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Original Research Article

Antibiotics Sensitivity of Bacterial Strains Isolated From Cold Meat in Saudi Markets

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Abstract: The objective research works on cold and ready-to-eat meat products from local markets and how they are affected by external factors and the transmission of microorganisms to them and their reproduction despite their preservation, despite the preservation of these meats in consumers' refrigerators, and how some of these microbes are pathogenic or cause diseases by excreting toxins. The result of our research centers on displaying the total number of bacteria in all samples, identifying the strain contained in these ready-to-eat meats, and comparing them with the permissible limits imposed by most food and drug authorities in the world to preserve the safety of consumers. Also, we test the microbes' sensitivity to antibiotics. The types of bacteria were identified through approved protocols. The total count of bacteria was not in the range of the FDA limits rate, and we found some strains that should not be in the product as *E. coli* and *Salmonella. spp*. These strains can cause severe gastrointestinal tract diseases. The results of the Antibiotic test were positive. Only two of the 30 species we identified were resistant to all the antibiotics we used. They were *S. aureus.* The microbiological quality of cold meats in Jeddah, Saudi Arabia, is barely within the limits of the FDA. In terms of coliforms, *Staphylococcus, and streptococci.* So, all the food handlers should receive training to increase the level of hygiene in the supermarkets in Jeddah, Saudi Arabia.

Keywords: Antibiotics, bacterial strain, colds meat, Gram stain.

1. INTRODUCTION

There are quite a few meat diseases that you should be aware of. These diseases are caused mainly by bacteria, which harm human health. From this research, will be learning more about meat diseases. Therefore, the meat that buys is well-cooked and refrigerated. The information in this article will help avoid getting sick. Meat disease is common and can be pretty severe. It is one of the reasons why taking precautions with your food is so important. The information below will help you protect yourself from meat illnesses. Meat diseases aren't the most common food-related illnesses, but they're still pretty standard (Scharff, 2020). Meat diseases are caused by bacteria and viruses that can be passed on to humans by the contact with uncooked meat (Richardson *et al.,* 2017). The meat-borne illnesses are the leading cause of foodborne illnesses. Bacteria like Salmonella, E. coli, and norovirus can be controlled by disposing of contaminated meats (Jaroenporn *et al.,* 2022). Prevent these diseases and get rid of them if they occur about the risks of eating that meat.

Meat can be unhealthy to consume. There are ways to reduce risk and protect humans from illness when they eat meat. Diseases in cold meat are some of the biggest problems confronting retailers and consumers alike. The fact is that despite being confined to our refrigerators, most of these bacterial diseases thrive at a constant temperature of 5 degrees, which makes them hard to detect and easy for consumers to ignore. Streptococcus, E. coli, Salmonella, and Listeria are pathogens that can cause serious foodborne illnesses. Other common food poisoning microbes include Campylobacter and Staphylococcus (Pal 2022). Antibiotic resistance is a mismatch between the sensitivities observed in clinical practice and the strains of bacteria to which they have been shown to be effective in treating. The increasing loss of antibiotic efficacy, particularly in humans, demands that new approaches have be developed. Cases of gastrointestinal infections

have been reported to be at an average of 40 million globally annually. There has been a responsive change in the food processing industries to overcome the problem of food poisoning. Many companies have upgraded to deter foodborne pathogen infection from rising. However, the bacteria *Escherichia coli* and *Salmonella spp*. have adjusted to the conditions through evolution, threatening food safety. They have adapted to the chemicals used and become resistant, resulting in diarrhoea and stomach upset when these foods are eventually taken (Nazari 2017).

