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# Detection of Antibacterial Susceptible *Salmonella* spp. in Cured Beef with Different Shelf-Life Expectancy

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**Abstract**

Meat is currently one of the food products with the highest demand ever since 1980, where demand has reached 24.8 kg per year per person as of 2020. With high demand comes intensive farming which causes overuse of antimicrobials for both therapeutic and non-therapeutic reasons, allowing more antimicrobial resistant (AMR) strains of bacteria to occur. In the case of meat products, salmonella is considered one of the more commonly occurring bacteria found in raw meat products. However, with meat's short shelf-life expectancy, the likeliness of consumers suffering from salmonellosis increases. Thus, preservation methods have been implemented to reduce this likeliness, primarily through curing beef. Although curing may reduce the likeliness of excessive microbial growth, AMR salmonella has been detected in cured beef samples. The scope of this research determines whether there is *Salmonella* spp. within the cured beef samples, conduct AMR (azithromycin, ciprofloxacin, and ceftriaxone) analysis of the *Salmonella* spp. isolated from the cured beef samples and enumeration was conducted. The longer the shelf-life expectancy of cured beef samples, the lower the overall CFU/mL per sample was (control: 17,800,000 CFU/mL, >1 year: 0 CFU/mL). It was also discovered that *Salmonella* spp. has potential resistance towards ciprofloxacin (33.33% intermediate) and susceptibility towards azithromycin and ceftriaxone (100.00% sensitive). This research implies the agricultural industry and safety for consumers of cured beef products with different shelf-life expectancies.

**Keywords:** antimicrobial resistance; cured beef; *Salmonella* spp.; shelf-life expectancy.

**Abbreviations:** AMR: antimicrobial resistant; SS: *Salmonella-Shigella*; DNA: deoxyrubonucleic acid; PMQR: plasmid-mediated quinolone resistance; ESBL: extended spectrum β-lactamase; NA: nutrient agar; BPW: buffer peptone water; TPC: total plate count.

## INTRODUCTION

Since 1980 until 2010, the global average demand for livestock-derived food per person has increased by a total of 8 grams, from 22 grams of protein per day to 30 grams of protein per day. This includes the increased demand for meat, with an estimated 25 grams per day per person (24.8 kg per year per person) as of 2020. Annual percent change in per person demand is more prevalent within countries with rising incomes and falling prices on demand, which is most prevalent in South Asia and sub-Saharan Africa. Overall, demands for livestock-derived food demands are estimated to increase by 14% per person and 38% in total from 2020 and 2050 (Komarek *et al*., 2021).

One of the main issues with raw meat as a food product is its short shelf-life due to its natural microflora. However, many preservation methods have been implemented to increase their shelf-life, including curing. Cured meat, where there is salt, sugar, and nitrites/nitrates added to the meat, has proven to be an effective method in reducing spoilage of meat products and reducing the total aerobic and anaerobic plate count within samples, allowing meat products to be stored for longer periods without experiencing signs of spoilage. A study conducted by Bower *et al*. (2018) concluded that a slight decrease in water activity within cured meat samples has been proven to extend the shelf life of said product (Bower *et al*., 2018).

Curing meat is an alternative to allowing easier storage and distribution of meat. However, with the increasing demand for meat products, intensive farming is a necessary solution for supply to meet demands. Intensive farming may lead to overcrowding of farm animals, pushing the usage of antimicrobials in animal feeds to reduce the risks of diseases spreading amongst livestock (Manyi-Loh *et al*., 2018). Based on research conducted by Landers *et* al. (2012), antimicrobial resistance (AMR) has been an emerging public health crisis where most AMR cases occur in agricultural settings (Lander *et al*., 2012). Many of these antimicrobials are used irrationally within the

agricultural setting, proving more dangerous than helpful (Manyi-Loh *et al*., 2017).

Due to constant exposure to new lines of antimicrobials, the bacterial population will adapt faster and mutate, making antimicrobials ineffective towards them (Lander *et al*., 2012). The first recorded case of antibiotic resistance in bacterial strains was discovered in 1975. By 1984, different strains of *Escherichia*, *Salmonella*, and *Klebsiella* displayed resistance to several commonly used antimicrobials, such as aminoglycosides (Galhano *et al*., 2021). There is a high probability of the AMR gene being transferred from livestock to humans, due to the commercial distribution of meat products where AMR bacteria are present (Lander *et al*., 2012).

