Int.J.Curr.Microbiol.App.Sci (2023) *12*(08): 7-13

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 8 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1208.002

Assessment of Microbial Isolation from Refrigerated Food and their Response to Antimicrobial Agents

Subhabrata Goswami[1], Satyabrata Manna[1], Tapati Adak[1], Raktim Chakraborty[1], Gora Chand Ghosh[1], Ajit Pradhhan[1], Sumana Dolui[1], Ranita Maji[1] and Somnath De[2]*

[1]Department of Microbiology, Panskura Banamali College (Autonomous), Purba Medinipur-721 152, West Bengal, India,
[2]Department of Biotechnology, Panskura Banamali College (Autonomous), Purba Medinipur-721 152, West Bengal, India,
**Corresponding author*

Keywords

Food-borne diseases, antimicrobial agents, microbial load, refrigerator management

Article Info

Received:
04 July 2023
Accepted:
05 August 2023
Available Online:
10 August 2023

A B S T R A C T

Food-borne illnesses are becoming more prevalent worldwide as a result of eating contaminated refrigerated foods. The present study was undertaken to investigate the microbial load of different refrigerated food and their response to antimicrobial agents. A total of five processed food samples were taken aseptically, stored in the refrigerator for three days, and examined for microbial loads every day. Spread plate technique and biochemical characterisation had been used for the microbial screening of diverse food samples. The findings indicated that there were numerous contaminating bacteria present, which can cause a variety of food-borne diseases. Samples were analysed for the presence of bacteria while antibiotic resistance patterns of bacterial isolates were determined using standard methods. According to these studies, consumer refrigerator management and hygiene are of extremely low standards, endangering their health. In order to ensure prompt food safety, the study's findings emphasise the significance of proper temperature management and thorough, regular cleaning of their refrigerators.

Introduction

An increasing public health issue worldwide, food-borne diseases cover a broad spectrum of ailments. They include illnesses brought on by a wide variety of microorganisms and are brought on by eating contaminated food (WHO, 2008). Foods that have been refrigerated might spread food-borne illness when they are contaminated at grocery shops, processing facilities, or consumers' homes (Kosa *et al.*, 2007). These spoilage-causing or pathogenic microbes can frequently flourish at low temperatures, resulting in a reduction in shelf life or even having an adverse effect on the health of consumers (Kramer, 2002 and Kreyenschmidt, 2003). Three times as often as in industrial refrigerators, it has been claimed, household refrigerators are where food-borne illnesses begin

(Borneff *et al.,* 1988). Unsuitable food storage practises, such as improper refrigerator management and inefficient chill storage, are to blame for a large number of incidents (Ryan *et al.,* 1996). Storage locations and temperatures must vary depending on the food (Mitchell *et al.,* 2004).

As an instance, fresh meat, fish, and poultry are kept chilled by being placed in freezers and containers with crack ice, much like they do with fish that is bought at the market (Arora, 2003).

Fruits and vegetables are stored in cool, ventilated areas or the refrigerator's vegetable drawer (Prescott *et al.,* 2005). Refrigerators can support the growth of psychoactive pathogens like Listeria monocytogenes and Yersinia enterocolitica, which can grow to clinically relevant levels in foods stored in refrigerators even when properly adjusted (Flynn *et al.,* 1992 and Johnson *et al.,* 1998). The aim of this study is to isolate, characterize and determine the prevalence of microorganisms associated with refrigerated foods from selected sample food.

Materials and Methods

Selection of food samples

The collected food samples are milk, home-made rice, home-made curry, sweet, tamarind water. Samples are collected from different places and different time. Milk, is collected in the month of February, 2023 from Purusattampur market, East Medinipur District (Latitude 22.8101° N, Longitude 88.2632° E, Altitude- 7 meters from mean sea level) West Bengal, India. Home-made rice and curry were collected in the month of March, 2023 from Khirai market area, East Medinipur District (Latitude 22.3750° N, Longitude 87.6971° E, Altitude- 7meters from mean sea level) West Bengal, India.

