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Microbial Load of Canned Foods Imported Through Ibrahim Khalil International Border, Iraq

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HIGHLIGHTS

- *Bacillus coagulans* with *Klebsiella* spp. were the most isolated bacteria.
- The study found no growth of aerobic and anaerobic bacteria in the tested samples at 15 °C incubation.
- Canned tomatoes and fishes relatively had more microorganisms than the meat products.

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Acronyms and abbreviations
CFU=Colony Forming Unit
TPCs=Total Plate Counts

ABSTRACT

Background: Canned foods may be contaminated with microbes and primarily with spore-forming bacteria. This study was designed to give information about microbial load of canned foods imported through Ibrahim Khalil International Border, Iraq.

Methods: Total of 119 samples of canned foods comprising 35 poultry meats, 40 fishes, and 44 tomato pastes were collected from Ibrahim Khalil International Border. Using conventional protocols, samples were evaluated for total plate counts (aerobic and anaerobic microorganisms), spoilage pathogenic, and coliform organisms. The obtained results were analysed by One-Way Analysis of Variance (ANOVA) suing GraphPad Prism (V.5.01).

Results: The total aerobic plate counts at 37 °C incubation were 1.30±0.2 log Colony Forming Unit (CFU)/g in canned meats, 1.32±0.3 log CFU/g for fishes, and tomato paste accounts for 2.11±0.5 log CFU/g. On the other hand, the counts of anaerobic plate were 0.95±0.2 log CFU/g in meat samples, 1.08±0.2 log CFU/g for fishes, and tomatoes were scored at 0.95±0.2 log CFU/g. *Bacillus subtilis*, *B. coagulans*, *Clostridium perfringens*, and *Klebsiella* spp. were recovered from some of the canned samples.

Conclusion: Canned tomatoes and fishes relatively had more microorganisms than the poultry meat products. These data suggested that poor hygiene standards in the processing line may result in microbial control loss.

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Introduction

Canned meat products are popular meals when compared to other food meals since they are easy to make and hence fit most working ladies and families, as well as canteens and fast service cafeterias. They are indeed appropriate for camping and also other outdoor activities when refrigeration is not accessible (Maheswara et al., 2011). The purpose of the canning is to kill the hazardous microorganisms in food; yet, improperly handled cans provide breeding grounds for microorganisms. In fact, canning eliminates microbiological pollutants (FDA, 2021). However, canned foods are susceptible to microbial decomposition

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and may cause food-borne illness due to poor processing, inadequate chilling, contamination of the can due to leakage, and pre-process spoiling. Moreover, certain canned foods are susceptible to contamination by a variety of bacteria as a result of their low-heat processing (Lorenzo et al., 2018).

Canned foods are reported in various cases that they contaminated with spore-forming bacteria particularly by *Clostridium* (Jamroskovic et al., 2016), *Bacillus*, and *Desulfotomaculum* (Oranusi et al., 2012). *B. coagulans* and *Geobacillus stearothermophilus* are associated with the flat sour spoilage (unappealing flavour) with increasing the acidity of the canned food but without much gas generation (Bintsis, 2017; Rigaux et al., 2014). *B. cereus* and *B. licheniformis* contaminate milk, resulting in fractured cream and soft coagulum with ruptured cans (Awasti et al., 2019; Gopal et al., 2015). Toxins from *C. perfringens* lead to food poisoning by consumption of contaminated meat and gravies.

It is essential for low acid canned foods that do not undergo a full botulinum cook. The bacteriological analysis of canned meat is performed to assess the presence of potentially harmful microorganisms as well as the sanitary conditions of the canned meat, including temperature abuse and cleanliness during handling, processing, and storage. Although, aerobic plate count is not a reliable indicator of its safety for ingestion, it is critical in appraising the sanitary conditions of manufacturing, handling, and storage (Ali et al., 2018). This study was designed to give information about microbial load of canned foods imported through Ibrahim Khalil International Border, Iraq.

Materials and methods

Sampling

In 2022, a total of 119 samples of imported canned foods were collected at the Ibrahim Khalil international border between Iraq and Turkey. The canned samples that comprising of 35 poultry meats, 40 fishes, and 44 tomato paste were directly taken to the central laboratory of New Standard Company for analysis. The appropriate information on the cans including manufacture and expiry dates, manufacturer's address and country, batch number, and compositions were recorded properly. The cans were checked for evidence of any leaking, physical damage, and bloating.

Microbial analysis

Prior to examination, the container's surface was cleaned with 70% ethanol and iodine tincture. To prevent contamination, containers were handled under aseptic condition within the laboratory. The cans were opened near the heat of the Bunsen burner using a sterile can opener to induce small opening. Firstly, the pH of the samples was determined and recorded by a pH meter (Jenway 3,505, England). Subsequently, 10 g of each food were taken and mixed in sterile mixing blender containing 90 ml Brain Heart Infusion (BHI) broth (Oxoid, UK) and Cooked Meat Medium (CMM) (Oxoid, UK) in duplicate tubes. The tubes were then incubated aerobically and anaerobically at 15 °C for psychrophiles, 37 °C for mesophiles, and 55 °C for thermophiles for 24-48 h. After pre-enrichment, triplicate plates of BHI agar (Oxoid, UK) and Nutrient agar (LabM, UK) were inoculated and incubated (Oxoid anaerobic Jar, UK) at the same temperature's pre-enrichment broths.

