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# Isolation and identification of microorganisms in processed chicken products and determination of antibiotic susceptibility

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## Abstract

On a global level, there is a rising demand for a steady supply of animal protein, and consumption of processed meat. Meat is the most perishable food type since it has the most nutrients that enables microbial development. To enhance flavor and improve shelf life, processed chicken products are produced by smoking, fermenting, curing, salting, and adding chemical preservatives. *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis* are the most frequent bacteria found in processed chicken products. The processed chicken meat may become contaminated by a range of bacteria during meat processing facility operations. One of the largest challenges to development, food security, and global health is antibiotic resistance. Studies on the horizontal transfer of drug resistance determinants have focused mostly on the primary pathogenic and opportunistic microorganisms. The food chain can be viewed as the primary means of transmission of microorganisms resistant to antibiotics between the animal and human populations. This study aims to isolate and identify microorganisms in processed chicken products and determine the antibiotic susceptibility. Five commercially available processed chicken products from different manufacturers were collected from Sri Lankan supermarkets. Enrichment, plating methods, staining techniques, biochemical tests, DNA extraction and determining the antibiotic susceptibility using the disk diffusion method were the major procedures followed in the study. Microorganisms were predicted in all the samples using probabilistic neural network and multidimensional scaling method using MATLAB®. *Bacillus cereus, Listeria monocytogenes* and *Paenibacillus alvei* were predicted by the software. However, further confirmation using molecular biological techniques regarding the same is required to effectively conclude the results.

**Keywords:** Enrichment, DNA quantification, Food microbiology, Food quality and Antibiotic susceptibility

## 1. Introduction

Presently, there is a gradually increasing demand for a consistent supply of animal protein on a global scale, and processed meat consumption is currently expanding. Since meat contains enough nutrients to support the growth of microorganisms, it is the most perishable of all food types. Microbial infection can reduce the quality of meat, reduce its shelf life, cause financial loss, as well as present health risks. Meat is an essential component of the growth, repair, and maintenance of body cells because it is the main source of protein and important vitamins for most people in many parts of the world. Processed chicken products are made by smoking, fermenting, curing, salting, and adding chemical preservatives to improve flavor and increase shelf life. There is a worldwide interest in preservation to maintain the safety and bioavailability of processed meat for a longer shelf life.[1]

Typically, the first evidence of chicken meat dates from 4,000 to 10,500 years ago. It was claimed to have originated in India, Northern China, and Southeast Asia.[2] In 2020, Sri Lanka's population consumed 10.4 kg of poultry meat annually. This is 19.2% increase from the previous year (2019).[3] In comparison to 9.8 kg in 2000, the global per capita consumption of poultry increased to 14.8 kg in 2019. In 13 countries, the total amount of poultry consumed per person is more than doubled. In 30 of the 35

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studied countries, there was a linear increase in per capita poultry consumption between 2000 and 2019.[4] In Sri Lanka, there were 7.09 kg of chicken available annually per person as meat and meat-related products and the total amount of meat produced increased from 119,620 million tons in 2004 to 185,490 million tons in 2013.[5] 130 million tons of poultry were produced globally in 2019, an increase of 3.7% from the previous year (2018). It is anticipated that this growth will continue, accounting for 45% of global production. Nearly 40% of the meat produced worldwide in 2020 was poultry meat.[6]

Microorganisms, which include bacteria, fungi, and protozoans, are defined as organisms or agents of infection that are microscopic or submicroscopic in size.[7] The common microorganisms that are present in processed chicken products are *Escherichia coli, Bacillus subtilis, Aspergillus brasiliensis, Salmonella* and *Campylobacter*.[8] Many foodborne illnesses are caused by these bacteria. *E. coli* is a typical resident of intestinal tracts and is present in dust, litter, chicken droppings, and rodent droppings.[9] An alternative to antibiotic growth promoters in the poultry industry could be *B. subtilis* as in-feed antibiotics.[10] *Salmonella gallinarum* and *Salmonella pullorum* are particularly adapted to poultry. They are responsible for the pullorum disease (*S. Pullorum*) and fowl typhoid (*S. Gallinarum*).[11] *E. coli* infections, salmonellosis and paratyphoid infections, and fowl cholera are other common infectious diseases in poultry animals.[12]

