumulation of various antibiotics in bacterial cells by pumping out from the cell. Finally, some antibiotics (e.g., sulfonamides) work by inhibiting the synthesis of molecules essential for bacterial survival, such as folic acid.[12]

The general objective of this study was to biochemically characterize the microorganisms present in five processed meat products available in the local market and to test their antibiotic sensitivity. To achieve this objective, microorganisms were cultured under necessary conditions and staining techniques were performed to observe the morphology. After that, isolated organisms were tested against selected antibiotics to assess sensitivity.

## 2. Methodology

*2.1 Sample collection.* Commercially available, chicken meatballs from five (05) different brands were purchased from local supermarkets in Sri Lanka.

**Table 1.** Meatball sample details

| Sample ID | Packaging size (g) | Standards used for the production |
|---|---|---|
| **CM1** | 200 | SLS |
| **CM2** | 200 | HACCP |
| **CM3** | 200 | SLS |
| **CM4** | 200 | HACCP |
| **CM5** | 200 | SLS |
| **Positive Controls** | *S. aureus* (ATCC 6538)<br>*E. coli* (ATCC 8739)<br>*B. subtilis* (ATCC 6633) | |
| **Negative Control** | | |

*2.2 Sample Enrichment.* The products were surface sterilized before transferring to the bio safety cabinet (BSC) (HPsafe-1200LC/ Class, Type: II, A2). Packages were opened and approximate 01 g of each sample was weighed in to 10 ml sterile peptone water added Uricol™ containers. Aseptic conditions were maintained during the transfer.

For negative control 10 ml of peptone water (HIMEDIA®/ Ref: M028-500G) was used. The containers were incubated at 37 °C for 24 hours.

*2.3 Screening and isolation of microorganisms*

*2.3.1 Streak plate method using Nutrient Agar (NA).* The streak plate technique was used and incubated at 37 °C for 24 hours. Well isolated colonies were selected for further analysis. Gram staining, motility testing, and endospore staining were performed on these selected colonies from each sample.

*2.4 Microscopic analysis*

*2.4.1 Motility test (hanging drop method).* A loopful of isolated colony inoculum from each sample was examined under a microscope.

*2.4.2 Gram's staining.* Gram staining was carried out according to the ASM protocol. [36]

*2.4.3 Endospores staining.* Endospore staining was carried out following the Schaeffer-Fulton procedure.[37]

*2.5 Biochemical tests*

*2.5.1 IMViC test*

*2.5.1.1 Indole test.* A loopful of pure culture inoculum was inoculated in tryptophan broth (HIMEDIA®/ Ref: M1339-500G) 37°C for 24 hours. 2-3 drops of Kovac's reagent were added into each test tube, and results were obtained after 15 minutes.

*2.5.1.2. Methyl-red test.* MR-VP broths (HIMEDIA®/ Ref: M070-500G) were inoculated and incubated at 37 °C for 24 hours. 2-3 drops of methyl-red indicator were added into each test tube, and results were taken after 15 minutes.

*2.5.1.3 VP test.* As sample ID, five test tubes containing VP broth (HIMEDIA®/ Ref: M070F-500G) were inoculated and were incubated at 37 °C for 24 hours. 2-3 drops of Barritt's reagent were added into each test tube, and results were taken after 15 minutes.

*2.5.1.4 Citrate test.* Test was performed using citrate agar slants (HIMEDIA®/ Ref: M099-500G) Bacterial colonies were streaked throughout the test tubes. The test tubes were incubated at 37°C for 24 hours.

*2.5.2 TSI test.* 5 slanted test tubes with TSI agar (HIMEDIA®/ Ref: M021-500G) were used. Bacterial colonies were streaked throughout the test tubes. The test tubes were incubated at 37°C for 24 hours.

*2.5.3 Catalase test.* Catalase was tested using drop of hydrogen peroxide (20% V/V) on a bacterial smear.

*2.6 Hi-chrome E. coli agar inoculation.* A loopful of the bacterial inoculum was straked on Hi-chrome (HIMEDIA® /Ref: M1295-500G) to check whether the isolated organism was *E. coli*. Plates were incubated at 37°C for 24 hours.

*2.7 Streak plate method using TCBS agar*. The streak plate technique was then used and incubated at 37°C for 24 hours.

*2.8 DNA extraction.* Pure colonies were transferred to 1.5 ml tubes. 1ml of saline water (0.9% NaCl) was added and vortexed. It was centrifuged for 5 minutes at 13000 rpm. The supernatant was discarded. The preceding stages were carried out twice. The pellet was vortexed after 20 µl of TAE buffer was added. The tubes were placed on ice for 15 minutes before being placed in a 95°C water bath for 15 minutes. It was then centrifuged for 5 minutes at 13000 rpm. The supernatants were transferred to fresh microcentrifuge tubes. A total of 20 µl of 99% ethanol was added. The tubes were then placed on ice.