Although curing meat may reduce the natural growth of microflora, the presence of microbes in the food product is inevitable. Therefore, the possibility of antibiotic resistant bacteria being present in the cured meat is still prevalent (Bower *et al*., 2018). One of the commonly occurring bacteria in meat products is *Salmonella* which causes salmonellosis, a foodborne infection that is responsible for an estimated 80.3 million cases per year. Contamination by *Salmonella*, specifically *Salmonella enterica*, can originate from the raw meat product itself or during the production stage (Mutz *et al*., 2019). With the possibility of commercial bacteria being present in cured meat products and acting as a reservoir for AMR genes, there is potential for AMR *Salmonella* spp. to be transferred to humans (Lander *et al*., 2012).

## MATERIALS AND METHODS

### Study area

Samples were taken from cured beef samples of the origins, which had different shelf-life expectancies, where they varied from control, <1 year, 1 year, and >1 year.

### Procedures

*Preparation of Cured Beef Samples (replace with your sub-sub-title of procedures)*

A total of 5 grams of control/cured beef sample was weighted on a scale. The meat was pounded using the mortar and pestle until smooth, with the help of sterilized distilled water, and then suspended into 45 mL of BPW, a non-selective enrichment broth, making the original stock culture ($10^0$). The sample in the BPW was incubated at 37ºC for 18±1 hours (Temelli et al., 2012). Serial dilution is made from level $10^{-2}$ until $10^{-6}$. To make the serial dilution, 1 mL is added to 9 mL of sterilized saline, making the first level of dilution ($10^{-1}$). This is repeated for all dilution levels where 1 mL of the previous dilution level is added into 9 mL of sterilized saline (Cappuccino & Welsh, 2017).

*Identification of Salmonella spp. In Cured Beef Sample*

SS agar plate and the sample enriched in BPW are prepared. Streak plate is conducted, the sample is inoculated on the SS agar plate using a disposable swab with a quadrant streak method. This is done until there are three repetitions. The petri dish is sealed, turned upside down and incubated for 24±1 hours at 37ºC. After incubation, the sample is observed for transparent colonies with black $H_2S$ precipitate in the center, indicating the presence of Salmonella spp. Gram staining was then conducted for the isolated Salmonella spp. colonies and observed underneath a microscope at 10×100 (Nabbut, 1973).

*Enumeration of Cured Beef Sample*

SS agar plates are prepared for enumeration. Spread plate is conducted where 0.1 mL of $10^{-2}$ suspension is taken using a micropipette and inoculated on the SS agar plate. The suspension is spread evenly on the surface of the agar using a Drigalski spatula and then the petri dish is flipped. This methodology is repeated for dilution levels $10^{-3}$ until $10^{-6}$ for all samples. The Sample is incubated for 24±1 hours at 37ºC. After incubation, total plate count (TPC) is conducted twice, once for all colonies on the SS agar and a second time for the Salmonella spp. colonies, indicated with transparent colonies and black $H_2S$ precipitate in the center (Nabbut, 1973; Cappuccino and Welsh, 2017).

*Antimicrobial Susceptibility Test*

*Salmonella* spp. cultures are isolated from the initial identification of it from the cured beef samples. The isolated colonies are cultured on slant agar using NA and incubated for 24±1 hours at 37ºC. Then, 9 mL of sterilized saline was added into the culture and swabbed onto NA plates using the dense streaking method. Dense streaking was done three times where the petri dish was rotated 60º before doing the next pass of dense streaking. Once dense streaking was done, the perimeter of the petri dish was swabbed to pick up any excess amounts of liquid (Hudzicki, 2009). The antimicrobial disk was infused with their respective antimicrobial, as seen below.

### Data analysis

Once the data had been collected, confirmation was done to determine whether *Salmonella* spp. was isolated from the samples. Alongside the confirmation, a Total Plate Count was conducted to determine the correlation between shelf-life expectancy and overall CFU/mL detected. Once that was done, an antibacterial susceptibility test was done for the isolated *Salmonella* spp. strains.