However, classifying the bacteria through this method is difficult and hectic since they are further grouped into biotypes and serotypes. Therefore, this method is appropriate to be used to sufficient to give clear information on differences arising from the categories of bacteria. During the study, our aim was to determine the effects antibiotics have on the bacteria strains in the meat samples obtained from the Jeddah markets, Saudi Arabia. This involved screening the meat samples from various sections of the market. Twenty-five samples were taken to the laboratory after previous interviews during the study. Cases of the dominant bacteria *Escherichia coli* and *Salmonella spp.* were observed and recorded. A series of reactions were carried in the laboratory to categorise the bacteria from the fresh meats. Meat samples were placed on separate slides, and stains were added to make the specimen distinct. Counter-stains were also added, and the experiment was left for some time allowing time for the reactions. The slides were then put under a light microscope for the observations. It was found that stains of unknown microorganisms were present in the samples. The contaminated meat samples were subjected to strains of antibiotics, and observations were later made. Antibiotic dyes were added at different concentrations to observe the variations in the reactions of the bacteria. Different dyes of the antibiotics were used in the reaction to compare the reactions of the two pathogens' sensitivity.

Antibacterial resistance is the ability of bacteria to either grow fast or continue to survive in the presence of known antibiotics effectively degrading the common antibiotics and the power of new antibiotics to prevent bacterial growth (Wang-Lin 2019). The commonly used antibiotics, such as tetracycline, penicillin, and erythromycin, have seen their effectiveness decrease due to the evolution of bacteria that are resistant to these antibiotics.

2. MATERIALS AND METHODS

2.1. Sample Preparations

The basic methodology of sampling was used to collect specimens. Bacteria were subjected to media culture preparations to test bacterial growth. Nutrient agar and Mac Conkey agar can differentiate negative and positive bacterial growth. Two supermarkets in Jeddah, Saudi Arabia, were made from six cold cuts. After searching about the most popular brand, C1 and C2 were chosen. Stomachers are also assessed for weight accuracy because they are heavy and difficult to sample on a scale. This helps us establish the sample quantity needed. One of our graduate students will measure 200 cc into a graduated cylinder, and then spin the stomacher to make a sample solution. Then we turn the petri dish and let it sit at 37 degrees Celsius for 24 hours. Isolations and examined into two period of time from the zero day and after seven days from collected samples. The experiment was repeated it three time.

2.2. Identification of Bacteria

After isolating samples from colds meat and growing them, many different bacterial strains appeared that we needed to stain with grams. Harmful bacteria are stained blue, while positive ones are stained pink (positive bacilli) or red (positive spore), (Giri 2021). To identify the bacteria species, isolation is necessary for the second time, and the use of selective media for positive gram, which is called Mannitol Salt Agar (MSA). To isolate bacterial colonies and add contamination, all isolation steps must be carried out in a safety-proof culture laboratory (Lorenz 2016). On another hand, selective medium Eosin methylene blue (EMB) was used to identify the gram negative bacteria.

2.3. Antibiotic Sensitivity Test

Muller Hinton agar is used to apply an antimicrobial sensitivity test. The antibiotic desk is constructed from cotton swabs and includes six different antibiotics (Shanmugapriya *et al.*, 2021). The antibiotic desk is a plate reader for measuring the inhibition zone of antibiotics against bacteria. The antibiotic desk has six different antibiotics, which are Penicillin G (PG), Erythromycin (E), Ampicillin (AP), Cephalothin (KF), Clindamycin (CD), and Cotrimoxazole (TS).

3. RESULTS

3.1. Total Bacteria Count

The total bacterial count in all fresh cut cold meats were placed in the Table 1, the ones for the seven days are in the Table 2. All the fresh samples for the cold meats in the study were all tested within the acceptable limits as recommended by the FDA. Samples are diluted three times to read clear colonies. Salami and the pastrami were found to be having the same growths as compared by other samples. All the samples have exceeded the acceptable limits. As shown in Table 2, all different colonies of bacteria have been isolated. More than one kind of bacteria is found in each sample, which makes the conclusion that there are several kinds of bacteria that can cause food contamination.