*2.9 DNA quantification.* Quartz cuvette was used to measure optical density of extracted DNA samples. At 225 nm, 260 nm, 280 nm, 330 nm, and 450 nm.

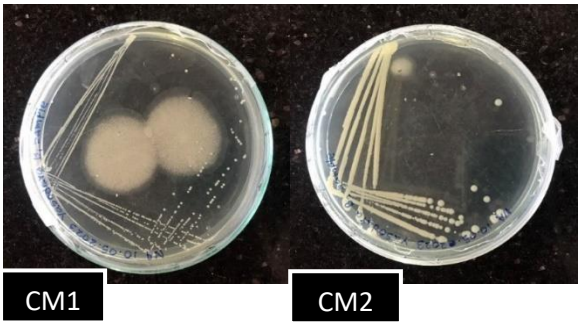
*2.10 Antibiotic susceptibility test*

*2.10.1 Disc diffusion method.* A loopful of pure isolated colony from each sample was swabbed on Mueller Hinton Agar (MHA) (HIMEDIA®/ Ref: M173-500G) plates with sterile cotton swabs. Selected antibiotic discs were placed on the plates using sterile forceps and incubated at 37°C for 24 hours.

*2.11 Organism prediction using MATLAB.* Observed biochemical test results were fed into MATLAB annbis algorithm interface to generate predictions.[32]

## 3. Results

*3.1 Streak plate method.* The colony morphologies observed indicated the presence of a single type of organism. In some of the plates pigment formation could be observed.



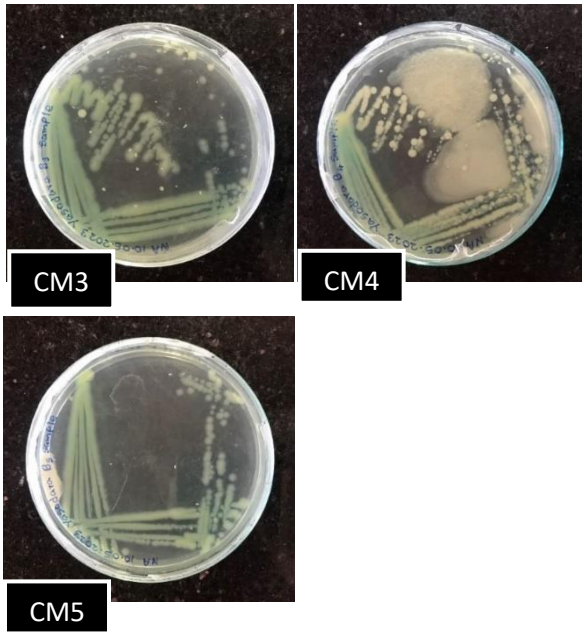




**Figure 1.** Results of 3rd subculture nutrient agar plates

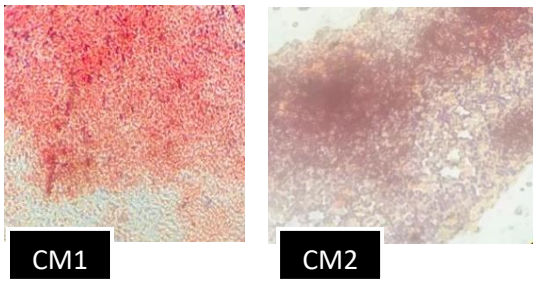
*3.2 Microscopic analysis*

*3.2.1 Motility test.* The results of the motility test are summarized in table 2. Selected bacteria from each sample were motile in all the samples, and rod-shaped bacteria could be observed in sample CM3 while others were cocci.

**Table 2.** Motility test results

| Sample ID | Shape |
|---|---|
| **CM1** | Cocci |
| **CM2** | Cocci |
| **CM3** | Bacilli |
| **CM4** | Cocci |
| **CM5** | Cocci |

*3.2.2. Gram's staining*



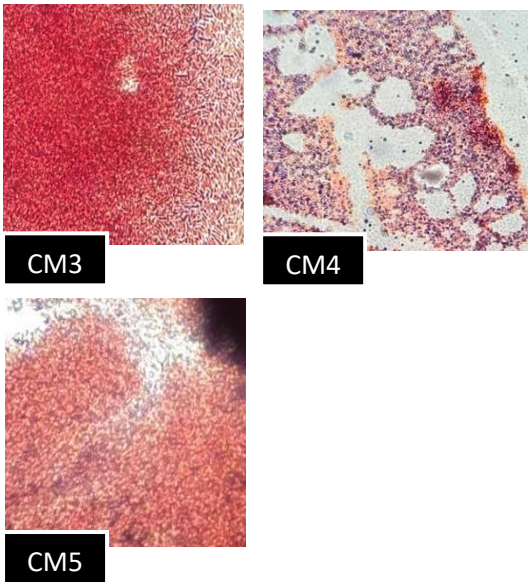



**Figure 2.** Gram's-stained bacteria under 100X magnification

When observed under the microscope (10X 100X1), samples CM1, CM3, CM5 appeared pink in color indicating Grams negative nature, while samples CM2 and CM4 appeared purple in color indicating Grams positive.