## RESULTS AND DISCUSSION

### Confirmation of *Salmonella* spp.

Confirmation of the presence of *Salmonella* spp. is significant within this research to allow pure culturing of the targeted bacteria. To do so, a confirmation test was conducted with the beef samples that were non-selectively enriched using Buffer Peptone Water (BPW) to increase the accuracy of the media (Ruiz *et al*., 1996). Based on the methodology and independent variables utilized, confirmation of *Salmonella* spp. was done for four samples with different shelf-life expectancies, which are; control, <1 year, 1 year, and >1 year. These beef samples were most likely sourced from *Bos taurus indicus* Linnaeus, 1758, or also known as beef cattle (GBIF, 2024). Quadrant streaking was done to isolate single colonies of *Salmonella* spp. and, with the help of the biochemical and selective properties of SS agar, single colonies with black $H_2S$ precipitate were easily identifiable and isolated. Following the isolation of single colonies, it was further cultured on NA slants to produce pure culture isolates of *Salmonella* spp. and was moved onto glass slides for microscopical analysis. In total, two outputs can confirm the presence of *Salmonella* spp., through the bacteria's cell morphology and whether it is gram positive or negative.

**Table 1.** Confirmation and basic characterization of *Salmonella* spp. and unconfirmed colonies isolate from cured beef samples.

| | SS Agar | Cell Morphology | Gram positive/ negative |
|---|---|---|---|
| **Control** | + | bacillus | − |
| **< 1 year** | + | bacillus | − |
| **1 year*** | − | bacillus | − |
| **> 1 year**** | − | invalid | invalid |

***no presence of *Salmonella* spp. but other microbes were detected
****no presence of bacterial growth

Based on the results that have been obtained from the confirmation test (Table 1), *Salmonella* spp. was detectable in both the control and in the <1 year sample. On the other hand, *Salmonella* spp. was not detectable in 1 year and >1 year samples but other forms of microbes were detectable. Due to the selective nature of the SS agar, only a select few types of bacteria can be grown on the agar, which are *Salmonella* spp., *Shigella* spp., *Escherichia coli*, *Enterobacter* spp., and *Klebsiella* spp. transparent colonies with black $H_2S$ precipitate indicated the positive presence of *Salmonella* spp. (Yanestria *et al*., 2019). the Formation of transparent colonies on SS agar is due to the inability of *Salmonella* spp to ferment lactose within the medium. In addition to that, with the help of sodium thiosulfate and ferric citrate, the removal of sulfur from hydrogen sulfide, which is produced by the bacteria reacts with the thiosulfate from the sodium thiosulfate and iron ions from the ferric citrate to create the black precipitate, differentiating its colonies from the rest of the gram-negative bacteria that can grow on SS agar (Tortora *et al*., 2010; Remel, 2010).

Further confirmation was done through gram staining, where the clear colonies with black precipitate were isolated and stained. For samples with no clear colonies with black precipitate, isolation of available colonies was done. Microscopic observation was done with a magnification of 10×100. Based on the results obtained (Table 1), all the bacteria isolated were gram-negative bacteria, including *Salmonella* spp., as seen in Figure 2 (Ray & Bhunia, 2013). This is due to the selective properties of SS agar, where sodium citrate and brilliant green inhibited the growth of gram-positive bacteria (Remel, 2010). Therefore, not only does it confirm the transparent colonies with black precipitate are indeed *Salmonella* spp. colonies, but it also limits what strains of bacteria the unknown colonies are, which are limited to only gram-negative bacteria. Gram staining also provided insight to the cell morphology, where all microscopic observation confirms that both confirmed *Salmonella* spp. colonies and unconfirmed colonies were bacillus or rod shaped (Figure 2). This aligns with the fact that the enteric bacteria that can grow on SS agar are all rod-shaped bacteria. Thus, it can be conclusive that SS agar is effective in being a selective media and its confirmation of *Salmonella* spp. is reliable (Madigan *et al*., 2015)