Ten g portions of the samples were homogenized in 90 ml tryptone soya broth water (LabM, UK). According to ICMSF and Iraqi standard specification guidelines, the solutions were diluted into 1×10^{-2} and plated on triplicate plates of Nutrient agar, Violet Red Bile Salt agar (HiMedia, India), and reinforced Clostridial agar (HiMedia, India) using the pour plate method and spread on Baird-parker agar (Oxoid, UK). The plates were incubated at three different temperature conditions as described above. At the end of the incubation period, a colony counter was used to count the colonies (Stuart Scientific, UK). The results were given in log Colony Forming Unit (CFU)/g. The suspected colonies on plates were picked up and stained with Gram stain. In addition, the colonies were purified by repeated subculturing, and stored on agar slants or agar stabs, if they were anaerobes for further analysis. It is worth mentioning that the isolates were identified and confirmed by VITEK 2 system (bioMérieux VITEK® 2, UK) according to Tayeb et al. (2020).

Statistical analysis

The values obtained for all aerobic plate counts, Total Plate Counts (TPC) and microorganisms were subjected to analysis of variance, One Way Analysis of Variance (ANOVA), by using GraphPad Prism 5.01 (GraphPad Software, San Diego, CA, USA).

Results and discussion

As indicated in Table 1, the total aerobic plate counts at 37 °C incubation were 1.30±0.2 log CFU/g in canned poultry meats, 1.32±0.3 log CFU/g for fishes, and tomato paste accounts for 2.11±0.5 log CFU/g. On the other hand, the counts of anaerobic plate were 0.95±0.2 log CFU/g in poultry meat samples, 1.08±0.2 log CFU/g for fishes, and tomatoes were scored at 0.95±0.2 log CFU/g. *Bacillus subtilis*, *B. coagulans*, *Clostridium perfringens*,

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and *Klebsiella* spp. were recovered from some of the canned samples.

Canning is a technique for keeping foods, and the business grew via trial and error and the talent of individual canners. This process was subjected to extensive scientific scrutiny in the 1990s and now evolves into a sound and proven technology for producing commercially sterilized safe foods with a nearly unlimited shelf life (Maheswara et al., 2011). However, canned food can be a potential source of contamination with several aerobic and anaerobic microorganisms (Lorenzo et al., 2018; Oranusi et al., 2012). Therefore, the results of our study revealed that colonies of aerobic were more than the anaerobic microorganisms. Our data are compatible with a previous study by Oranusi et al. (2012) who found that the total aerobic plate count in 30 canned food samples in Nigeria ranged from <10 to 1.4×10^3 CFU/g. Another research was performed by Hamasalim (2012) and showed that the aerobic bacteria were totally absent in canned meat. Surprisingly, Hamasalim (2012) detected very low number of anaerobic microorganisms. This could be because of pH of the materials and presence of food additives are diminish the bacterial growth. For example, adding nitrate to a canned item reduces microorganism especially *Clostridium* (Hamasalim, 2012).

Table 1: Total Plate Counts (TPCs) of canned foods imported through Ibrahim Khalil International Border, Iraq

Canned Food	Aerobic incubation			Anaerobic incubation			Coliform Count
	15 ºC	37 ºC	55 ºC	15 ºC	37 ºC	55 ºC	
Poultry meat	-	1.30±0.2	0.95±0.2	-	0.95±0.2	0.95±0.24	-
Fish	-	1.32±0.3	1.04±0.3	-	1.08±0.2	0.95±0.22	0.954
Tomato paste	-	2.11±0.5	0.95±0.2	-	0.95±0.2	0.95±0.21	-

Microbial counts presented as log Colony Forming Unit (CFU)/g (mean±SD)
- : No growth at expiry of incubation time
p value= >0.05 (statistically non-significant)

The current study discovered that canned tomatoes and fishes had more microorganisms than the poultry meat products (Table 1). Based on Khalafalla et al. (2020), there is more *Clostridium* spp. in corned beef and sausages than in canned meat. In a similar research by Hamasalim (2012) on imported canned beef sold in Sulaimani markets (Iraq), it was shown that anaerobic bacterial counts were within the acceptable limits and no aerobic bacteria was found in the samples. Also, the author found that *Bacillus* spp. was the predominant organism which is in agreement with findings of our study. In addition, *B. cereus* and *C. perfringens* were also detected in canned meat. Determining *B. coagulans*, *B. subtilis*, *C. sporogenes*, and *C. perfringens* in samples could be explained as they were known to be the most common environmental contaminants. These pathogens are notorious for infection and food poisoning, and it is well known that their growth temperature ranges from 20 to 50 °C (Tewari and Abdullah, 2015). Furthermore, the lower incubation temperature (at 15 °C) produces less CFU in both aerobic and anaerobic incubations than incubation at higher temperatures, at 37 and 55 °C. This is also confirmed in a newly published study carried out by Cruz et al. (2022).

Turning to the confirmation, the result reports from the VITEK 2 system displayed the confidence from most selected colonies as excellent identification of confirmed microorganism from above 97% probability (data not shown). All other isolates from the suspected colonies of aerobic or anaerobic revealed identification value between 92-97% of the pathogens. This clearly revealed of successful identification of the organisms from the canned foods.

Conclusion

Canned tomatoes and fishes relatively had more microorganisms than the poultry meat products. It can be concluded that microbiological evaluation of canned foods is critical for determining the efficiency of the processing. Consequently, risk assessment of food products, contamination levels, and prevent transmission of this bacterium could be evaluated and brought about safer food for communities. Therefore, for proper food safety, regular monitoring, and inspections to track canned food sales are required.

Author contributions

Y.H.M.S. and B.A.T. designed the study; N.A.M. and P.J.Y. collected samples and conducted the experimental

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work; Y.H.M.S. conceptualized, supervised, and also analyzed the data; B.A.T conceptualized, drafted, and reviewed the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

All authors declare no conflicts of interest.

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