*3.2.3 Endospore staining*

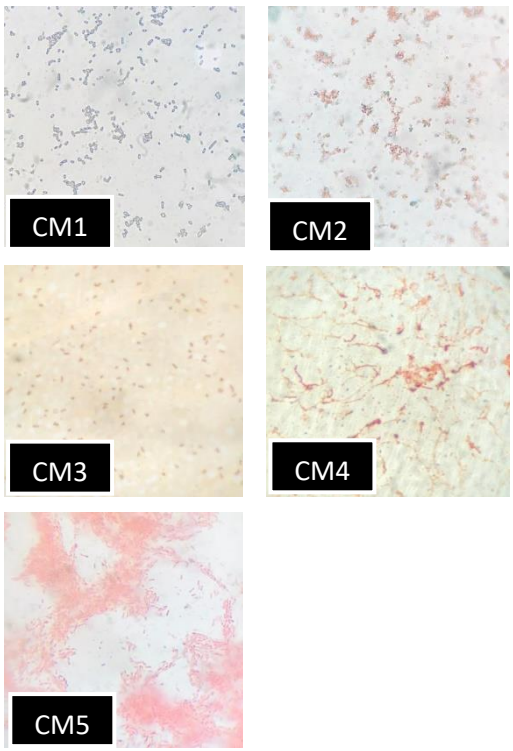



**Figure 3.** Endospore-stained bacteria under 100X magnification.

When observed under the microscope (10X 100X1), the spores of CM1 sample were appeared green and the other samples except CM1 was appeared red. The results of the endospore's staining test are summarized in the table 3.

**Table 3.** Observation of selected organisms from sample

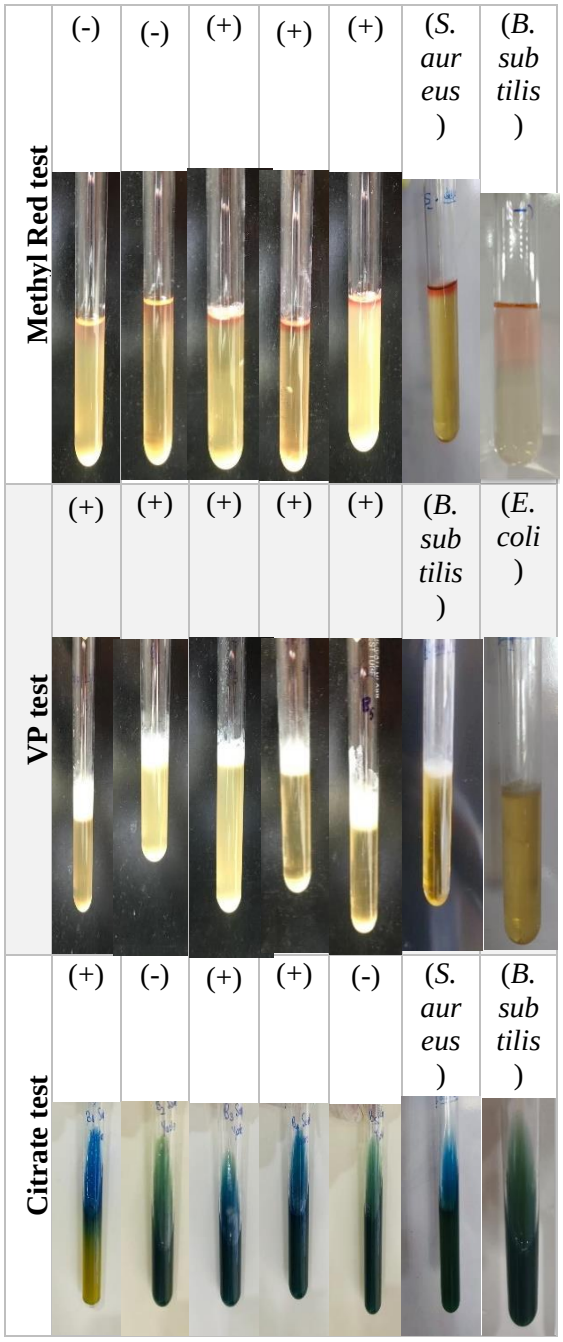
| Sample ID | Gram nature | Endospore | Motility |
|---|---|---|---|
| **CM1** | G- | observed | Motile |
| **CM2** | G+ | Not observed | |
| **CM3** | G- | Not observed | |
| **CM4** | G+ | Not observed | |
| **CM5** | G- | Not observed | |