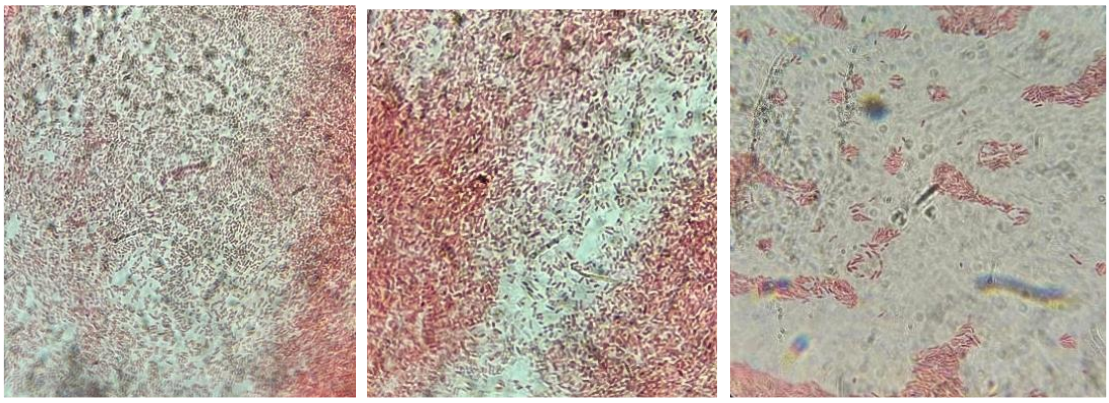

**Figure 2.** Microscopic observation at 10×100 for *Salmonella* spp. from control (a) and <1 year (b), and unknown isolate for sample 1 year (c) isolated from cured beef samples.

**Total Plate Count of Cured Beef Sample**

Following the confirmation of *Salmonella* spp. presence in cured beef samples with different shelf-life expectancy, Total Plate Count (TPC) was conducted to determine the overall presence of bacteria within the beef samples alongside the percentage of *Salmonella* spp. compared to the overall enumeration of bacteria, as seen in Table 2. Same as previously, there were four samples used for the enumeration of *Salmonella* spp. with the consideration that the samples can only be enumerated for bacteria that can grow on SS agar, which were previously mentioned.

**Table 2.** Enumeration of the overall presence of bacteria in comparison to the percentage of *Salmonella* spp. in the cured beef samples.

| | **Overall CFU/mL** | ***Salmonella* spp. CFU/mL** | **% of *Salmonella* spp.** |
|---|---|---|---|
| **Control** | 17,800,000 | 2,165,000 | 12.16% |
| **< 1 year*** | 6,000,000 | 0 | 0.00% |
| **1 year**** | 14,100 | 0 | 0.00% |
| **> 1 year***** | 0 | 0 | 0.00% |

*presence of *Salmonella* spp. but not detectable in TPC
****no presence of *Salmonella* spp. but other microbes were detected
*****no presence of bacterial growth

Based on the results obtained for the enumeration (Table 2), we can observe that samples with shorter shelf-lives tend to have a higher overall CFU/mL due to the lack of curing, and samples with longer curing time have lower overall CFU/mL. This can be seen when comparing the control with the >1 year sample, where the control has an overall CFU/mL of 17,800,000 CFU/mL while the >1 year sample has an overall CFU/mL of 0 CFU/mL. This trend is also observable with the percentage of *Salmonella* spp. within the sample where *Salmonella* spp. makes up 12.16% (2,165,000 CFU/mL) of the overall CFU/mL of the control. In addition, *Salmonella* spp. was detected in the confirmation test for the <1 year sample but was very sparse when TPC was conducted for this sample and could not be calculated, leading to *Salmonella* spp. making up 0.00% of the overall CFU/mL (Attachment 1). For the one year sample, TPC growth was very minimal but based on the morphological characteristics of the colonies, colonies with pink centers, it is likely that the unconfirmed colonies are *E. coli* (Remel, 2010). considering that the TPC was done on SS agar, this research's scope is limited to the growth of enteric gram-negative bacteria. Thus, the TPC does not consider that other microbes outside of the enteric gram-negative group may have been present in the samples.