During operations at meat-processing plants, a variety of microorganisms may contaminate the processed chicken meat.[13] Microbial contamination can reduce the quality of meat, minimize its shelf life, lead to financial loss, and possibly available health hazards. Meat spoilage and afterward decomposition are caused by the practically unavoidable infection, which is carried by the animal itself, by those handling the meat, and by their tools.[14] The spoilage from bacteria is very low immediately after processing, but they can quickly grow and produce spoilage slime and odors. The primary causes of poultry products spoilage are prolonged distribution, inappropriate storage temperature and time, and high post-rigor meat pH.[15] The careful regulation and oversight of the slaughtering and processing facilities, proper handling and storage, and adequate cooking of raw and processed poultry products are all necessary for the prevention of microbial contamination.[16] Patients with weakened immunity are more vulnerable to the serious effects of foodborne infections, which can range from long-term illness to death. Typically, bacteria and their metabolites, parasites, viruses, or toxins are responsible for microbiological food-borne illnesses. Depending on the foods consumed, the methods used for food processing, preparation, handling, and storage, as well as the population's sensitivity, different food borne diseases have varying levels of importance in different countries.[17]

Antibiotic resistance (AR) is currently one of the biggest threats to development, food security, and global health. When bacteria learn to resist the medications meant to kill them, antibiotic resistance occurs. AR quickens, when bacteria are compelled to respond to the presence of antibiotics.[18] The primary pathogenic and opportunistic bacteria have received most of the attention in studies on the horizontal transfer of drug resistance determinants. The main way of transmission of antibiotic resistant bacteria between the animal and human populations can be thought of as the food chain.[19] At least 1.27 million people are dying from antibiotic resistance-related causes worldwide and this number is expected to reach almost 5 million in 2019.

**Table 1.** The common microorganisms in processed chicken products and antibiotic resistance.[20]

| Microorganisms | Resistant antibiotics |
|---|---|
| *Escherichia coli* | Penicillins, aminopenicillins, and cephalosporins |
| *Bacillus subtilis* | Lincomycin, streptogramin A, and antibiotic virginiamycin M |
| *Aspergillus brasiliensis* | Polyenes, triazoles, and echinocandin |
| *Salmonella* | Ampicillin, chloramphenicol, florfenicol, |

| | |
|---|---|
| | streptomycin, sulfonamides, and tetracycline |
| *Campylobacter* | Ciprofloxacin, and fluoroquinolone antibiotic |

This study is aimed to isolate and identify microorganisms in processed chicken products and determine the antibiotic susceptibility. The main techniques used in the study are plating methods, staining methods, biochemical tests, DNA extraction, and investigating the antibiotic susceptibility using disk diffusion method.

## 2. Methodology

*2.1 Sample collection.* Commercially available five processed chicken products (sausages, meatballs, ham, bacon and lingus) from different manufacturers were collected from local supermarkets in Sri Lanka. Surface sterilization of intact packaging was done using 70% ethanol to minimize environmental contamination and stored at -20 ℃. *E. coli* (ATCC 8739)*, S. aureus* (ATCC 6538) and *B. subtilis* (ATCC 6633) were used as positive controls.

**Table 2.** Processed chicken samples and the product type used for the study.

| Sample number | Product type |
|---|---|
| **1** | Chicken sausages |
| **2** | Chicken meatballs |
| **3** | Chicken ham |
| **4** | Chicken bacon |
| **5** | Chicken lingus |

*2.2 Enrichment.* Products were transferred to biosafety cabinet after surface sterilization. They were opened inside the biosafety cabinet to minimize contamination. Sterile surgical blades were used to sample the meat products. Approximately 1 g of sample was taken and transferred to sterile labelled Uricol™ containers containing 10 ml of sterile peptone water (HIMEDIA®/Ref: M028-500G). The containers were incubated at 37 ℃ for 48-72 hours. Positive and negative controls were also subjected to the same conditions.

*2.3 Culturing of microorganisms*

*2.3.1. Streak plate method.* After incubation, a loopful of samples from each container was inoculated in nutrient agar (NA) (HIMEDIA®/Ref: M57-500G) and soybean casein digest agar (SCDA) (HIMEDIA®/Ref: M290-500G) under aseptic conditions. The plates were incubated at 37 °C for 24 hours.

*2.3.2 Liquid culturing.* The selected colonies of bacteria were inoculated into 2 ml microcentrifuge tubes containing 1.5 ml sterile nutrient broth (HIMEDIA®/Ref: M002-500G). The tubes were incubated at 37 °C for 24-72 hours. The organisms were propagated by subculturing from time to time.