*3.3 Biochemical Test results*

*3.3.1 IMViC test*

**Table 4.** IMViC test results

| Test | CM1 | CM2 | CM3 | CM4 | CM5 | Positive | Negative |
|---|---|---|---|---|---|---|---|
| Indole test | (+) | (+) | (+) | (+) | (+) | (*E. coli*) | (*B. subtilis*) |
| |  |  |  |  |  |  |  |




Positive Results- (+), Negative Results- (-)

*3.3.2 TSI test*

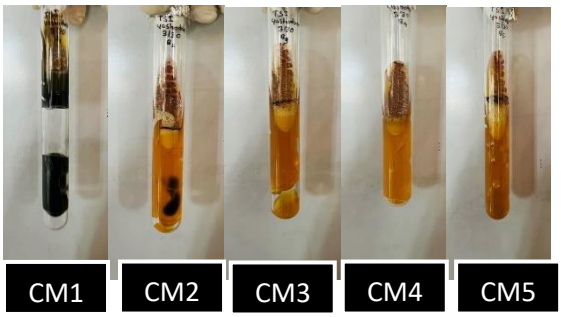



**Figure 4.** Results of TSI test

**Table 5.** TSI test results

| Sample ID | Slant | Butt | Gas production | Blackening of the medium/ black pigments |
|---|---|---|---|---|
| **CM1** | | | ✓ | ✓ |
| **CM2** | R | Y | ✓ | ✓ |
| **CM3** | R | Y | ✓ | |
| **CM4** | R | Y | ✓ | |
| **CM5** | R | Y | ✓ | |

R- Red, Y- Yellow

*3.3.3 Catalase test*

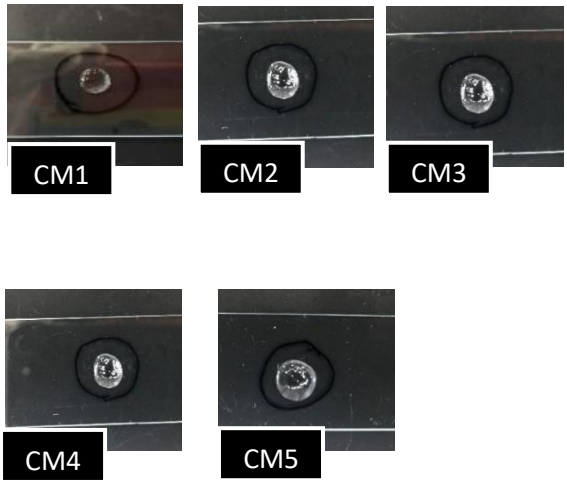



**Figure 5.** Results of catalase test

Bubbles appeared in all the samples except CM1.

*3.4 Isolation on Hi-chrome E. coli agar*

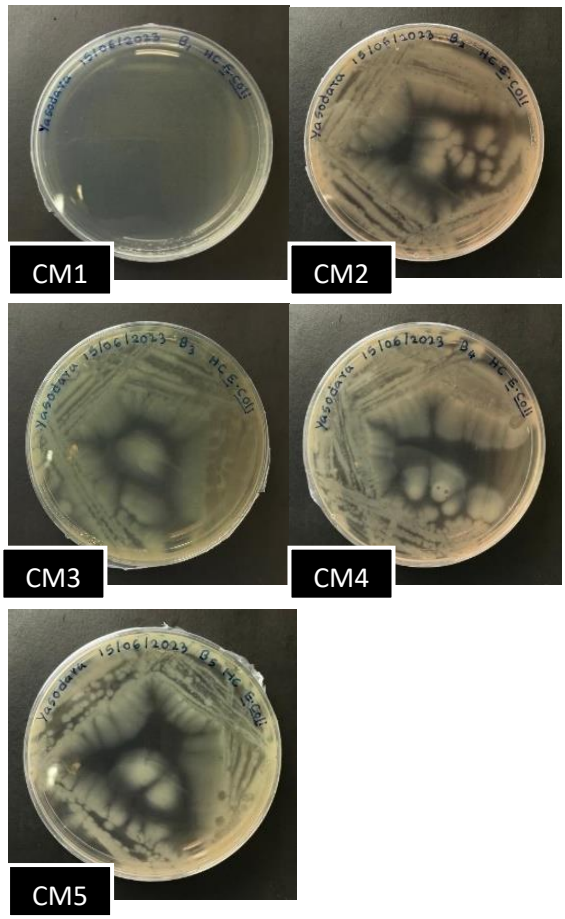
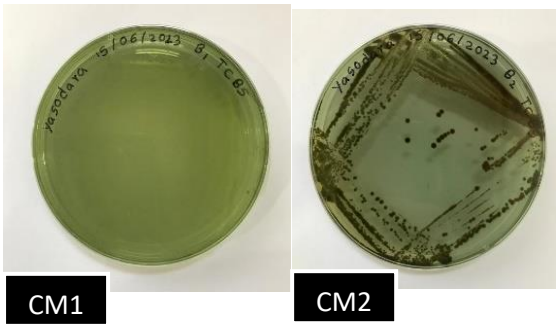