Curing can be seen as an effective method in reducing the growth of unwanted microflora, in this case bacteria. As mentioned previously, curing is a process that utilizes salt in a dry mixture that envelops the meat. This can be cured over different time ranges to create cured meat products. Adding salt, it preserves the flavor, color and quality of the product while also reducing the water activity level of the meat. Thus, with reduced water activity levels, bacteria will not have access to unbounded water necessary for their biochemical and metabolic pathways. Therefore, the present bacteria within the meats are eliminated. Furthermore, the bacteria cells will experience osmotic shock and undergo lysis due to the movement of water within their cells to the outside environment, which has a low water concentration, killing off the present bacteria cells from lysis caused by osmotic shock (Nummer & Andress, 2015; Henny *et al*., 2010). The sodium content within the cured beef is also a factor that can contribute to the effectiveness of the curing process. As seen in Table 3, the longer the shelf-life expectancy of a sample is, the higher the sodium content per 100 grams will be.

**Table 3.** Sodium content of cured beef samples (mg/100g) as per their nutrition facts.

| **Cured Beef Sample** | **Sodium Content (mg/100g)** |
|---|---|
| Control (Uncured Beef) | 0 |
| <1 year (Pepperoni) | 500 |
| 1 year (Corned Beef) | 1,134 |
| >1 year (Jerky) | 1,642 |

When comparing the overall CFU/mL and the sodium content of the samples, the results are parallel to one another, where the higher the salt content, the lower the overall CFU/mL will become. This is seen with the control and 1 year sample. In the control, with a sodium content of 0 mg/100g, the overall CFU/mL is 17,800,000 CFU/mL. On the other hand, the 1 year sample, with a sodium content of 1,134 mg/100g, the overall CFU/mL is 14,100 CFU/mL. The effectiveness of a higher sodium content can clearly be seen with the >1 year sample as the overall CFU/mL is at 0 CFU/mL, proving that the curing method is an effective way to eliminate unwanted growth of bacteria. In addition to that, with *Salmonella* spp., dry curing is highly effective at reducing the percentage of *Salmonella* spp. from the overall CFU/mL. An occurrence such as this is because of *Salmonella* spp. present in the samples had not adapted to the hypertonic environment of cured meats, proving them to be sensitive to osmotic shock. Thus, the extreme decline in the *Salmonella* spp. a percentage from the overall CFU/mL (Li, *et al*., 2023). However, the unconfirmed colonies in the one year sample may have been present in the TPC, although the sodium content of the sample is high, because hyperosmotic environments caused by the high sodium content may cause bacteria, or in this case, *E. coli*, to experience accelerated constriction rates. This will lead to an increased overall CFU/mL due to faster division rates within the small number of bacteria present in the sample (Sun *et al*., 2021).

**Antimicrobial Susceptibility Test**

Once confirmation and enumeration of the bacteria in cured beef samples were done, the pure cultures isolated from the confirmation tests were used for the antimicrobial susceptibility test. Based on the results that have been obtained from the confirmation test, the samples that underwent antimicrobial susceptibility testing were only the control and the <1 year sample, as they were the only ones that had a confirmed presence of *Salmonella* spp. To do so, the test was done on NA plates, where the Kirby Bauer Disk Diffusion Method was implemented. This methodology requires the creation of a suspension of the targeted isolate, in this case, *Salmonella* spp., where the suspension were made with sterilized saline solution and vortexed. Following this, three passes of dense swabbing of this suspension was done using disposable swabs on NA plates to ensure even swabbing of the suspension. Theoretically, Mueller-Hinton Agar is required for this method, but from trial and error, NA plates proved to be more effective in reading apparent zone formations from the antimicrobial susceptibility testing. Once swabbed evenly, the loaded and sterilized antimicrobial disks are placed evenly on the surface and incubated. Apparent zone formations were measured using a caliper and interpreted using the CLSI guidelines (Hudzicki, 2009).