Table 1: The CFU of day one cold meats isolation

Cold meats Day one	Number of different colonies x10^3
Turkey C 1	0
Turkey C 2	0
Roast beef C1	3x10^3
Roast beef C2	2x10^3
Plain beef mortadella C1	2x10^3
Plain beef mortadella C2	0
Plain Chicken mortadella C1	2x10^3
Plain Chicken mortadella C2	1x10^3
Pastrami C1	2x10^3
Pastrami C2	6x10^3
Salami C1	1x10^3
Salami C2	2x10^3

Table 2: The CFU after seven days in refrigerator cold meats isolation

Cold meat after 7 days in refrigerator	Number of different colonies x10^3
Turkey C 1	0
Turkey C 2	0
Roast beef C1	3x10^3
Roast beef C2	3x10^3
Plain beef mortadella C1	3x10^3
Plain beef mortadella C2	2x10^3
Plain Chicken mortadella C1	2x10^3
Plain Chicken mortadella C2	2x10^3
Pastrima C1	1x10^3
Pastrima C2	3x10^3
Slamai C1	5x10^3
Slamai C2	2x10^3

3.2. Isolation and Identification of Bacteria by Mannitol Salt Agar (MSA)

The bacteria will grow and ferment mannitol to turn entirely yellow Figure 3A. This shows a meaning that bacteria are coagulase positive as *Staphylococcus aureus*. In a case where there is a yellow haze in the media, it means the bacteria are coagulase-negative as *Staphylococcus saprophyticus*, or the colour change is absent but good growth Figure 3B; this gives the meaning that the bacteria are coagulase-negative staphylococci epidermis. In Figure 3C, if the results are different from the three situations, we could tell the bacteria are *Streptococci*. Figure 3D.

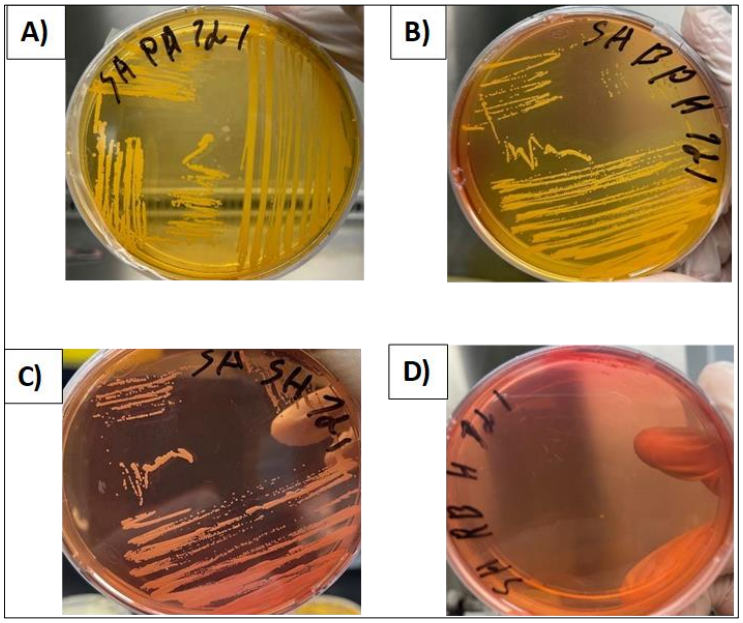


Figure 3: Shown Staphylococcus strain grow on MSA. A) and B) coagulase positive *Staphylococcus,* C) and D) coagulase negative *Staphylococcus*

3.3. Isolation and Identification of Bacteria by Eosin Methylene Blue (EMB) Agar and Macconkey Agar

On the Figure 4 A below show that our negative-gram bacteria are mainly *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes* producing gram-negative *enterobacteria* grown on EMB agar. On the other hand, MacConkey agar to confirm the presence of *Enterobacteriaceae*. As shown in Figure 4B.