Sweet and tamarind-water is collected in the month of April, 2023 from Panskura Station Market (Latitude 22.3933° N, Longitude 87.7425° E, Altitude- 7 meters from mean sea level) West Bengal, India.

Food Sample Collection Process

These food samples were kept in a sterilized container with a proper label with the samples name respectively and then taken to the laboratory for analysis and processed as soon as possible.

Isolation of Microorganisms

From the collected samples 1gm (for solid food item) or 1ml (for liquid food item) was taken and serially diluted up to 10^{-8} dilution in sterile water. After serial dilution, 10^{-4}, 10^{-5}, 10^{-6} dilutions of the samples were used in petri plates for isolation of microorganism.

All the glasses wares used for this work was completely sterilized under hot air oven at 160^{o} C for 10 minutes. Isolation was done on Nutrient Agar medium consisting of 10g Peptone, 10g Meat extracts, 8g Sodium Chloride (NaCl), 15g Agar, pH 7.0 for 1000ml distilled water. The medium was prepared and sterilized, Isolation was done as per the procedures of spread plate method. 0.1 ml of sample from 10^{-4}, 10^{-5}, 10^{-6} dilution was inoculated in the medium and were allowed to solidify. The petri plates were incubated at 37 ± 2^{o} Cfor 24 hours and observations were recorded.

Detection of Microbial Load

This procedure which is given in upwards continued up to 3 days and collect the data of 3 times or 72 hours for each sample to detect the bacterial load by colony counting.

Morphological and Biochemical Characterization

Morphological characteristic was measured on the basis of stain colour only (Chauhan *et al.,* 2015 and Aneja, 2003).

Gram Staining

This test helps in differentiating the bacteria into Gram Positive and Gram Negative. A thin smear of

bacteria was prepared on a clean slide and air dried. Then heat fixed. The smear was then covered with crystal violet and allowed it for 20 sec and rinsed with water. Then it was covered with gram's iodine for 1 min and washed with decolorizer.

Then covered with safranin for 20 sec and washed in water and observed under microscope. The bacteria stained with pink or red is Gram Negative and bacteria with purple or violet is Gram Positive.

Indole Test

The indole production test is a biochemical test used to determine the ability of certain bacteria to break down the amino acid tryptophan and produce indole as a by-product. Indole is a compound that accumulates in the growth medium when the bacteria express the enzyme tryptophanase, which catalysis the deamination of tryptophan.

The test is significant in the identification of Enterobacteria, and it is part of the IMVIC procedures designed to distinguish among members of the family Enterobacteriaceae.

Tryptophan, an amino acid, can be hydrolyzed by the enzyme tryptophanase to produce three end products: indole, pyruvate, and ammonia. The presence of indole is detected using chemical reagents such as Kovac's reagent or Ehrlich's reagent. When indole is present, it reacts with the aldehyde in the reagent to form a pink to red-violet quinoidal compound (with benzaldehyde reagent) or a blue to green colour (with cinnamaldehyde reagent). If the enzyme tryptophanase is absent, there will be no colour production, indicating a negative result for indole production.

Methyl Red

A biochemical test used to determine whether an organism can perform mixed acid fermentation and produce stable acid end products from glucose. It is commonly used to differentiate members of the family Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae*, based on their metabolic capabilities. The test relies on the use of the pH indicator methyl red to detect the pH level after an organism has fermented glucose to completion. Here's a concise summary of the principle, procedure, and interpretation of the methyl red test.

Voges-Proskauer Test

A microbiological test used to determine if an organism produces acetyl methyl carbinol (also known as acetoin) from glucose fermentation. The test is part of a group of tests known as IMVIC, which aid in the differentiation of Enterobacteria, particularly members of the family Enterobacteriaceae. It is also used to characterize other groups of bacteria, including Actinobacteria.

The Voges-Proskauer test is based on the production of acetyl methyl carbinol during glucose fermentation. If the organism produces acetyl methyl carbinol, it is converted to diacetyl in the presence of alpha-naphthol, strong alkali (40% KOH), and atmospheric oxygen. The diacetyl, along with quinidine-containing compounds found in the peptones of the broth, then condenses to form a pinkish-red polymer.

