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Culture-Based Isolation and Identification of Bacteria Associated with Three Rice Varieties under Different Storage Conditions in the Kurdistan Region

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Abstract

This study sought to assess bacterial contamination in three frequently consumed rice varieties (Kurdish, Mahmood, and Local) under various processing and storage circumstances. Methods: A total of 75 samples were obtained from three rice kinds, including five batches with five repetitions each. Samples were analyzed under three conditions: uncooked, cooked at ambient temperature (30 ± 2 °C), and chilled (5–7 °C). Bacterial isolation was conducted utilizing standard culture media, succeeded by identification through colony morphology, Gram staining, and biochemical assays. The data were subjected to descriptive analysis. Results: Most raw and refrigerated samples exhibited no bacterial growth, with the exception of Kurdish rice, which contained *Bacillus* spp. and *Staphylococcus* spp. Conversely, rice maintained at ambient temperature displayed heightened bacterial diversity. Kurdish rice exhibited the greatest levels of contamination, including *Bacillus* spp., *Streptococcus* spp., and *Staphylococcus* spp. Mahmood rice was found to include *Staphylococcu*s spp. and *Klebsiella* spp., whereas Local rice exhibited *Streptococcus* spp. and *Staphylococcus* spp.Conclusion: Storage temperature markedly affects bacterial proliferation in cooked rice. Refrigeration significantly mitigates contamination, while room temperature storage encourages bacterial growth, underscoring the necessity of appropriate storage methods to diminish food safety hazards.

1. Introduction

Rice (Oryza sativa L.) is a vital cereal crop globally and serves as a primary food supply for numerous individuals [1]. Rice possesses a virtually neutral pH (about 7) and is predominantly composed of carbs (around 79%), with lesser amounts of protein (roughly 7%) and fat (about 2%). These attributes establish conducive conditions for the proliferation of specific bacteria [2]. Rice is categorized into three types: long-grain, medium-grain, and short-grain. The type of rice is determined by the size of the grains. Long-grain rice is the most widely consumed variety of rice globally. Upon cooking, the grains remain distinct and airy. Conversely, short-grain rice contains a higher starch content and cooks more uniformly [3].

The structure and function of bacterial communities in rice are affected by various parameters, such as temperature, humidity, storage time, and initial microbial load. Collectively, these factors influence microbial growth, survival, and metabolic activity, eventually determining the microbial community structure of stored rice [4]. The microbial population found in rice grains is extremely diverse and includes both helpful and harmful microbes. Molds, mycotoxin-producing fungi, and pathogenic bacteria are examples of damaging microorganisms that can lead to rotting, deterioration of quality, and health problems [5].

Microorganisms exploit rice nutrients, resulting in the degradation of grain integrity and diminished germination potential. Microbial activity can lead to the emergence of undesirable scents and mold-associated off-flavors. The activities of the microbial community influence the shape, color, and appearance of rice. Studies have demonstrated that the microbial population dynamics in rice are greatly impacted by various storage conditions [6]. Elevated temperatures and humidity foster optimal circumstances for microbial proliferation, resulting in heightened microbial activity and food degradation. Prior research has shown that microbial proliferation is influenced by temperature and relative humidity [7].

Previous studies show that high temperatures and humidity during storage increase microbial activity, which speeds up decomposition and may pose health risks [8]. In contrast, cooling suppresses microbial growth, helping retain the quality and safety of cooked rice for longer periods [9]. It is essential to comprehend how different storage circumstances impact the equilibrium between advantageous and harmful bacteria in order to preserve the safety and quality of rice. Additionally, the qualitative characteristics of rice can be significantly impacted by the presence of particular microbes [10]. Microbial contamination, mainly bacterial contamination, can happen at any moment in the process of producing, milling, harvesting, storing, and handling rice after it has been harvested. Eating cooked rice increases the water activity because it undergoes hydrothermal treatment and gelatinization. Because of this, cooked rice is extremely perishable and susceptible to microbial development, particularly at room temperature [11].

Prior research from different parts of the globe has demonstrated that rice frequently harbors a variety of bacterial species. Members of the *Pseudomonadaceae* family, *coliform* bacteria, endospore-forming bacteria, yeasts, and molds are typically associated with rice microflora. These microorganisms can drastically impair the quality and safety of both raw and cooked rice [12]. Prior research has clarified the microbiological variables impacting the shelf life and storage stability of cooked rice, supplying crucial fundamental data for creating ways to reduce rice degradation. An investigation in Korea on numerous rice varieties found that the predominant bacterial taxa discovered from rice samples were *Bacillus*, *Pectobacterium*, *Pantoea*, and *Microbacterium* [13]. Similar outcomes from Pakistani research showed that the most often found microorganisms were *Aspergillus* and *Penicillium* species, as well as *Bacillus cereus*, coliform bacteria, Pseudomonadaceae family members, and other endospore-forming bacteria [14].

