

ISOLATION OF VARIOUS BACTERIAL PATHOGENS FROM DOMESTIC REFRIGERATORS

M. ROHINI KUMAR, APURVA, BHAWNA, RISHU, JABEZ W. OSBORNE*

School of Bio Sciences & Technology, Vellore Institute of Technology, University, Vellore 632014, India. Email: jabez.vit@gmail.com

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ABSTRACT

The people of South India are often suffering from food poisoning and other health diseases. The isolation of pathogens from domestic refrigerators was performed to determine the prevalence of pathogenic microorganisms. Samples were obtained from domestic refrigerators of various parts of South India (Vellore district). The swabs were inoculated onto Mannitol salt agar, EMB agar, Mac conkey agar and were incubated for 24 hours at 37°C. After incubation 10 bacterial cultures were obtained using various cultivation medium. Gram's staining revealed 10 isolates were Gram negative. For the Gram negative isolates biochemical tests were performed. Catalase test showed positive results for all the isolates. From the morphological and biochemical characteristics the isolates were identified as *Salmonella sp.*, *Citrobactor sp.*, *Proteus sp.*, *E. coli*. These findings underline the need for greater consumer's education regarding proper cleaning of their refrigerators and safe food handling practices.

Keywords: Refrigerator, pathogenic bacteria, food contact surface, Vellore.

INTRODUCTION

The reported incidence of pathogens found in domestic refrigerator in south India is unacceptably high, even though it is also significant underestimation the true magnitude of the problem¹. It has been suggested that the food borne illness is initiated in domestic refrigerator three times more frequently than in commercial refrigerator². Many cases are attributable to inappropriate food storage including ineffective chill storage and refrigerator management³. Failure to follow correct practices in the adjustment maintenance use or cleaning of domestic refrigerator poses a number of risks to consumers.

Refrigerator form an important link in wide chain of cross contamination which leads to the outbreak of domestic food borne disease³. Many domestic refrigerators incorrectly adjusted capable of supporting sub optimum but significant growth of mesophilic organisms such as *Staphylococcus aureus* and *Salmonella sp.*⁴. Bacterial contamination caused by unwashed raw foods, hands,

leaking packages, utensil surface, etc are introduced to domestic refrigerator would directly contaminate other stored foods⁵.

Bacteria can colonize a wide range of substrates like food preparation surface, utensils, domestic dish clothes, sponges and others cleaning materials from which they can be transfers into food and further could cause harmful effects on human^{6,10}. The study examined the incidence of the pathogen (from chart) on the surface of domestic refrigerator to provide insights and the risk posed by, these pathogens in domestic refrigeration system.

METHODS

Location/household distribution

Sampling locations were chosen from different part of Vellore district (Katpadi and Ambur) (Fig.1) for the isolation of pathogens from domestic refrigerators.

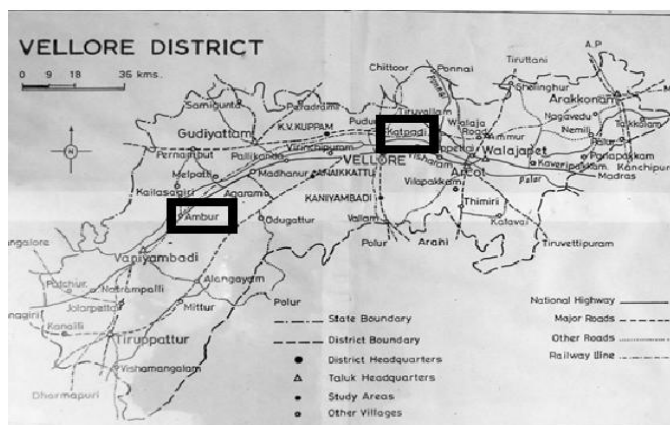


Fig 1: Map of Vellore district: Above two blocks representing two cities of Vellore district (Katpadi and Ambur) from where the samples were collected.

Sampling procedure

With the consent of householders swabs were collected from base, shelves and sides of refrigerator and were transported to laboratory within 2h and were processed. The swabs obtained were used for culturing of pathogenic bacterial isolates¹¹.

Isolation

For the isolation of bacterial cultures various media were used. Salmonella shigella agar, Eosine Methylene Blue (EMB) agar, Mac conkey agar and Mannitol salt agar were used for the isolation of pathogens⁴.

Characterization techniques

The isolates were characterized based on morphological and biochemical analysis and was compared with standards using Bergey's manual.

Morphological Characterization

The cultures were morphologically characterized by Gram staining, methylene blue, Hanging drop and Endospore staining were performed for examination of culture¹².

Biochemical Characterization

The bacterial isolates were characterized by the various biochemical (IMVIC Test) i.e. Indole, Methyl red, Voges Proskauer, Citrate, TSI, Mannitol motility, Catalase¹³. From the biochemical analysis the pathogenic microbes was identified by comparing the results with Bergey's manual¹⁴.

RESULTS

Isolation

Bacterial cultures were isolated from different media. Two sampling locations were chosen for the study and from both the locations five bacterial strains were isolated, respectively.

Morphological Characterization

In the morphological characterization from both the sampling location, five bacterial colonies were isolated.

