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Microbial Load and Prevalence of Multidrug Resistant Bacteria in Various Frozen Raw Meat Products

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KEYWORDS

Frozen meat, Multi-drug resistant bacteria, ESBL producers, Carbapenem resistance, Enteric pathogens.

ABSTRACT:

Introduction: Bacteria are ubiquitous to cause various infections and bring about a huge impact on public health. Contaminated fresh and frozen foods can act as main source of infection for humans. Therefore, proper storage and maintenance of frozen products is of key importance. In the recent days there is an increased rate of infections caused by Gram negative enteric pathogens. The emergence of antimicrobial resistant organisms is also on the rise since the livestock are also fed with antibiotic incorporated foods. This study is to evaluate the microbial load in the frozen raw meat collected from various supermarkets.

Objectives: The emergence of antimicrobial resistant organisms is also on the rise since the livestock are also fed with antibiotic incorporated foods. This has greatly led to the emergence of antibiotic resistant bacteria.

Methods: The bacterial isolates from various frozen food samples of animal origin were subjected to routine bacterial identification and susceptibility testing by standard microbiological procedures.

Results: A total of 48 frozen meat samples were analysed. Type of frozen meat analysed include, poultry meat, lamb, beef, pork, fish, and shellfish. A plethora of organisms was isolated from various frozen meat samples. A total of 83 organisms were isolated from various frozen samples out of which 7% of the isolates were Extended spectrum beta-lactamase (ESBL) producers.

Conclusions: To conclude, though freezing the foods have great impact in preventing the microbial growth, safety of frozen foods mainly depend upon the proper handling and storing them in appropriate temperatures.

1. Introduction

Bacteria are ubiquitous to cause various infections and bring about a huge impact on public health (Doron et.al, 2008). It affects the economy of both developing and developed countries. Contaminated fresh and frozen foods can act as main source of infection for humans. Contamination of frozen foods can occur at any point in time from handling to storage. Therefore, proper storage and maintenance of frozen products is of key importance as it can greatly affect the overall acceptability of the product not only in the terms of Flavors, texture, color, and appearance but the microbiological safety and nutritional quality. There are many epidemiological reports clearly implicating that food of animal origin act as vehicles for food borne pathogens.

The microorganisms that occur as enteric pathogens include bacteria, parasites, and viruses. The common bacteria that occur as human enteric pathogens belong to the family Enterobacteriaceae especially the microorganisms of the genera – Salmonella, Shigella and diarrheagenic Escherichia coli. Other enteric pathogens include – Vibrio cholerae, Campylobacter jejuni, Vibrio parahemolyticus, Clostridium difficile, Staphylococcus aureus, Bacillus cereus etc., Meat stored in frozen form when not handled properly can be contaminated and can serve as a main source of food-borne illness (Kanaan et.al 2018).

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2. Objectives

To assess the microbial load present in frozen raw meat obtained from different supermarkets.

To identify and characterize multidrug-resistant bacteria isolated from frozen food samples of animal origin.

3. Methods:

This prospective study was carried out in the Department of Microbiology of a teaching medical college hospital. Samples were collected from the various supermarkets were included in this study. The samples were collected between, February 2023 to May 2023. Institutional Ethical Committee clearance was obtained before initiating the study. IHEC reference No: IHEC-I/1457/22.

Inclusion criteria: Various frozen meat samples from supermarkets

Exclusion criteria: Frozen vegetables, frozen fruits, non-frozen meat samples, meat samples from wet markets.

The frozen meat samples were collected from various supermarkets and meat selling outlets. The samples were transported at proper storage conditions to the microbiology laboratory. If any delay in sample processing was anticipated, they were stored in refrigerator between 20 to 80C

Once transported to the laboratory, under sterile conditions, the frozen meat after thawing, about 25g of each sample was inoculated into thioglycollate broth and onto to enriched medium like blood agar and differential medium like MacConkey agar. The samples were also inoculated onto Sabouraud's Dextrose Agar to isolate fungi. The plates were incubated at 370C for 24 hours.