*2.4 Microscopic observation.* Microscopic observations were performed to identify the selected pure colony from each sample. The colonies were propagated by subculturing from time to time.

*2.4.1. Gram's staining technique.* The isolated colonies of bacteria were placed on microscopic slides using sterile inoculation loop. The staining was carried out following the ASM gram staining protocol.[46] The specimens were observed under the microscope.

*2.4.2 Endospore staining.* A loopful of bacteria were placed on the microscopic slides using sterilized inoculation loop. The staining was carried out following the ASM spore staining protocol.[46]

*2.4.3 Motility test.* Bacterial motility was observed using hanging drop method.

Fungus or molds that were present on the media plates were not selected for further analysis.

*2.5 Biochemical testing*

2.5.1. IMViC test

*2.5.1.1. Indole test.* A loopful from liquid culture tubes were inoculated in 10ml of tryptophan broth (HIMEDIA®/Ref: M1339-500G). The tubes were incubated for 24 to 48 hours at 37 °C.

2-3 drops of Kovacs reagent were added to it after incubation.

*2.5.1.2. Methyl-red test.* A loopful from liquid culture tubes were inoculated in 10ml of MR-VP broth (HIMEDIA®/Ref: M070-500G). The tubes were incubated for 24 to 48 hours at 37 °C. 2-3 drops of Methyl-Red indicator were added to it after incubation.

*2.5.1.3. VP test.* A loopful from liquid culture tubes were inoculated in 10ml of VP broth (HIMEDIA®/Ref: M070F-500G). The tubes were incubated for 24 to 48 hours at 37 °C. 2-3 drops of Barritt reagent were added to it after incubation.

*2.5.1.4. Citrate utilization test.* The selected colonies from streak plates were streaked on 10 ml citrate agar slant. The tubes were incubated for 24 hours at 37 °C.

*2.5.2 TSI test.* The selected colonies from streak plates were streaked on 10 ml TSI agar (HIMEDIA®/Ref: M021-500G) slant. The tubes were incubated for 24 hours at 37 °C.

*2.5.3. Catalase test*. A small amount of bacteria was placed onto the microscopic slides using sterilized inoculating loop. A drop of hydrogen peroxide (20% V/V) was placed on top of the bacterial smear.

*2.6 DNA extraction.* The pure culture plates were prepared by streaking a loopful from the liquid culture. After incubation at 37 ℃ for 48 hours, bacterial colonies were transferred to 1.5 ml microcentrifuge tubes using sterile blades and inoculation loop. 1 ml of saline water (0.9% NaCl) was added to the tubes. They were vortexed thoroughly. The tubes were centrifuged at 13 000 rpm for 5 minutes. The supernatant was discarded. The washing step was repeated once again. Residual supernatant was discarded. 20 µl of TAE buffer was added to the pellet. The tubes were vortexed again and were transferred to ice for 15 minutes. The tubes were immediately kept in 95 ℃ water bath for 15 minutes. The tubes were centrifuged at 13 000 rpm for 5 minutes. The supernatants were transferred to 1.5 ml new sterile microcentrifuge tubes. 20 µl of 100% ethanol was added to each tube containing the supernatant. The tubes were stored at -20 ℃ until further analysis.

*2.7 DNA quantification*

*2.7.1. Spectrophotometer.* Quartz cuvette was used to measure absorbance at 225 nm, 260 nm, 280 nm, 330 nm and 450 nm. Distilled water was used as the blank. Various dilutions were used to measure the absorbance.

*2.8 Antibiotic susceptibility test*

*2.8.1 Disc diffusion method.* The microbial inoculum was spread on the surface of Mueller Hinton Agar (MHA) (HIMEDIA®/Ref: M173-500G) using sterile cotton swabs. Ampicillin (SD002), Erythromycin (SD083), Vancomycin (SD163), Tetracycline (SD037) and Chloramphenicol (SD006) discs were used as antibiotics. The plates were incubated for 24 hours at 37 °C. Zone of inhibition was measured using a vernier caliper after incubation.

The organisms tested from each sample were predicted using Probabilistic Neural Network (PNN) structure and classical Multidimensional Scaling (MDS) method in MATLAB® software.[21] Annbis GUI was used for this purpose. Antibiotic susceptibility was determined after referring to the CLSI M100 guidelines.[22]

## 3. Results

*3.1 Streak plate method.* Growth was observed in all the pre-culture plates indicating the presence of organisms in all the samples. Various colony morphologies could be observed.