Biochemical Characterization

Location 1

In the biochemical characterization, five strains were negative for the Indole test and VP test. For the MR test all five strains were shown the positive result. In the citrate test two strains were shown the negative result. For the TSI test four strains were shown the positive results and for the Catalase test all the strains were shown the positive result and for the Mannitol motility test two strains

were showing the negative result (Table 1).

Location 2

The gram negative strains were identified by the IMVIC tests. In the Indole test two strains were shown the positive result. For the MR test all the strains were shown the positive results where as in the VP test all the strains were shown the negative results. In the Citrate test two of the strains were shown negative result. For the TSI test one strain was showing the negative result and for the Catalase test all bacterial strains were shown the positive results and for the Mannitol motility test all the strains were shown the positive result (Table 2).

In this study *Salmonella sp.* was detected in many of the refrigerators which were observed previously¹⁵. The morphological and biochemical characterization shows the presence of one isolates of *Shigella sp.*, six isolates of *Salmonella sp.* and two isolates of *E. coli* were identified.

DISCUSSION

E.coli and *Salmonella sp.* were detected in the refrigerator examined by various or different morphological and biochemical characterization. *Salmonella sp.* was the most frequently recovered from domestic refrigerator in this study. Few *Shigella sp.* and *Citrobacter sp.* were also recovered from refrigerator surface. By the help of the tables 1 and 2, it can be understand that different types of strains are shown with different type of activity.

Table 1: Biochemical tests for the isolates of Katpadi region

Strain	Grams	Endospore	Catalase	Indole	M.R	V.P	Citrate	TSI	Mannitol motility	Organism
1	-	-	+	-	+	-	+	+	+	<i>Salmonella</i>
2	-	-	+	-	+	-	+	+	-	<i>Salmonella</i>
3	-	-	+	-	+	-	-	-	-	<i>Shigella</i>
4	-	-	+	-	+	-	+	+	+	<i>Salmonella</i>
5	-	-	+	-	+	-	-	+	+	<i>Salmonella</i>

Table 2: Biochemical tests for the isolates of Ambur region

Strain	Grams	Endospore	Catalase	Indole	M.R	V.P	Citrate	TSI	Mannitol motility	Organism
1	-	-	+	+	+	-	-	+	+	<i>E. coli</i>
2	-	-	+	-	+	-	+	+	+	<i>Salmonella</i>
3	-	-	+	-	+	-	+	+	+	<i>Salmonella</i>
4	-	-	+	+	+	-	-	-	+	<i>Escherichia</i>
5	-	-	+	-	+	-	+	+	+	<i>Citrobacter</i>

Where MR= Methyl red test, V.P= Voges proskauer test, TSI= Triple sugar ion test.

Five *Salmonella sp.* were detected in most of the refrigerators sampled. In a study by Spiers et al. (1995) reported the failure to detect these pathogens, not only in refrigerator, but in a wide range of sites examined in domestic kitchens¹⁵. However, *Salmonella sp.* was found to be easily spread throughout the domestic environment¹⁸. *E. coli* was identified in this study, which is relatively rare occurrence of the low infective dose pathogens in the human food chain and its ability to form viable non-culturable forms^{16,17}. *E. coli* strains were isolated from almost every refrigerator surface. These result supported the report of Scott et al. (1982) that such organisms are common contaminant in refrigerators¹⁸. Additional bacteria identified, but not quantified included *Klebsiella sp.*, *Citrobacter sp.* (*Citrobacter freundii*, *Citrobacter diversus*) and *Shigella sp.*

Salmonella sp. is a frequent contaminant of many retail foods, and poses public health challenges in terms of potential cross contamination to food and food preparation surfaces during routine food preparation. *Salmonella sp.* are equally easily spread through the domestic environment where they can persist for upto four days. Surface associated *Salmonella sp.* still because a significant cross contamination risk, means this pathogen can multiply under condition of mild temperature abuse in cross contaminated foods²².

E. coli was identified in this study, which is much unexpected pathogen which founds in mainly meat products and raw food contact surfaces. The levels of contamination observed in domestic refrigerators are likely to be influenced by a range of factors

including the nature and levels of initial contamination introduced on contaminated foods, the presence and absence of effective packaging, the hygiene of those preparing and placing foods into the refrigerators and the efficiency and frequency of refrigerator maintenance and cleaning.

E. coli is widely accepted indicator of fecal contamination suggesting that the refrigerator internal surfaces are frequently contaminated by import of contaminated raw foods or by poor personal hygiene.

It is impossible to completely exclude food pathogens from the kitchens; however their spread, growth in survival can be controlled with correct food storage and preparation of practices and regular cleaning and disinfection of food contact site. As we rely more and more on refrigeration as a means of food preservation it is crucial to aware the public about the refrigeration better handlings. The importance of temperature control and regular efficient cleaning should be communicated to the public.

CONCLUSION

The major factor contributing to food borne illness, especially in the home, is the mishandling of food in the final preparation steps. This study has shown that the pathogenic bacteria can survive in refrigerator surfaces and can cause cross contamination. Thus a number of undesirable pathogens such as *Salmonella sp.*, *Citrobacter sp.*, *Shigella sp.*, and *Proteus sp.*, were isolated from the refrigerators of Vellore district.

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