Bacterial identification and antibiotic susceptibility testing

After incubation the plates were inspected for bacterial growth. The plates showing growth were subjected to the preliminary identification tests such as Gram stain, Catalase, and Oxidase test. Tube coagulase test was performed for the catalase positive Gram-positive cocci, to differentiate Staphylococcus aureus from Coagulase-negative Staphylococci (CoNS). The organisms were speciated according to the standard microbiological procedures.

The biochemically characterized bacterial isolates were subjected to antibiotic susceptibility testing. The antimicrobial susceptibility testing was carried out following Kirby Bauer disc diffusion technique for all the bacterial isolates according to CLSI guidelines.

The following antibiotics procured from HI media Labs Mumbai; India were used. These include amikacin (30µg), ampicillin (10µg), cefazolin (30µg), gentamicin (10µg), ciprofloxacin (5µg), cefuroxime (30µg), cotrimoxazole (1.25/23.75µg), piperacillin- tazobactam (100/10µg), cefotaxime (30 µg), cefepime (30µg), meropenem (10 µg), tobramycin (10µg), penicillin (10 units), erythromycin (15µg), clindamycin (2 µg) chloramphenicol (30 µg), linezolid (30 µg), tetracycline (30 µg), teicoplanin and vancomycin.

4. Results

A total of 48 samples were included in the study. 14 samples of poultry meat, 11 samples of lamb, 11 samples of fish, 5 samples of beef, 5 samples of pork and 2 samples of shellfish. All 48 samples showed growth of single or mixture of organisms.

Of the total 8 frozen meat samples processed, 63% of the samples yielded single type of organism, 28% of the samples yielded two types of organism and 13% of the samples yielded three types of organisms.

Organisms isolated from the 14 frozen poultry meat included – Citrobacter freundii 4 (17%), Citrobacter koseri 4 (17%), Proteus mirabilis 4(17%), Proteus vulgaris 1(4%), Morganella morganii 4(17%), Enterobacter species 2 (9%) and Escherichia coli 4(17%). 7 (50%) poultry meat samples yielded single type of organism, 5(36%) samples yielded two types of organism and 2 (14%) samples yielded three types of organism.

Organisms isolated from 11 frozen lamb samples include –Proteus mirabilis 6(33%), Escherichia coli 4(22%), Acinetobacter species 3(17%) and Enterococcus species 5(28%).

7(64%) of the lamb meat samples has yielded single type of organism and 4 (36%) of the samples yielded two types of organisms.

Organisms isolated from 11 samples from frozen fish include Citrobacter koseri 2 (11%), Citrobacter freundii 2(11%), Acinetobacter species 1(5%), Escherichia coli 2(11%), Klebsiella species 2(11%), Morganella morganii 2(11%), Proteus vulgaris 1(5%), Proteus

Journal of Chemical Health Risks

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mirabilis 2(11%) Staphylococcus aureus 2(11%) and Enterococcus species 3(16%). 8(73%) of the samples has yielded single type of organism, 2(18%) of the samples yielded two types of organisms and 1(9%) samples has yielded three different types of organism.

Organisms isolated from frozen pork include – Klebsiella species 2(18%), Escherichia coli 2(18%), Proteus mirabilis 2(18%), Proteus vulgaris 1(9%), Citrobacter koseri 2(18%) and Enterococcus species 2(18%). The distribution of the above-mentioned organisms was found to be uniform. 2(40%) of the samples yielded single type of bacteria, another 2(40%) samples yielded two types of organism and 1(20%) sample yielded three types of bacteria.

Organisms isolated from frozen beef include – Acinetobacter species 1(11%), Serratia marcescens 2 (22%), Escherichia coli -3 (33%), Citrobacter freundii 2 (22%) and Enterococcus species 1(11%). Escherichia coli was isolated most from the frozen beef samples processed. In the frozen beef samples, 3(60%) yielded single type of organism and 2(40%) samples yielded three types of organisms.

Organisms isolated from frozen shell fish include – Enterobacter species- 2 (67%), and Citrobacter koserii - 1 (33%).