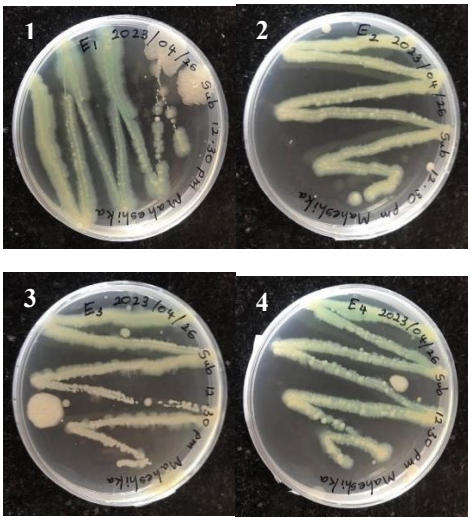



**Figure 1.** Third Sub-culture in Nutrient Agar.

The colonies observed had relatively similar morphologies indicated the presence of a single type of colony.

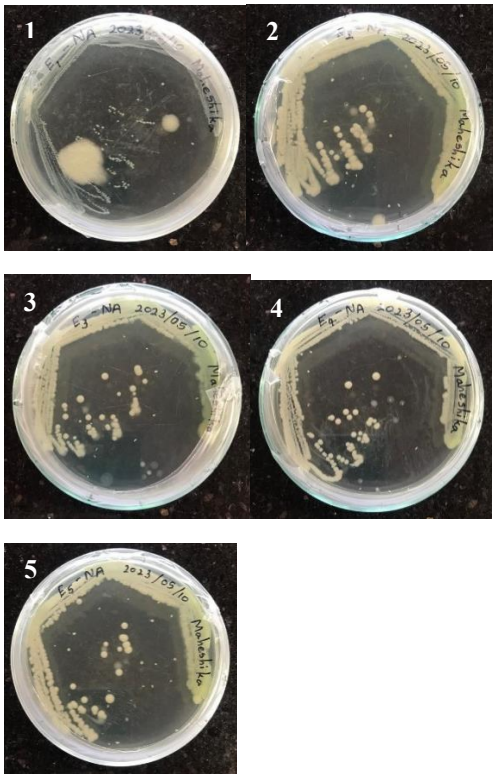
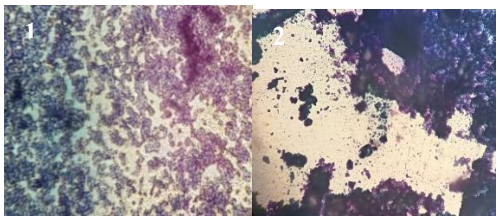



**Figure 2.** Fourth Sub-culture in Nutrient Agar.

After subsequent culturing pure colonies could be isolated. This was confirmed by having same colony morphologies.

3.2 Microscopic observation

*3.2.1. Gram's staining technique*



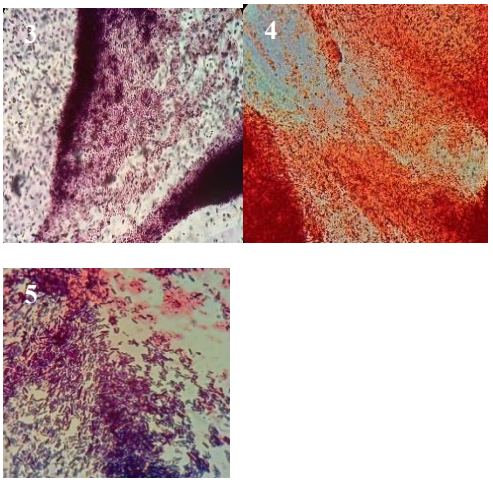




**Figure 3.** Gram's-stained bacteria under 100x magnification.

Gram-positive bacteria were observed in purple colour and Gram-negative bacteria in pink colour. Out of all 5 samples, 1, 2, 3 and 5 were Gram-positive and only sample 4 was Gram-negative.