**Figure 6.** Hi-chrome *E. coli* agar test results

**Table 6.** Results of Hi-chrome *E. coli* agar test

| Sample ID | Blue green appearance | colorless |
|---|---|---|
| **CM1** | | ✓ |
| **CM2** | | ✓ |
| **CM3** | | ✓ |
| **CM4** | | ✓ |
| **CM5** | | ✓ |

*3.5 Inoculation on TCBS agar*



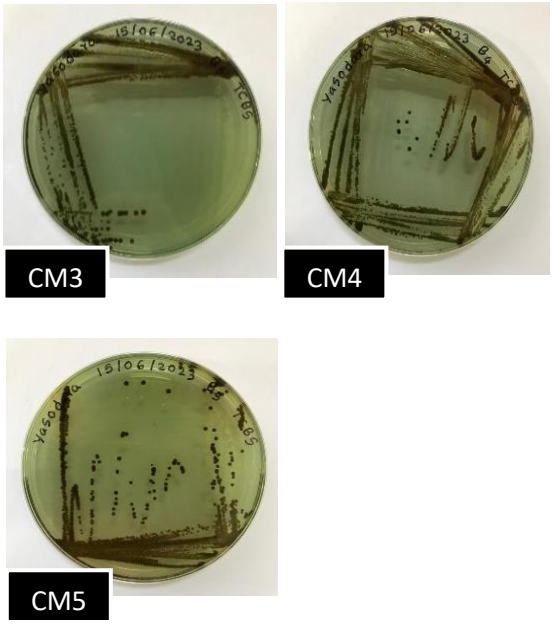



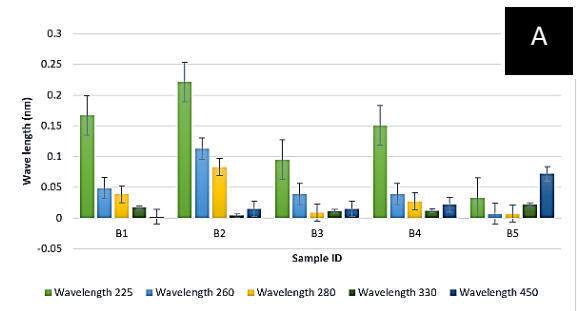

**Figure 7.** TCBS agar test results

**Table 7.** Results of TCBS agar test

| Sample ID | Growth inhibited | Bacterial Growth | Colony color |
|---|---|---|---|
| **CM1** | ✓ | | |
| **CM2** | | ✓ | Dark green |
| **CM3** | | ✓ | Dark green |
| **CM4** | | ✓ | Dark green |
| **CM5** | | ✓ | Dark green |

*3.6 DNA quantification*

*3.6.1 Spectrophotometer*



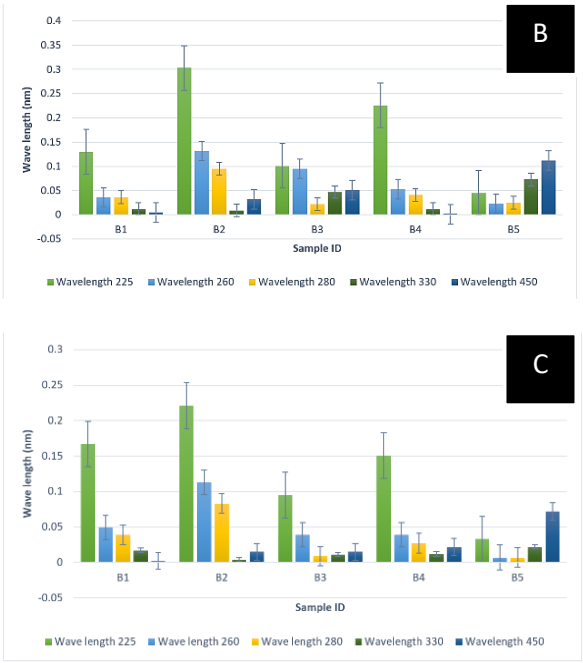
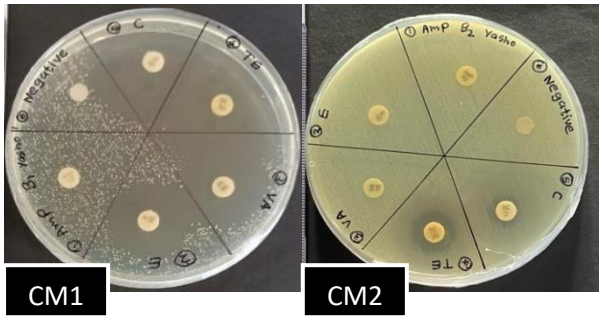
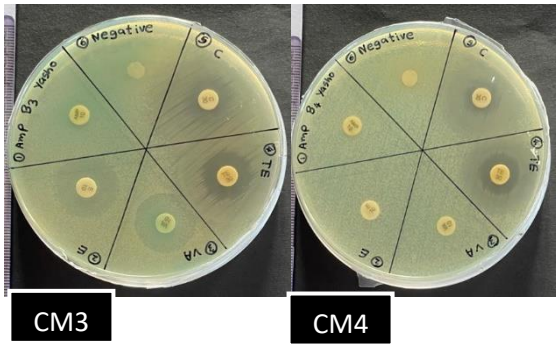