Among the total 48 frozen food samples, 29 (63%) samples yielded single type of organism, 13(28%) samples has yielded two different organisms and 6(13%) samples yielded three types of organisms.

Most of the organisms isolated from frozen foods were found to susceptible to aminoglycosides and carbapenem. However reduced susceptibility was noted to first and second generation cephalosporins and quinolones. (Fig:1 to 5)

Among the Gram-negative bacilli isolated, 8.3% (6) of the isolates were found to be ESBL producers.

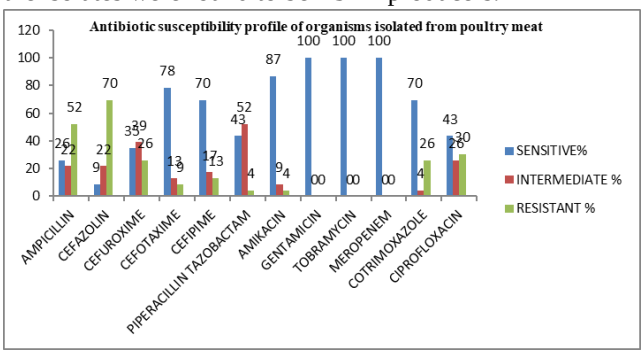

Figure 1 Antibiotic susceptibility profile of organisms isolated from frozen poultry meat.

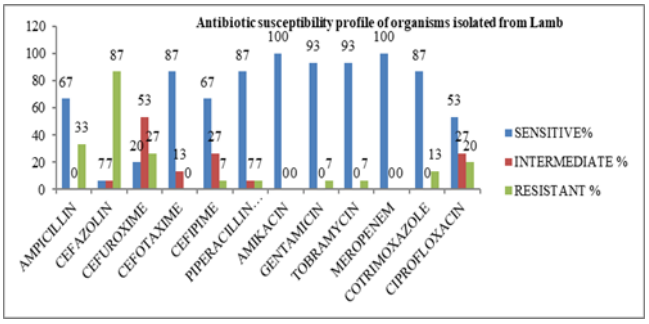

Figure 2 Antibiotic susceptibility profile of organisms isolated from frozen Lamb.

Among the 72 Gram negative bacilli isolated from frozen foods, 8.3% (6) isolates were found to be ESBL producers. Gram positive organisms like Enterococcus species and Staphylococcus aureus were isolated from frozen meat. (Fig:6)

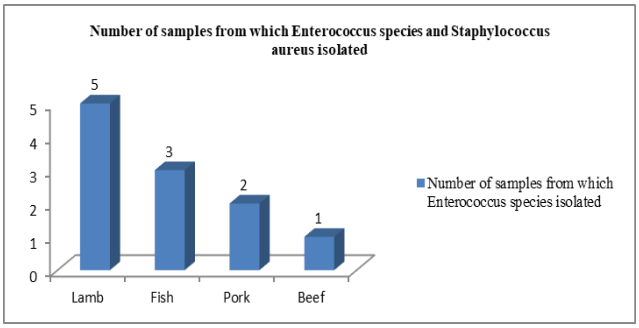

Figure 1: Frozen samples from which Enterococcus species isolated.

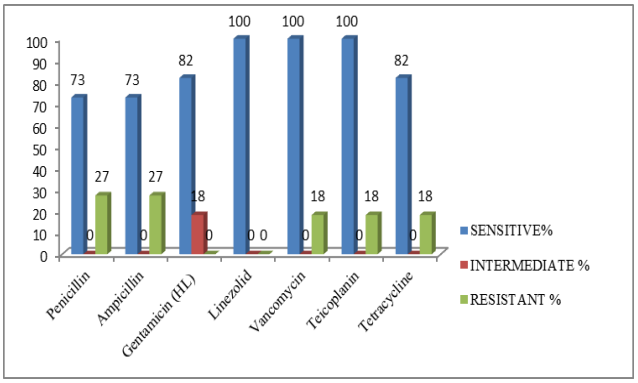

Figure 2 Antibiotic susceptibility pattern of Enterococcus species isolated from frozen meat.