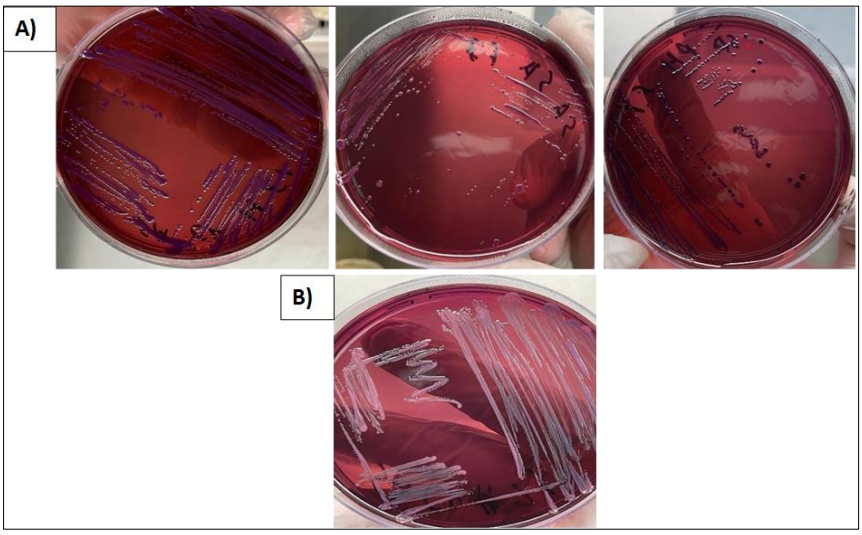
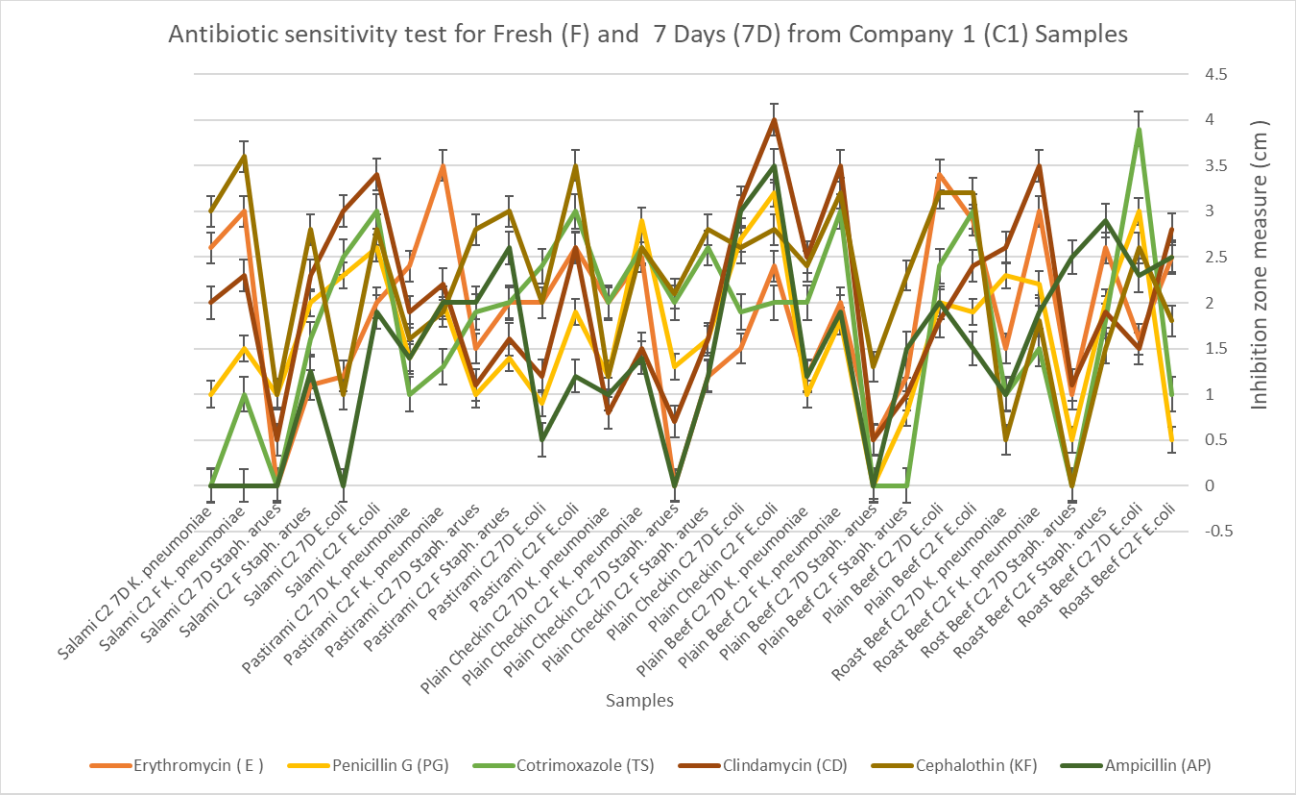