*3.2.2. Endospore staining*

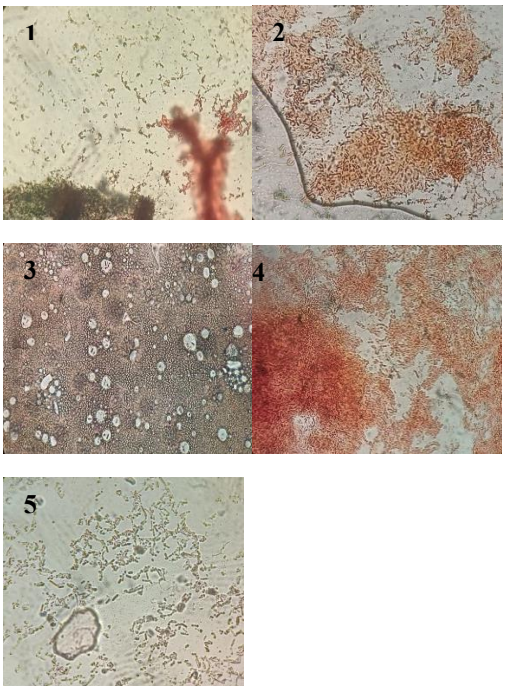



**Figure 4.** Endospore-stained bacteria under 100x magnification.

Bacteria containing spores were observed in green colour while vegetative bacteria were observed in red colour. Out of all 5 selected organisms, 1, 2, 3 and 5 could be

identified as endospore formers while sample 4 had vegetative cells.

*3.2.3 Motility test.* Out of all 5 samples, 1, 2, 3, 4 and 5 had motile bacteria. Cocci shaped bacteria were observed from 1, 2, 3 and 5 samples. Bacilli shaped bacteria were observed from 1, 3, 4 and 5 samples.

**Table 3.** Observations of selected organisms from samples.

| Sample | Gram nature | Endospore | Motility |
|---|---|---|---|
| **1** | Positive | Present | M |
| **2** | Positive | Present | M |
| **3** | Positive | Present | M |
| **4** | Negative | Absent | M |
| **5** | Positive | Present | M |

M- Motile

*3.3 Biochemical testing*

*3.3.1 IMViC test*

**Table 4.** IMViC test results.

| Test | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Positive | Negative |
|---|---|---|---|---|---|---|---|
| **Indole test** | + | + | + | + | + | | |
| **MR test** | + | + | + | + | + | | |
| **VP test** | + | + | + | + | + | | |
| **Citrate utilization test** | + | + | - | + | + | | |

(+) - Positive, (-) - negative

*3.3.2 TSI test*

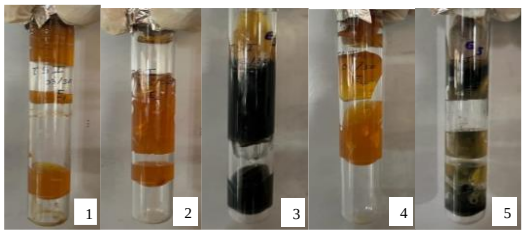



**Figure 5.** TSI test results.

Yellow slant and yellow butt were observed from sample 1, 2 and 4. It indicated the fermentation of glucose, sucrose and lactose. Black colour medium was observed from 3 and 5. It indicated the presence of $H_2$.

**Table 5.** The results of TSI test.

| Sample | $H_2$ gas production (black) | Acid/ Acid reaction (Y/S, Y/B) | Alkaline /Acid reaction (R/S, Y/B) | Gas production / ($H_2S$) |
|---|---|---|---|---|
| **1** | | √ | | √ |
| **2** | | √ | | √ |
| **3** | √ | | | √ |
| **4** | | √ | | √ |
| **5** | √ | | | √ |

Y/S: Yellow slant, Y/B: Yellow butt, R/S: Red slant

*3.3.3. Catalase test*

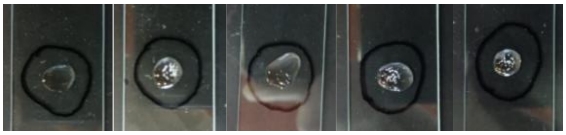


**Figure 6.** Catalase test results.

The positive reaction was indicated by the presence of bubbles upon addition of $H_2O_2$. Out of all 5 samples, 2, 3, 4 and 5 had positive results and only 1 had a negative result.

**Table 6.** The results of catalase test.

| Sample | Positive | Negative |
|---|---|---|
| **1** | | √ |
| **2** | √ | |
| **3** | √ | |
| **4** | √ | |
| **5** | √ | |

*3.4 DNA quantification*

*3.4.1. Spectrophotometer*

Wavelengths were measured at different wavelengths and significant amount of DNA was present in each sample.