**Figure 8.** DNA OD measurement using 5 µl (A), 10 µl (B) and 15 µl (C).

*3.7 Antibiotic susceptibility test*

*3.7.1 Disc diffusion method*



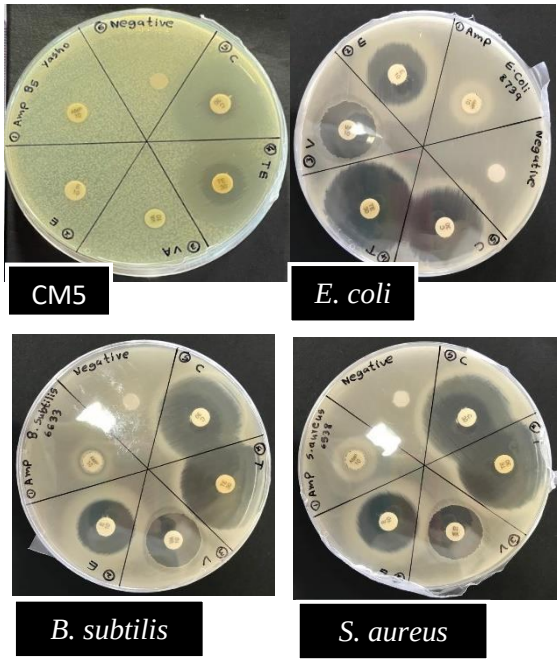




**Figure 9.** ABST on MHA the zone of inhibition (ZOI), incubated at 37 °C for 24 hrs.

**Table 8.** ZOI of inhibition (mm).

| Sample ID | Amp | Ery | Van | Tet | Chl |
|---|---|---|---|---|---|
| **CM1** | - | 28.4 | 24.8 | 39.5 | 36.5 |
| **CM2** | - | - | - | 21.8 | 31.5 |
| **CM3** | - | 11.6 | 18.2 | 28.5 | 35.3 |
| **CM4** | - | - | - | 24.8 | 22.3 |
| **CM5** | - | - | - | 22.3 | 12.5 |

Amp – Ampicillin, Ery – Erythromycin, Van – Vancomycin, Tet – Tetracycline, Chl – Chloramphenicol

**Table 9.** Organism prediction using MATLAB®

| Sample ID | Organism | Evaluation | Similarity |
|---|---|---|---|
| **CM1** | *Bacillus cereus* | Good identification | VP (+), IND (+) |
| **CM2** | *Aeromonas salmonicida subsp. Masoucida* | Acceptable identification | VP (+), IND (+), CIT (+) |
| **CM3** | *Vibrio nigripilchritudo* | Acceptable identification | VP (+), IND (+), CIT (+) |

| | | | |
|---|---|---|---|
| **CM4** | *Vibrio nigripilchritudo* | Acceptable identification | VP (+), IND (+), CIT (+) |
| **CM5** | *Paenibacillus alvei* | Good identification | VP (+), IND (+), CIT (+) |

**Table 10.** Susceptibility of the predicted organisms to the selected antibiotics according to Bergey's Manual.

| **Sample ID** | **Predicted Organism** | **Amp** | **Ery** | **Van** | **Tet** | **Chl** |
|---|---|---|---|---|---|---|
| **CM 1** | *Bacillus cereus* | R | R | S | R | S |
| **CM 2** | *Aeromonas salmonicida subsp. Masoucida* | R | I | R | R | S |
| **CM 3** | *Vibrio nigripilchritudo* | R | R | R | S | I |
| **CM 4** | *Vibrio nigripilchritudo* | R | R | R | S | I |
| **CM 5** | *Paenibacillus alvei* | R | R | S | S | S |

R - resistant, S - sensitive, I – intermediate

## 4. Discussion

The purpose of this study was to isolate and biochemically characterize the bacteria present in selected processed chicken meat ball products and ascertain whether or not they were susceptible to antibiotics. The samples were collected from the Kalubowila area in Sri Lanka. After the enrichment process, the turbidity was observed in the sample containers indicating the presence of microorganisms. Overgrowth could be seen in some culture plates. A series of biochemical tests were performed to characterize the bacteria present in the samples.