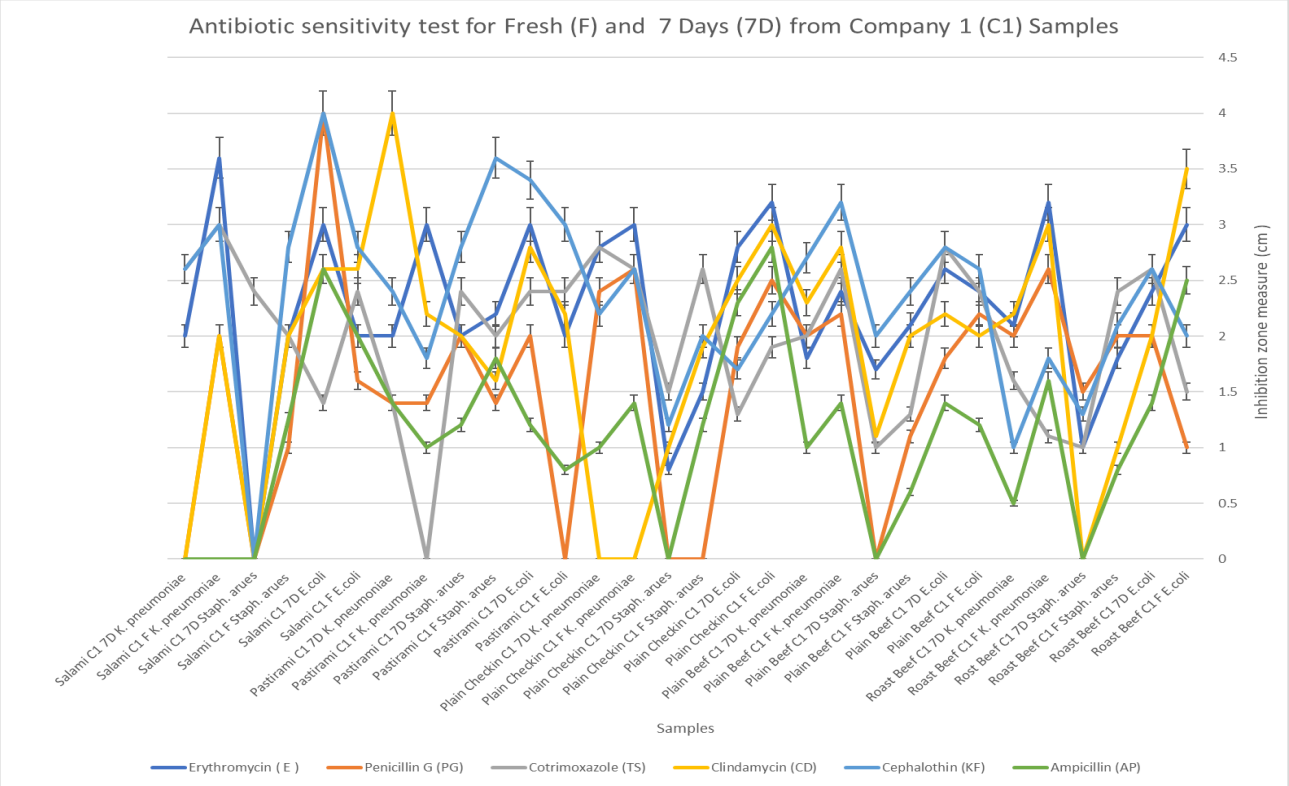
Figure 4: Shown the Gram negative *Enterobacteriaceae* grown on A) EMB agar and B) MacConkey agar