**Table 4.** Antimicrobial susceptibility testing with azithromycin (15 µg), ciprofloxacin (5 µg), and ceftriaxone (30 µg) against *Salmonella* spp. Isolated from cured beef samples of control and <1 year.

| Antimicrobial tested | Dose (µg) | Categories | | |
|---|---|---|---|---|
| | | Sensitive | Intermediate | Resistant |
| Azithromycin | 15 | 100.00% | 0.00% | 0.00% |
| Ceftriaxone | 30 | 100.00% | 0.00% | 0.00% |
| Ciprofloxacin | 5 | 66.67% | 33.33% | 0.00% |

The results obtained above were limited to only the control and the <1 year sample due to the presence of *Salmonella* spp. within these two samples. The one year and >1 year samples did not undergo the antimicrobial resistance test due to the lack of *Salmonella* spp. detected within the samples. From the tested samples, *Salmonella* spp. is 100.00% sensitive to azithromycin and ceftriaxone while only being 66.67% sensitive to ciprofloxacin. It was discovered that *Salmonella* spp. is intermediate to ciprofloxacin with 33.33% of the tests reacting that way. However, the results state that *Salmonella* spp. is not resistant to all three antimicrobials tested in this research, which are: azithromycin, ceftriaxone, and ciprofloxacin. These results a contradict the initial hypothesis created for this research, where resistance was expected towards azithromycin and sensitivity was expected for ciprofloxacin. Results obtained completely went against the hypothesis and instead the most resistance is towards ciprofloxacin (33.33% intermediate) and most sensitive towards both azithromycin and ceftriaxone (100.00% sensitive). Although the results did not follow the hypothesis, the research problem and objectives were achieved. Where it is determined that *Salmonella* spp. shows a degree of resistance to ciprofloxacin and sensitivity to azithromycin and ceftriaxone. Furthermore, antimicrobial susceptibility was conducted effectively towards the *Salmonella* spp. strains that were able to be isolated from the sample.

These results may be due to a few possible scenarios. Firstly, ciprofloxacin's 66.67% sensitivity is lower than the other two may be caused by the occurrence of mutations within the gene that encodes for gyrase (*gryA* and *gryB*) and the occurrence of the PMQR gene. Mutation in this gene will prevent the segregation of the daughter chromosomes ahead of the replication fork. Malfunction of the gyrase enzyme will prevent topoisomerase IV from segregating the chromosomes at the replication fork and lead to rapid cell death due to failure of cell division (Nair *et al*., 2018; Shariati *et al*., 2022; Blondeau, 2004). However, *Salmonella* spp. did not show complete resistance to ciprofloxacin which may be due to the ineffective transmission of the AMR gene within the beef cattle's intestinal tract. One of the ways to transmit genes between bacteria cells is through horizontal transmission, specifically conjugation, entailing cell-to-cell contact through the sex pilus, a structure detectable within *Salmonella* spp. (Sanseverino *et al*., 2018).

Secondly, azithromycin and ceftriaxone's 100.00% sensitivity can be due to a few reasons. For azithromycin, sensitivity of *Salmonella* spp. can be due to the absence of mutation in the *Erm42* and *ErmB* and no methylation of the 23s rRNA thus, total effectivity of the 50S ribosomal subunits. Furthermore, there may have been no modification of the AcrAB-TolC efflux pump, allowing full retention time of the antimicrobial within the *Salmonella* spp. cells (Chiou *et al*., 2023). On the other hand, sensitivity of *Salmonella* spp. to ceftriaxone may be due to the presence of the ESBLs enzyme. This enzyme functions explicitly to hydrolyzing the β-lactam ring within the antibiotics, rendering them inactive and inefficient in fighting off bacterial infections (Nair *et al*., 2018). It is possible that certain *Salmonella* spp. cells within the beef cattle did have these genes but there was ineffective conjugation.

Failure of the conjugation process can be due to multiple reasons. One of these is due to the environment in which conjugation occurs, which is in the rumen of beef cattle. Previous research has indicated the diets of beef cattle affect gut resistomes in livestock, where high grain diets, although economically efficient, have proven to increase the chances of AMR gene transfer within the rumen. It is possible that the beef cattle, where the cured beef is sourced from, has a mixture of a regular diet and a high grain diet, which makes it more likely for conjugation to occur between present microbes within its rumen. Thus, the beef cattle where the samples for this

research were sourced from may have been on regular diets while the samples from the literature review may have been sourced from beef cattle with high grain diets (Zhang *et al*., 2023).