The hanging drop method was used to study bacterial motility along with the size, shape, and arrangement of bacteria.[13] According to research performed by Supriya in 2023, if there are motile bacteria, the bacterial structure, shape, and number of bacteria can be predicted to some extent. When there is no movement, the result indicates absence of flagella or other locomotive structures.[14] Selected organisms from B1 to B5, bacteria swam erratically with the kinetic energy held by the fluid molecules surrounding the microparticles in the fluid. Therefore, those bacteria were motile in CM1, CM2, CM4, and CM5, bacteria appeared in the form of spheres, so the bacterial shape were recognized as cocci.

Gram staining was performed to determine the ability of bacterial cell walls to retain crystal violet dye during solvent.[15] Microorganisms selected from samples CM1, CM3, and CM5 stained pink and were gram-negative. Gram-negative bacteria have a thin peptidoglycan layer and an outer lipid membrane. In CM2, CM4 samples the bacteria stayed purple therefore they were gram positive. Presence of a thick cell wall, peptidoglycan layer and no outer lipid membrane, those bacteria are capable of retaining crystal violet and iodine complex when decolorized by ethanol, which appeared as blue or purple. The CM3 sample was rod-shaped and was identified as bacilli. All the other 4 samples were round-shaped and were identified as cocci. According to study done by WU and YANG in 2020, the quality of the bacterial cell wall determines whether the organism will be Gram-negative or Gram-positive.[16] Fresh bacteria cultures were used for staining.

Endospore staining test helps to identify endospore-containing bacteria.[17] According to a research performed by Hussey and Zayaitz in 2007, the endospore retains malachite green color and appears green. The cells could appear red-brown or pink in the absence of spores.[17] Samples CM2, CM3, CM4, and CM5 appeared red-brown in color. Therefore, it can be concluded that spores were absent in those bacteria during the time of testing. The CM1 sample appeared green and it was concluded that they were spores. However, the bacterial samples were not specifically stressed to induce spore formation prior to the endospore staining.

The indole test is used to measure the ability of an organism to degrade the amino acid

tryptophan and produce indole.[18] *E. coli* was used as the positive control and *Bacillus subtilis* was used for the negative control. A cherry red ring appeared at the top of the positive control medium and no color change in the negative control. A pink to red was formed in the treatment layer above the medium in all the samples (CM1 to CM5) indicating positive results for the indole test. This concludes that all isolated bacterial samples were capable of producing indole.[19]

The Methyl-red test was performed to test the ability of the organism to produce, maintain stable acids as an end product of glucose fermentation and to overcome the buffering capacity of the system.[20] For Methyl-Red test, *S. aureus* was used for positive control and *B. subtilis* was used as the negative. The culture medium of the positive control turned red. This is because the pH was 4.4 or lower due to glucose fermentation. No color change occurred in the negative control. CM1 and CM2 did not exert any color change, but CM3, CM4, and CM5 samples turned red indicating positive reaction. It was concluded that the selected microorganisms in samples CM3, CM4, and CM5 have the ability to produce and maintain stable acids as the end product of glucose fermentation. Microorganisms selected in CM1 and CM2 samples are not able to do so. According to a research performed by Shanmugaraj, Anokhe and Kalia in 2021, two (02) of the three (03) bacterial samples they had studied was negative for Methyl Red, and one sample was positive.[21]

VP test determines whether organisms produce acetylmethyl carbinol by fermenting glucose.[22] *B. subtilis* and *E. coli* was used for positive and negative controls for VP test. When the results were analyzed it could be concluded that all 5 samples were positive.