Citrate utilization Test

A biochemical test commonly used to distinguish between members of the Enterobacteriaceae family based on their ability to utilize citrate as the sole carbon source and inorganic ammonium dihydrogen phosphate as the sole nitrogen source. This test is part of a group of tests called IMVIC (Indole, Methyl Red, Voges-Proskauer, and Citrate) tests that aid in the identification of Gram-negative bacilli within the Enterobacteriaceae family.

The citrate utilization test determines whether a bacterium possesses the enzyme citrate-permease, which is capable of converting citrate to pyruvate. Bacteria that can utilize citrate as a source of energy can enzymatically break down citrate into oxaloacetate and acetate.

Oxaloacetate is then further metabolized to pyruvate and carbon dioxide. The production of carbon dioxide increases the alkalinity of the medium, leading to a colour change of the pH indicator bromothymol blue from green to blue when the pH rises above 7.6.

Antibiotic sensitivity test against Isolated Microbes

Antibiotic sensitivity was performed by moderate cork borer method against the isolated bacteria. Two standard antibiotics namely Chloramphenicol and Streptomycin was used against isolated bacteria. Briefly, 20 ml quantities of nutrient agar were plated in petri dish, spread with 0.1 ml of bacterial culture. The sensitivity was determined after 24hours of incubation at 37°C. The diameters of zone of inhibition produced by the antibiotics were then measured in mm scale.

Results and Discussion

Microbial Load of Food Samples

For all samples, increasing the microbial load depends on the time period and shown in Table 1 and shown the graphical representation in Figure 1. It can create food-borne disease or food poisoning in our body.

Morphological and Biochemical Identification

Gram-positive bacteria were isolated from milk, home-made rice and sweet. Gram-negative bacteria were isolated from home-made curry and Tamarind water (Table 2). For indole test all isolated microbes has shown negative response. For MR test, only milk, home-made rice and curry's isolated microbe has shown positive response, but sweet and tamarind water' isolated microbes showed negative results. For VP test, all isolated microbes showed negative results. For citrate utilization test only, milk's isolated has shown positive response and others sample's isolated microbes shown negative result (Table 3).

Antibiotic Sensitivity Test

Every isolated bacterium is sensitive to both groups of antibiotics, except the bacteria which was isolated from milk only against with Streptomycin group, because it shows a very low clear zone area respective to others bacteria. That mean we can resist these microbes if they make any type food poisoning or any type of diseases. Results show in below (Table 4, Figure 2 and Figure 3).

Table.1 The detection of microbial load by colony count method

Sample Name	No. of colonies after 24 hours	No. of colonies after 48 hours	No. of colonies after 72 hours
Milk	19	55	**76**
Home-made rice	61	70	**85**
Home-made curry	75	77	**92**
Sweet	65	87	**126**
Tamarind water	**67**	**93**	**110**

Table.2 Gram staining results of isolated microbes

Sample Name	Gram Positive	Gram Negative
Milk	+	-
Home-made rice	+	-
Home-made curry	-	+
Sweet	+	-
Tamarind water	+	-

N.B + = Present, - = Absent

Table.3 Biochemical test for isolated microbes

Characteristics	Samples				
	Milk	Home-made rice	Home-made curry	Sweet	Tamarind water
Indole Test	-	-	-	-	-
MR Test	+	+	+	-	-
VP Test	-	-	-	-	-
Citrate utilization test	+	-	-	-	-

N.B + = Positive, - = Negative

Table.4 Antibiotic sensitivity test against antimicrobial agent

Sample name	Inhibition-zone by Streptomycin(mm)	Inhibition-zone by Chloramphenicol(mm)
Milk	5.7	**10**
Home-made rice	14	**16**
Home-made curry	12.7	**16**
Sweet	14	**16**
Tamarind water	**16**	**13**

Fig.1 Graphical arrangement increasing number of colonies with time.