Specific implications can be made based on the results obtained. The common usage of antimicrobials within the agricultural industry, to a certain extent does have minor effects on the detection of AMR bacterial strains. Specifically for the potential resistance of *Salmonella* spp. towards ciprofloxacin. This may prove to be problematic as there is the presence of *Salmonella* spp. detected within the control and <1 year sample. If there is potential for resistance towards ciprofloxacin (33.33% intermediate), this poses a threat if consumers were to be introduced to the *Salmonella* spp. present in within the samples, potentially exposing consumers to the chances of contracting salmonellosis, or even worse, typhoid fever. However, it can be noted that the samples tested were not cooked beforehand which may have increased the likelihood of detecting *Salmonella* spp. within the samples. For consumer safety, it is right to suggest thoroughly cooking the cured beef samples at temperatures above 50$^{o}$C will help lower the chances of exposure to *Salmonella* spp. (Ray and Bhunia, 2013).

## CONCLUSIONS

In conclusion, *Salmonella* spp. was detectable in the control and <1 year cured beef samples but was undetectable in the 1 year and >1 year cured beef samples. This indicates that cured beef samples with longer shelf-life expectancy have lower chances of *Salmonella* spp. detection. Additionally, it was discovered that *Salmonella* spp. show potential resistance towards ciprofloxacin (66.67% intermediate) and susceptibility towards azithromycin and ceftriaxone (100.00% sensitive). These discoveries have specific implications for the agricultural industry and the safety of consumers of cured beef samples.

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## REFERENCES

Blondeau, J. M. 2004. Fluoroquinolones: mechanism of action, classification, and development of resistance. *Survey of Ophthalmology*. 49(2): S73-S78. DOI: https://doi.org/10.1016/j.survophthal.2004.01.005.

Bower, C. G., Stanley, R. E., Fernando, S. C. & Sullivan, G. A. 2018. The effect of salt reduction on the microbial community structure and quality characteristics of sliced roast beef and turkey breast. *LWT – Food Science and Technology*. 90(2018): 583-591. DOI: https://doi.org/10.1016/j.lwt.2017.12.067.

Cappuccino, J. and Welsh, C. T. 2017. *Microbiology: A Laboratory Manual, Global Edition*. 11th Ed. Harlow, Essex: Pearson.

Chiou, C., Hong, Y., Wang, Y., Chen, B., Teng, R., Song, H. & Liao, Y. 2023. Antimicrobial Resistance and Mechanisms of Azithromycin Resistance in Nontyphoidal *Salmonella* Isolates in Taiwan, 2017 to 2018. *Microbiology Spectrum*. 11(1). DOI: 10.1128/spectrum.03364-22.

Clinical and Laboratory Standards Institute. 2020. *Performance Standards for Antimicrobial Susceptibility Testing*. CLSI M100-30. Pennsylvania: Clinical and Laboratory Standards Institute.

Galhano, B. S. P., Ferrarim R. G., Panzenhagen, P., Jesus, A. C., D. & Conte-Junior, C. A. 2021. Antimicrobial Resistance Gene Detection Methods for Bacteria in Animal-Based Foods: A Brief Review of Highlights and Advantages. *Microorganisms*. 2021(9):923. DOI: https://doi.org/10.3390/microorganisms9050923.

Global Biodiversity Information Facility (GBIF), (2024). www.gbif.org, CC0 https://www.gbif.org/species/5384334.

Henny, J. E., Taylor, C. L. & Boon, C. S. 2010. *Strategies to Reduce Sodium Intake in the United States*. 1st Ed. Washington, DC: National Academies Press.

Komarek, A. M., Dunston, S., Enahoro, D., Godfray, H. C. J., Herrero, M., Mason-D'Croz, D., Rich, K. M., Scarborough, P., Springmann, M., Sulser, T. B., Wiebe, K. & Willenbockel, D. 2021. Income, consumer preferences, and the future of livestock-derived food demand. *Global Environmental Change*. 70(2021): 102343. DOI: https://doi.org/10.1016/j.gloenvcha.2021.102343.

Landers, T. F., Cohen, B., Wittum, T. E. & Larson, E. L. 2012. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. *Public Health Reports*. 127(1): 4-22. DOI: 10.1177/003335491212700103.