The primary purpose of the Citrate test is to determine the ability of an organism to use citrate as a sole source of carbon for metabolism with alkalinity.[23] In the citrate test, *S. aureus* was used as the positive control and *B. subtilis* was used as the negative control. The color of the positive control slant changed from green to dark blue. The negative control did not show any color change. The color of samples CM1, CM3, and CM4 changed from green to dark blue after incubation at 37 °C for 24 hours. Therefore, CM1, CM3, and CM4 samples were positive and it was concluded that the selected microorganisms can use citrate as a carbon source. But there was no color change in CM2 and CM5 samples, so those samples were negative. It was concluded that those bacteria are not able to use citrate. The CM1 sample appeared in blue color above and yellow color below. The reason for this is the pH difference and the presence of anaerobic bacteria. According to a paper by Salauddin *et al* published in 2019, citrate testing has been done for two (02) samples of bacteria.[24]

The TSI test was performed to determine an organism's ability to produce hydrogen sulfide and to ferment glucose, sucrose, and lactose.[25] CM1 and CM2 samples blackened the medium after incubation at 37 °C for 24 hours. Hence the selected microorganisms in those samples were capable of producing $H_2S$In samples CM2, CM3, CM4, and CM5, the slant appeared red and the butt appeared yellow. Therefore, it can be concluded that these samples showed alkaline/acid conditions. Hence it was concluded that organisms fermented glucose in the medium. Cracks could be observed in all sample tubes. It was concluded that all 5 samples produced gas. According to a study done by Sultana *et al* in 2022, the TSI test has been done for 5 samples.[26]

Catalase test was performed to identify organisms that produce catalase enzyme. This enzyme breaks down hydrogen peroxide into oxygen and water.[27] No bubbles occurred in sample CM1 indicating a negative result. Other samples were positive for catalase test. It was concluded that the organisms in these samples produce catalase enzymes. According to a research performed by Khairullah *et al* in 2022, the catalase test has been done for *Staphylococcus aureus* and a positive result has been obtained.[28]

Hi-chrome *E. coli* agar test is used to detect *Escherichia coli* and total coliforms simultaneously.[29] There was no growth of colonies in the CM1 sample, while colonies were observed in the rest of the samples. However, desired colour change (Bluish-green colony appearance) could not be observed. It was concluded that there was no *E. coli* in the enriched samples. According to a research

performed by Antony in 2018, there were *E. coli* positive and negative samples they had analyzed.[29]

TCBS agar test was done to check whether *Vibrio* species were present in the samples.[30] CM1 sample did not show any growth on TCBS, while the rest of the samples were capable of making colonies on TCBS agar. Since growth was observed on the plates. It can be concluded that *Vibrio* presence in CM2 to CM5 samples. But, based on the manufacturer reference the colonies on the plate may probably not *Vibrio cholerae, Vibrio parahemolyticus* or *Vibrio alginolyticus*, and could be another subspecies of Vibrio, which needs to be confirmed by further genetic testing.

According to the obtained biochemical results Aeromonas salmonicida, Vibrio nigripulchritudo, Paenibacillus alvei in the samples were further predicted using the mentioned MATLAB platform. However, this datapoint has to be confirmed with further DNA sequencing. [30,32] The predictions obtained from MATLAB platform may not be completely accurate since the later versions of MATLAB does not support the annbiss algorithm. Predictions could not be further confirmed with genetic testing.

The antibiotic susceptibility test determines the sensitivity or resistance of bacteria to specific antibiotics.[31] *E. coli, B. subtilis*, and *S. aureus* were used as positive controls. The sensitivity to the ABST test was analyzed with reference to MATLAB® predictions. CLSI standards were used to determine the zone diameter around the predicted organism. The CM1 sample was *Bacillus cereus* and the CM2 sample was *Aeromonas salmonicida subsp. masoucida*, samples CM3 and CM4 were identified as *Vibrio nigripulchritudo* and sample CM5 as *Paenibacillus alvei*.[32] All samples from CM1 to CM5 were resistant to Amp. CM1, CM3, CM4 and CM5 samples were resistant to erythromycin. The CM2 sample was intermediate for Ery. CM2, CM3, and CM4 samples were resistant to vancomycin and CM1 and CM5 samples were sensitive. CM3, CM4, and CM5 samples were sensitive to tetracycline, and CM1 and CM2 samples were resistant. CM1, CM2, and CM5 samples were sensitive to chloramphenicol, and CM3 and CM4 samples were intermediate.[33] Studies conducted in 2019 show that *Bacillus cereus* is resistant to Erythromycin, and Tetracycline. The ABST test conducted for CM1 also gave similar results to the study.[34] A 2021 study found that *Aeromonas* is resistant to ampicillin. The ABST test conducted for the CM2 sample also obtained similar results to the study.[35]

The antibiotic sensitivity of this work was based on the MATLAB annbis algorithm predictions which should be further confirmed with DNA sequencing. However, it was confirmed that there are microorganisms capable of growing on hi-chrome and TCBS agar and carrying out various other biochemical reactions as mentioned in the context. It is suggested that the quality of processed meat products produced in Sri Lanka should be further investigated.

## 5. Conclusion

In conclusion, by examining the results of the tests conducted, it can be concluded that all five (05) samples of processed chicken meat ball products contained bacteria with various biochemical capacities. Although chicken meat balls are processed under food safety conditions, this work evidenced the presence of microorganisms in those products.

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