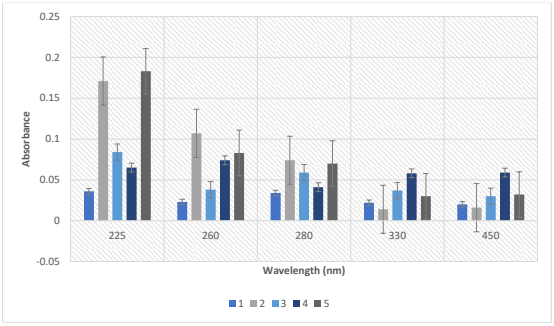



**Figure 7.** The readings of spectrophotometer in different wavelengths using 5 µl of DNA.

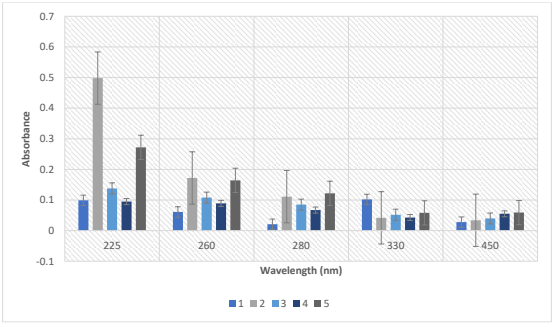



**Figure 8.** The readings of spectrophotometer in different wavelengths using 10 µl of DNA.

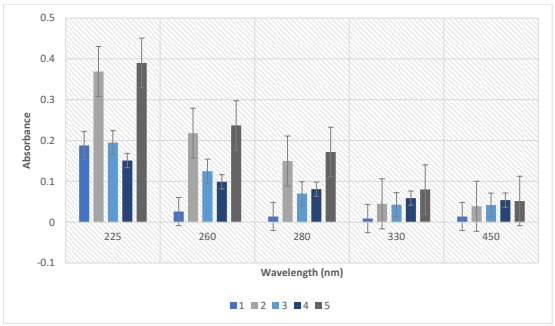



**Figure 9.** The readings of spectrophotometer in different wavelengths using 15 µl of DNA.

*3.5 Antibiotic susceptibility test*

*3.5.1. Disc diffusion method*

The zones of inhibition were observed in ABST.

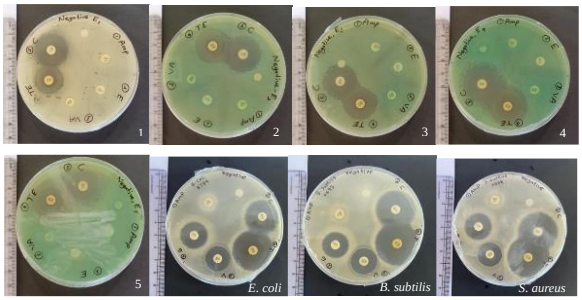



**Figure 10.** ABST results.

**Table 7.** The predicted organisms obtained from MATLAB® software by feeding biochemical characteristics.

| Sample number | Predicted organism |
|---|---|
| **1** | *Bacillus cereus* |
| **2** | *Listeria monocytogenes* |
| **3** | *Paenibacillus alvei* |
| **4** | *Listeria monocytogenes* |
| **5** | *Listeria monocytogenes* |

**Table 8.** The results of ABST.

| Sample | Antibiotics | Diameter of the ZOI | Sensitivity |
|---|---|---|---|
| **1** | Amp | - | R |

| | | | |
|---|---|---|---|
| | Ery | - | R |
| | Van | - | R |
| | Tet | 20.5 mm | I |
| | Chlor | 27.0 mm | I |
| **2** | Amp | - | R |
| | Ery | - | R |
| | Van | - | R |
| | Tet | 22.9 mm | S |
| | Chlor | 26.2 mm | S |
| **3** | Amp | - | R |
| | Ery | - | R |
| | Van | - | R |
| | Tet | 25.0 mm | S |
| | Chlor | 26.6 mm | I |
| **4** | Amp | - | R |
| | Ery | 15.6 mm | I |
| | Van | - | R |
| | Tet | 18.7 mm | S |
| | Chlor | 27.6 mm | I |
| **5** | Amp | - | R |
| | Ery | - | R |
| | Van | - | R |
| | Tet | 18.6 mm | S |
| | Chlor | 20.1 mm | I |

ZOI – Zone of inhibition, Amp- Ampicillin, Ery – Erythromycin, Van – Vancomycin, Tet – Tetracycline, Chlor – Chloramphenicol, R – resistant, I – intermediate, S – sensitive.