3.4. Antibiotic Sensitivity Test

The total of 30 samples was isolated from cold meats. They found the result is similarity between the two companies on two different of period. Excluding the *Staphylococcus*shows that 16 strains were sensitive except *Staphylococcus aureus*, which was still a few number were resistant in this study (Figure 5 A & B).



(A)



(B)

Figure 5: Shows the inhibition zone (cm) Antibiotics sensitivity test for 30 sample isolation from 4 type of colds meat from two company A) and B)

4. DISCUSSION

Antibiotic resistance is currently one of the most serious threats to global health and food security (Elbehiry *et al.,* 2022). Some bacterial strains will be tested for antibiotic sensitivity. Two samples contained antibiotic resistance genes, making the microbes resistant to six antibiotics. This makes us think most of these things may harm our health (Syafiuddin and Boopathy 2021). According to Hassan *et al.,* (2020), the mean Pseudomonas counts recovered from various chicken meat products (chilled breast, thigh, nuggets, and burger) ranged from $3.51 \times 10^3 \pm 0.76 \times 10^3$ to $8.44 \times 10^3 \pm 1.85 \times 10^3$. Resistance to earlier antimicrobials such as tetracycline, ampicillin, streptomycin, trimethoprim-sulfamethoxazole, and chloramphenicol was most frequent among the isolates collected in the current investigation. These findings are consistent with the findings for RTE foods in our preliminary study (Yang *et al.,* 2020) and are comparable to antimicrobial-resistance rates among Salmonella isolates from raw pork and chicken meat in China (Yang *et al.,* 2020).

This experiment was aimed to assess the antibacterial activity of six different antibiotics concerning their capacity to break down penicillinase. These antibiotics were tested against a strain of Staphylococcus epidermidis known to produce penicillin G (PG) when exposed to various concentrations of these antibiotics The results of these tests were analysed, and the findings indicate that, except for erythromycin, which has a lower activity level than cephalothin, all antibiotics showed a 100 percent inactivation rate at 32 hours. This demonstrates that penicillin G is not active against *S. epidermidis*. Disc diffusion was done by subjecting the medicines to a battery of tests to determine the extent of their antibacterial action against 24 different bacterial infections. In this investigation, the bacterial pathogens isolated from the six separate groups were utilized. The results of the tests indicated that 11 of the 24 strains were positive, whereas 13 of the 24 strains were negative(Krawczyk *et al.,* 2020). According to the findings, Penicillin G (PG) was effective against 14 of the 16 strains, whereas Erythromycin (E) had antibiotic activity against only 8 of the 16 strains. Because of the adverse effects that they had on certain bacteria strains when tested at low doses, the antibiotics ampicillin (AP), cephalothin (KF), clindamycin (CD), and cotrimoxazole (TS) did not demonstrate any meaningful efficacy.