Madigan, M. T., Martinko, J. M., Bender, D. H., Buckley, D. H. & Stahl, D. A. 2015. *Brock Biology of Microorganisms*. 14th Ed. Harlow, Essex: Pearson.

Manyi-Loh, C., Mamphweli, S., Meyer, E. & Okoh, A. 2018. Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules*. 23(4): 795. DOI: 10.3390/molecules23040795.

Mutz, Y. S., Rosario, D. K. A., Paschoalin, V. M. F. & Adam, C. 2019. Salmonella enterica: A hidden risk for dry-cured meat consumption? *Critical Review in Food Science and Nutrition*. 60(9): 1-15. DOI: 10.1080/10408398.2018.1555132.

Nabbut, N. H. 1973. Elevated temperature technique for the isolation of salmonellas from sewage and human faeces. *The*

*Journal of Hygiene*. 71(1): 49.54. DOI: 10.1017/s0022172400046209.

Nair, D. V. T., Venkitanarayanan, K. & Johny, A. K. 2018. Antibiotic-Resistant Salmonella in the Food Supply and the Potential Role of Antibiotic Alternatives for Control. *Foods*. 7(10): 167. DOI: 10.3390/foods7100167.

Nummer, B. A. & Andress, E. L. *Curing and Smoking Meats for Home Food Preservation: Literature Review and Critical Preservation Points*. Available at: https://nchfp.uga.edu/publications/nchfp/lit_rev/cure_smoke_cure.html#:~:text=Curing%20is%20the%20addition%20to,of%20salt%20with%20nitrates%2Fnitrites. [Accessed: 1 April 2023].

Ray, B & Bhunia, A. 2013. *Fundamental Food Microbiology*. 5th Ed. Florida: Taylor & Francis Group.

Remel. 2010. *Salmonella Shigella (SS) Agar*. Retrieved from https://assets.fishersci.com/TFS-Assets/LSG/manuals/IFU1840.pdf [Accessed: 13 July 2024].

Ruiz, J., Núñez, M., Díaz, J., Lorente, I., Pérez, J. & Gómez, J. 1996. Comparison of Five Plating Media for Isolation of *Salmonella* Species from Human Stools. *Journal of clinical Microbiology*. 34(3): 686-688. DOI: 10.1128/jcm.34.3.686-688.1996.

Sanseverino, I., Navarro, A., Loos, R., Marinov, D. & Lettieri, T. 2018. *State of the Art on the Contribution of Water to Antimicrobial Resistance*. Luxembourg: Publications Office of the European Union.

Shariati, A., Arshadi, M., Khosrojerdi, M. A., Abedinzadeh, M., Ganjalishahi, M., Maleki, A., Heidary, M. & Khoshnood, S. 2022. The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic. *Frontiers in Public Health*. 10. DOI: 10.3389/fpubh.2022.1025633.

Sun, J., Shi, H. & Huang, K. C. 2021. Hyperosmotic Shock Transiently Accelerates Constriction Rate in *Escherichia coli*. *Frontiers in Microbiology*. 12: 718600. DOI: 10.3389/fmicb.2021.718600.

Temelli, S., Eyigot, A. & Carli, T. 2012. *Salmonella* detection in poultry meat and meat products by the Vitek immunodiagnostic assay system easy Salmonella method, a LightCycler polymerase chain reaction system, and the International Organization for Standardization method 6579. *Poultry Science*. 91(3): 724-731. DOI: 10.3382/ps.2011-01863.

Tortora, G. J., Funke, B. R. & Case, C. L. 2012. *Microbiology: An Introduction*. 11th Ed. New York: Pearson.

Yanestria, S. M., Rahmaniar, R. P., Wibisono, F. J. & Effendi M. H. 2019. Detection of invA gene of Salmonella from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. *Veterinary World*. 12(1): 170-175. DOI:10.14202/vetworld.2019.170-175.

Zhang, T., Mu, Y., Gao, Y., Tang, Y., Mao, S. & Liu, J. 2023. Fecal microbial gene transfer contributes to the high-grain diet-induced augmentation of aminoglycoside resistance in dairy cattle. *Environmental Microbiology*. 9(1). DOI: 10.1128/msystems.00810-23.

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