**Table 9.** The susceptibility of the predicted organisms.

| Sample No. | Predicted organism | Ampicillin | Tetracycline | Erythromycin | Chloramphenicol | Vancomycin |
|---|---|---|---|---|---|---|
| **1** | *Bacillus cereus* | | | √ | √ | |
| **2** | *Listeria monocytogenes* | √ | √ | √ | √ | √ |
| **3** | *Paenibacillus alvei* | | √ | | | √ |
| **4** | *Listeria monocytogenes* | | √ | √ | √ | |
| **5** | *Listeria monocytogenes* | √ | √ | √ | √ | √ |

√ = Sensitive

## 4. Discussion

On a global level, there is a steadily rising demand for a reliable source of animal protein, and the use of processed meat is currently expanding. Meat is the most perishable food category because it contains enough nutrients to support the growth of bacteria. One of the main pathways for the spread of antibiotic resistance between animal and human populations has come to be identified as the food chain. In fact, it has been demonstrated that commensal bacteria can serve as repositories for antibiotic resistance genes, which may be transmitted to foodborne or enteric human pathogens through the food chain .[23] This study showed the antibiotic susceptibility of isolated microorganisms in processed chicken products. Microorganisms in processed chicken products were isolated and identified using various laboratory tests including the culturing of microorganisms, microscopic observation, biochemical tests and antibiotic susceptibility test.

Enrichment was followed to provide optimal conditions to organisms that could be

present in the meat in minute amounts.[24] Because it helps with exceptionally favorable growth for an interesting organism and an unfavourable environment for any competition. This makes it possible to detect and identify microorganisms with a range of nutritional requirements.[25] After incubation, because there was turbidity in the peptone water, it was predicted that microorganisms could be present in all samples.

Numerous species of bacteria can grow on NA, which also includes many of the nutrients required for bacterial growth.[26] SCDA is frequently used for the cultivation of microorganisms from environmental sources and promotes the growth of a wide range of microorganisms.[27] The streak plate method is important for the isolation of bacteria from a large population.[28] Appropriate aseptic procedures prevent the unintended release of microorganisms into the environment and the contamination.[29] The absence of a necessary nutrient, the toxicity of the culture medium itself, or the presence of other bacteria in the sample that create chemicals that are inhibitory to the target organism are the causes of bacteria not growing in a culture plate.[30] A wide range of fastidious and non-fastidious microorganisms with no nutritional requirements can be cultivated using nutrient broth.[31] The liquid culturing was carried out to ensure the luxurious growth of the selected colony and to avid overgrowth which is usually observed on the solid agar plates.

Gram-positive bacteria were stained in purple while gram-negative bacteria were stained in pink in gram's staining technique. The process involves staining bacteria samples with crystal violet, which binds to peptidoglycan layer of both Gram-positive and Gram-negative bacteria.[32] Gram's iodine fixes the crystal violet into the bacteria's cell wall. Crystal violet inside gram positive bacteria bind to the iodine and continue to be purple. Gram negative bacteria contains secondary plasma membrane and will be washed off when treated with decolorizer.[33] Gram-negative and gram-positive bacteria can be easily separated with safranin staining. Since there is no dye available at this point, gram negative cells bind the safranin and appear pink under the microscope.[34] In this study, colonies grown on NA were used for gram's staining technique. Gram-positive bacteria were observed in 4 samples and gram-negative bacteria were observed from only 1 sample. Cocci and bacilli shaped bacteria were observed.

The cells containing spores appeared as green coloured spot containing cells while vegetative cells appeared as red colour in endospore staining technique.[35,45] In this research, the first liquid culturing Eppendorf tubes made using NB were used for endospore staining technique. The spore forming bacteria were observed from 4 samples and non-spore forming bacteria were observed only 1 sample. The motility test can determine whether the bacteria are motile or non-motile and the shape of the bacteria. The second sub-culture plates made using SCDA were used for motility test. Motile bacteria were observed from all 5 samples.