After being subjected to an antibiotic resistance test, four different antibiotics were found to have excellent susceptibility. These included Penicillin G (PG), Erythromycin (E), Ampicillin (AP), Cephalothin (KF), and Clindamycin (CD). The results revealed that Penicillin G (PG) was found and showed excellent susceptibility towards all types of bacteria for the most part and is therefore considered safe for oral consumption. Erythromycin (E) also had excellent results, as it was highly resistant to gram-negative bacteria like *Pseudomonas aeroginosa*, *Staphylococcus aureus*, and

Enteric gram-negative bacteria but highly susceptible against gram-positive bacteria like Bacillus anthracis (Edwin MD 2018). Streptococcus, *E. coli*, Salmonella, and Listeria are pathogens that can cause serious foodborne illnesses. Other common food poisoning microbes include Campylobacter and Staphylococcus (Pal 2022). Gram staining and the Pastrami test showed more gram-positive than gram-negative bacteria. These results have multiple explanations. They cause meningitis, pneumonia, and diarrhoea. Staphylococcus aureus (also known as MRSA) and Staphylococcus epidermidis, two of the most infamous cocci-positive bacteria, thrive in cold meat because it is their natural home (Putri, Ramadhani and Wasito 2021). After being autoclaved, most samples of plain beef mortadella, plain chicken mortadella, and roast beef were found to be free of microbial. In meat products, the presence of a gram-positive bacterium is a reliable sign of the existence of contamination. Bacteria with a negative gram staining were found to infest pastrami, roast beef, chicken mortadella, and plain beef. On the other hand, Coli bacilli, which are typically found in sausages, had a positive gram staining for several different causes. For instance, our ancestors relied on raw and cold meat as their primary source of sustenance while they travelled throughout the world after leaving East Asia thousands of years ago. They have had a significant impact on the development of contemporary culinary traditions, such as the creation of salami in Europe and mortadella in Italy.

Cold meat is one of the biggest problems confronting retailers and consumers alike. Despite being confined to our refrigerators, most bacterial diseases thrive at a constant temperature of 5 degrees, making them hard to detect and easy for consumers to ignore. Whether you are a retailer or a consumer, diseases caused by bacteria in cold meat are among the biggest problems facing us. Despite having refrigerators, these bacterial diseases thrive at an ideal temperature of 5 degrees. They are hard to detect and easy for consumers to ignore.

With clean, healthy, and sustainable ways of preserving meat and dairy products, frozen meat could become a powerful new alternative solution in the food industry. Clean meat can be used for the following purposes: making bio digesters, making cultured meat (including the consumer version), and generating feedstock as a carbon source for their process (Pakseresht, Ahmadi Kaliji and Canavari 2022). Keeping meat clean is a great advantage for having a clean and healthy meal. The environment should also be kept healthy and clean by minimizing the carbon footprint from all the food you consume in your diet, including vegetables and fruits. Clean meat can be produced with a high-tech system that allows the growth of animal cells and food production while using very little waste (Srutee, R. S. and Uday S. 2021). Clean meat is a healthier, more environmentally friendly source of meat than the traditional ones from animals. Humans must consume healthy meat to live a healthy life. This also helps to increase the human living life span.

5. CONCLUSION

In conclusion, 30 sample were isolated that from 5 types of colds meat with two different company and we obtained 19 results from 30 which good results after repeated three time to measure. Although, cold meats in Jeddah, Saudi Arabia, are barely below the FDA's guidelines in terms of microbiological quality. *E. coli*, *Staphylococcus*, and *Streptococci* are the most dominant species. This means that food handlers should all be well-trained. To guarantee and prevent contamination of this kind, the public should be aware of the dangers of diseases from contaminated cold cuts and food poisoning. The microbiological quality of cold meats in Jeddah, Saudi Arabia, requires proper treatment against bacterial infections (Abdulsalam and Bakarman 2021).

Regarding coliforms, *Staphylococcus*, and *streptococci*, strict supervision must be carried out on the food handling for their disposal. Moreover, competent authorities should conduct awareness campaigns to educate people about the danger of diseases that may be transmitted through cold meats. We recommend that all food handlers receive training to prevent and reduce the risk of contamination (ERGOĞAN and Pamuk 2019). We also recommend conducting awareness campaigns within the communities.

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