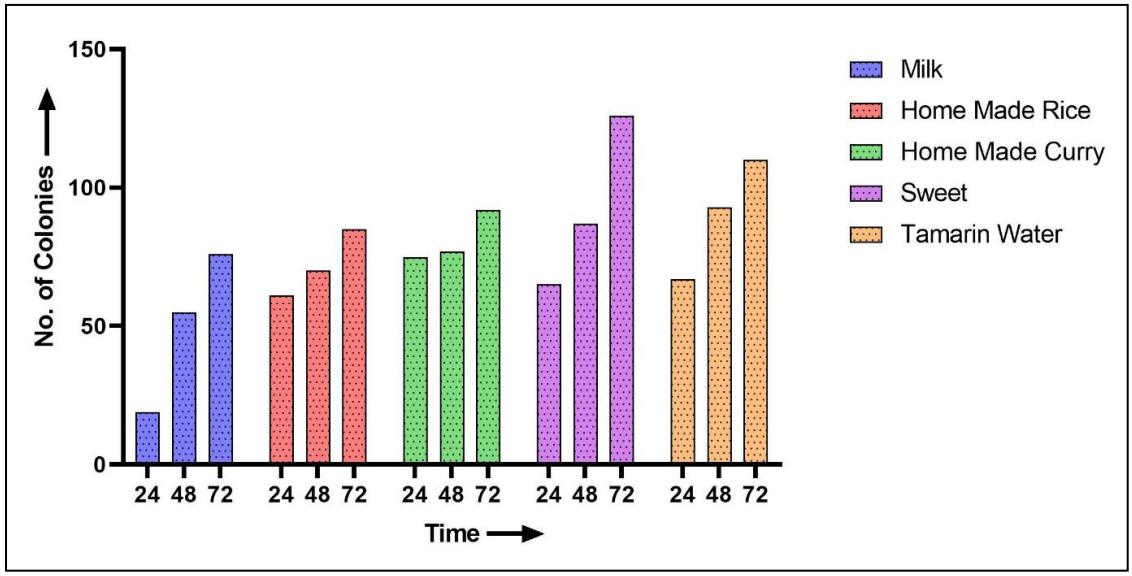
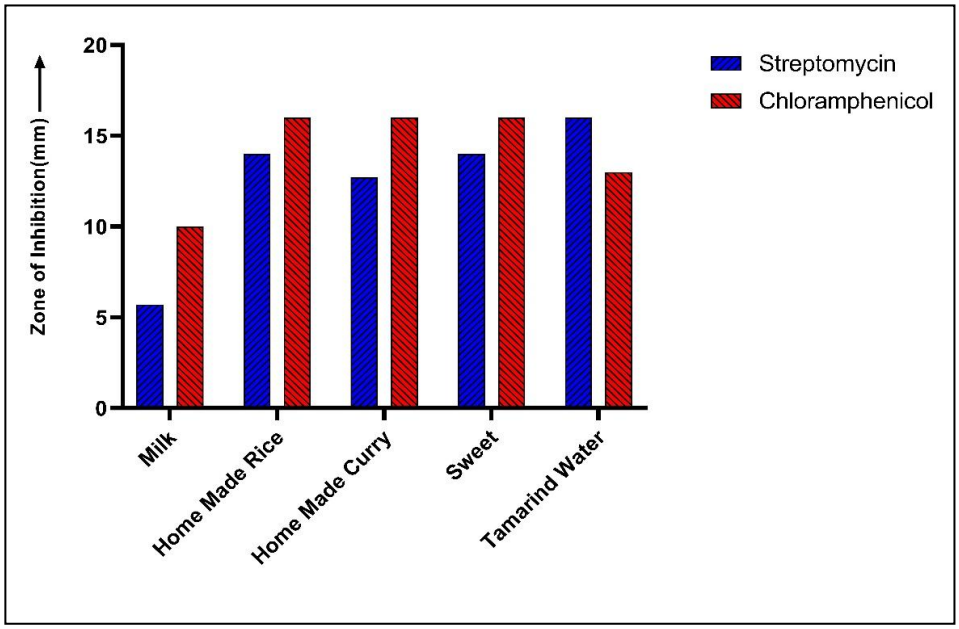


Fig.2 Zone of inhibition produced by the antibiotics against isolated colonies

Fig.3 Antibacterial sensitivity test against isolated strains



In conclusion, the assessment of microbial isolation from refrigerated food and their response to antimicrobial agents provides valuable insights into food safety and pathogen control. This study underscores the importance of proper refrigeration and handling practices to prevent the proliferation of harmful microorganisms in perishable foods.