Biochemical tests were performed to identify specific bacterial species based on their metabolic activities.[36] In this research, the first liquid culturing microcentrifuge tubes made using NB were used for IMViC testing. The indole test was performed to figure out whether the bacteria could synthesize tryptophanase enzyme. The appearance of cherry red ring is considered as positive while negative is interpreted with no color change. All the samples were positive for indole test. Bacteria that have a high capacity for producing acidic byproducts during the metabolism of glucose are recognized using the methyl-red test. The appearance of red colour is considered positive while negative is interpreted with yellow colour. All the samples were positive for methyl-red test. The VP test is used to assess whether the bacterium can use the enzyme acetoin reductase to create acetylmethylcarbinol from glucose. The appearance of red colour is considered as positive while negative is interpreted with no colour change. All the samples were positive for VP test. The ability of the bacteria to use citrate as its only carbon source is assessed using the citrate utilization test. The appearance of blue colour is considered positive while negative is interpreted with no colour change. Blue colour was observed from 4 samples and no colour change was observed from only 1 sample.

The presence of sodium thiosulphate and ferrous sulphate fills the need for sulphur, and phenol red serves as an indicator of changes in the media's environment brought on by the production of acid or alkali in TSI test.[37] Gas production ($H_2/H_2S$) was observed from all samples. An acid/acid reaction was observed from 3 samples. Catalase-producing microbes were found using catalase test. By this enzyme, hydrogen peroxide is detoxicated by converting it to water and oxygen gas. The creation of the bubbles is the positive result of the catalase test.[38] Except for 1 sample, bubbles were observed from 4 samples.

The antimicrobials that will prevent the growth of the bacteria causing the specific condition are identified by antibiotic susceptibility testing.[39] In comparison to most other media, MH agar is a soft agar that provides for improved antibiotic diffusion. A zone of inhibition is a section of media where bacteria cannot grow because this area contains a drug that prevents the growth of the bacteria.[40] In sample 1, 2, 3 and 5, a zone of inhibition could be seen only in tetracycline and chloramphenicol. In sample 4, a zone of inhibition could be seen only in erythromycin, tetracycline and chloramphenicol. In sample 1, ampicillin, erythromycin and vancomycin were resistant and tetracycline and chloramphenicol were intermediate. In sample 2, ampicillin, erythromycin and vancomycin were resistant and tetracycline and chloramphenicol were sensitive. In sample 3 and 5, ampicillin, erythromycin and vancomycin were resistant, tetracycline was sensitive and chloramphenicol was intermediate. In sample 4, ampicillin and vancomycin were resistant, erythromycin and chloramphenicol were intermediate and tetracycline was sensitive.

The "Annbis" is a newly created graphical user interface (GUI) for bacterial identification that is powered by MATLAB®. This GUI can differentiate 270 bacterial Operational Taxonomic Units (OTUS) by comparing some optional biochemical information with probability matrices.[41] When an appropriate distance range is selected, the unknown bacterium and its allied OTUS in biochemical phenotype within the range can be displayed in three-dimensional space using the standard Multidimensional Scaling method. Statistical Neural Network structure is used to obtain the most probable result of bacterial identification.[21] The article of Osman et al., 2018 has reported that *Bacillus cereus* is highly resistant to tetracycline antibiotic.[42] According to the results of this work, *B. cereus* was resistant to tetracycline, vancomycin and ampicillin and sensitive to erythromycin and chloramphenicol. The article of Lyon et al., 2008 has reported that *Listeria monocytogenes* is intermediate to tetracycline antibiotic.[43] According to the results of this work, *L. monocytogenes* was sensitive to ampicillin, tetracycline, erythromycin, chloramphenicol and vancomycin. The article of Nthenge, Rotich and Nahashon, 2013 has reported that *Paenibacillus alvei* is resistant to ampicillin antibiotic.[44] According to the results of this work, *P. alvei* was sensitive to tetracycline and vancomycin and resistant to ampicillin, erythromycin and chloramphenicol. However, studies with increased biochemical characteristics should be fed into MATLAB® platform to confirm above predictions.

## 5. Conclusion

Public health professionals cannot emphasize enough how important food is in the transmission of infections. Based on the results, processed chicken meat remains a potential source in transmitting pathogenic foodborne microorganisms. It was predicted that *Bacillus cereus, Listeria monocytogenes* and *Paenibacillus alvei* bacteria are present in processed chicken meat. These isolates were resistant to ampicillin, erythromycin and vancomycin while increased sensitivity toward tetracycline and chloramphenicol was recorded. Due to the rising threat of multidrug-resistant *B. cereus, L. monocytogenes* and *P. alvei* in both humans and animals, there is a need for adequate food processing, particularly at an appropriate temperature, as well as surveillance of and good hygiene practices by meat handlers. This highlights the significance of ongoing monitoring and the requirement to implement policies in the primary sector to reduce consumer risk.

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