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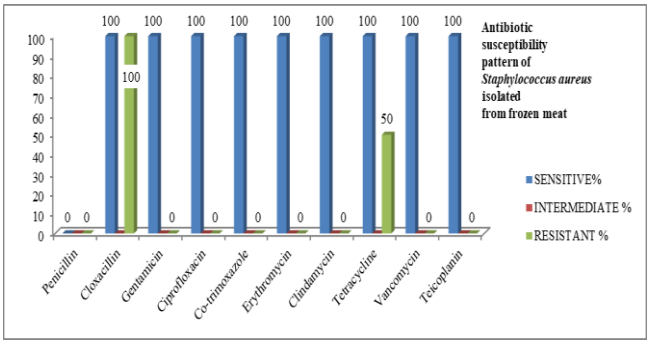

Figure 8 Antibiotic susceptibility pattern of Staphylococcus aureus isolated from frozen meat.

In our study none of the isolates were found to display methicillin resistance and none of the Enterococcus species demonstrated Vancomycin resistance.

5. Discussion

In our study we included the frozen meat products to analyze the microbial load and the presence of multidrug resistant bacteria. There are few studies carried out to analyze the microbial load in both plant based and meat based frozen foods. But in our study, we included only meat based frozen foods based on the assumption that meat based frozen foods have a greater microbial load when compared to plant-based foods (Shamimuzzaman M etal., 2022). In our study most of the isolates belonged to the family Enterobacteriaceae, the predominant genus being the important coliform Escherichia coli. This was similar to the study done by Oranusi et.al., where the coliform rate was found to be predominant. The presence of coliforms clearly indicates a caution that there could be a possibility of contamination with the enteric pathogens (Oranusi et al, 2014). The other predominant genera in the family Enterobacteriaceae included, Proteus, Morganella, Citrobacter and Enterobacter.

In our study we also isolated Gram positive cocci like Enterococcus species and Staphylococcus aureus. The presence of Staphylococcus aureus in fish could be due to contamination introduced from humans who handled the fish product (Sivaraman et.al 2012). In our study the next common member of the family Enterobacteriaceae was Proteus species. Other bacteria isolated were Acinetobacter species, Klebsiella species, Citrobacter species and Enterobacter species. The isolation of Gram-negative bacteria was found to be higher when compared to Gram positive bacteria. The results of our study were not consistent to the study done by Fouzia et.al., where they have isolated Gram-positive cocci like Enterococcus species and Staphylococcus species when compared to Gram negative bacilli (Sultana F 2014).

In our study we did not isolate enteric pathogens like Salmonella species, Shigella species, diarrheagenic Escherichia coli like E.coli O157:H7, Campylobacter jejuni, Vibrio cholerae, etc., This was found to be consistent with other studies were enteric pathogens were least isolated (Guha F et al 2020).

In our study mong the Gram-negative bacilli isolated, 8.3% (6) of the isolates were found to be ESBL producers. Most of the isolates were found to be sensitive to aminoglycosides & carbapenems. However, the highest resistance was noted to ampicillin and cephalosporins like cefazolin and cefuroxime. This was found to be consistent with other similar studies (Sultana F et.al 2014).

In our study we did not isolate any Methicillin Resistant Staphylococcus aureus (MRSA) or Vancomycin resistant Enterococci (VRE). However, few studies have reported MRSA from frozen meat.

6.Conclusion:

To conclude, though freezing the foods has great impact in preventing the microbial growth, safety of frozen foods mainly depends upon the proper handling and storing them in appropriate temperatures. In our study we isolated a plethora of organisms and few of them were ESBL producers. Therefore, a greater caution should be exercised, in the processing and preservation of these frozen foods, to avoid cross contamination with multidrug resistant organisms and enteric pathogens.

7.Conflict of interest:

Author has no conflict of interest in this article.

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Journal of Chemical Health Risks

www.jchr.org

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