The findings also highlight the presence of antimicrobial-resistant strains, emphasizing the need for targeted and effective treatment strategies. By understanding microbial behavior in refrigerated environments, we can better safeguard consumers from foodborne illnesses.

Continued research and surveillance in this area will aid in the development of innovative interventions to combat microbial contamination and ensure the

safety of food products. Overall, this assessment contributes to the ongoing efforts to enhance food safety protocols and protect public health.

References

Aneja, K. R. 2003. Staining and biochemical techniques. In: Experiments in microbiology, plant pathology and biotechnology, 4th edn. New Age International (p).Ltd., Publishers, New Delhi.

Arora, R. C. (2003). Mechanical Vapour Compression Refrigeration. In: Refrigeration and air conditioning. New Delhi, India, pp. 1096.

Borneff, J., Singer, H. R., Wittig, J., Harder, E. R. 1988. Distribution of microorganisms in household kitchens. 2. Critical-evaluation of the results and conclusions. ZblBakt MikHyg*B*. 186:30 44.

Chauhan, Neha and Uniyal, Veena and Singh, Devendra. (2015). Original Research Article Microbial profiling of street foods of different locations at Dehradun city, India. 340-347.

Flynn, O., Flynn, M. J., Blair, I and McDowell, D. 1992. The efficiency and consumer operation of domestic refrigerators, Int.J.Refrig. 15 5): 307-312. https://doi.org/10.1016/0140-7007(92)90046-W

Johnson, A. E., Donkin, A. J., Morgan, K., Lijley, J. M., Neale, R. J and Page, R. M. 1998. Food safety knowledge and practice among elderly people living at home, Journal of Epidemiology and Community Health. 52 (11): 745-748. https://doi.org/10.1136/jech.52.11.745

Kosa, K. M., Cates, C. S., Karnes, S., Godwin, S. L., and Chambers, D. (2007). Consumer home refrigeration practices: Results of a web-based survey. Journal of Food Protection, 70: 1640-1649. https://doi.org/10.4315/0362-028x-70.7.1640

Kramer, J. (2002). Lebensmittelmikrobiologie. 4. Auflage. Stuttgart: Eugen Ulmer Verlag.

Kreyenschmidt, J. (2003). Modellierung des Frischeverlustesvon Fleischsowiedes Entfa ¨rbungsprozessesvon Temperatur- ZeitInte-gratorenzur. Festlegung. Anforderungsro-filnfu¨rdieproduktbegleitend Temperaturu-berwachung. Bergen.

Mitchell, D., Finkelhor, W., and Rickert, G. (2004). Refrigeration and food safety, In: How to store vegetables you've grown, pp. 345-404.

Prescott, L. M., Harley, P. J., and Klein, A. D. (2005). Microbiology. 6th edition, McGraw Hill Publishers, New York.

Ryan, M. J., Wall, P. G., Gilbert, R. J., Griffin, M., Rowe, B. 1996. Risk factors for outbreaks of infectious intestinal disease linked to domestic catering. CDR Review. 13:179 182.

WHO. 2008. Initiative to estimation the Global Burden of Food-borne Diseases. Foodborne Diseases -a Growing Risk.

How to cite this article:

Subhabrata Goswami, Satyabrata Manna, Tapati Adak, Raktim Chakraborty, Gora Chand Ghosh, Ajit Pradhhan, Sumana Dolui, Ranita Maji and Somnath De. 2023. Assessment of Microbial Isolation from Refrigerated Food and their Response to Antimicrobial Agents. *Int.J.Curr.Microbiol.App.Sci.* 12(08): 7-13.
doi: https://doi.org/10.20546/ijcmas.2023.1208.002