The foodborne illness caused by Bacillus cereus, which produces toxins that cause diarrhea and vomiting, is strongly associated with cooked rice. There have been several reports of food illness linked to rice since 1971. When cooked rice is stored improperly, particularly at room temperature or with minimal chilling before reheating, *B. cereus* spores may withstand boiling temperatures and proliferate [15]. Microbial contamination can be extremely harmful to humans and animals and deteriorates rice quality in chemical, physical, and biological aspects [16]. Bacterial food spoilage denotes any auditory alteration (tactile, visual, olfactory, or gustatory) that renders food unsuitable for consumption. Symptoms of foodborne infections include diarrhea, vomiting, abdominal pains, and nausea, caused by *Staphylococcus aureus*, *Salmonella* spp., *Clostridium perfringens*, *Clostridium botulinum*, *Campylobacter*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and enteropathogenic *Escherichia coli* [17]. Although microbial contamination of rice has been thoroughly investigated globally, there remains a dearth of data from the Kurdistan Region concerning bacterial persistence in actual household environments along the entire processing continuum—from raw to cooked, stored, and reheated. Most studies overlook the diversity of grain varieties and the changes in bacterial populations post-cooking, focusing instead on raw grains or certain storage phases. Moreover, there is an absence of context-specific data concerning the impact of prevalent home practices, especially room temperature storage and reheating, on the survival and potential resurgence of harmful bacteria. The notable lack of local evidence limits the development of accurate, region-specific food safety guidelines and highlights the need for comprehensive research over the whole rice use continuum.

2. Materials and Methods

2.1. Sample Collection and Preparation of Cooked Rice Varieties

This cross-sectional laboratory study was conducted in the Microbiology Laboratory, Department of Biology, College of Science, Raparin University. We got three kinds of rice from stores in the Kurdistan Region of Iraq: Mahmood, Kurdish, and Local. Five biological replicates from five distinct batch numbers were obtained for each rice type under every experimental setting to guarantee representativeness and independence.

For analyzing the raw rice, 1 g of each uncooked sample was weighed and combined in a sterile mortar and pestle. To create a uniform microbial suspension, 9 mL of sterile water for injection was included into the homogenate. Serial dilutions were prepared as necessary, and aliquots were used to inoculate blood agar, MacConkey agar, and nutrient agar. The plates were maintained at 30 ± 2 °C for 24 hours, after which colony shape and growth characteristics were examined.

To study cooked rice, 1 g of each sample was put into sterile test tubes with 9 mL of sterile water and heated at 100 °C for 60 minutes to mimic cooking conditions. After cooling in a sterile environment, the samples were split into two groups for storage: one was kept at ambient temperature (30 ± 2 °C) and the other was stored in the fridge (5–7 °C) for 24 hours. After being stored, samples were grown on the same media in the same circumstances.

A total of 75 rice samples were collected from three varieties: Mahmood, Kurdish, and Local. For each variety, five independent batches were obtained from different sources, and five replicate samples were collected from each batch, yielding 25 samples per variety. This sampling protocol was applied uniformly across all varieties, resulting in a total of 75 samples included in the study. They were subsequently evaluated in three conditions: raw, held at ambient temperature, and refrigerated. Bacterial identification was conducted using colony morphology, Gram staining, and conventional biochemical assays. This method enabled the identification of bacterial types present; however, it lacked quantitative measurements (CFU/g), which constitutes a limitation of the study.

2.2. Identification of Bacteria

Bacterial isolates were isolated from different colonies and purified by repeatedly subculturing using the spread plate technique in order to ensure the isolation of pure cultures. Based on colony morphology, which includes color, size, shape, and surface qualities, the bacterial isolates were initially identified. Standard microbiological and biochemical testing were utilized to carry out additional identification. These included catalase and oxidase assays, as well as Gram staining to identify characteristics of the cell wall. Moreover, biochemical characterization was done using the triple sugar iron agar test and the citrate utilization test. The combined findings of morphological, staining, and biochemical investigations were employed for the tentative identification of the bacterial species isolated from the rice samples.

2.3. Statistical Analysis

Data were examined with GraphPad Prism version 10.4.2 (GraphPad Software, USA). The qualitative analysis documented bacterial isolates as either present or absent, categorized by rice variety, processing stage, and storage environment. Results were encapsulated through descriptive statistics and displayed in tabular format. Comparisons of bacterial prevalence among groups were conducted, and the Chi-square (χ^2) test was utilized where necessary to evaluate relationships between factors. Quantitative studies were not conducted owing to the lack of CFU measurements.

Table (1): List of media and reagents used in this study.

Media and chemical reagents	Purpose
Nutrient agar	Used for cultivation of bacteria
Mannitol salt agar	Used for cultivation of certain bacteria as well as inhibit others
MacConkey agar	Selective and differential media used for cultivation of bacteria
Blood agar	Used for cultivation of bacteria.
Triple sugar agar	Used to ferment sugar ,producing of hydrogen sulfide to selective identification of bacteria
Hydrogen peroxide	Used to distinguish *Staphylococcus* from *Streptococcus* species.

3. Results

3.1. Bacterial Isolation from Uncooked Rice Samples

In uncooked rice samples, bacterial contamination was seen solely in Kurdish rice, whereas Mahmood and Local varieties exhibited no bacterial growth. Four different types of bacteria were found in Kurdish rice, and they were from both the Gram-positive and Gram-negative categories.

The Gram-positive isolates were *Bacillus cereus* and *Staphylococcus* spp., while the Gram-negative isolates were *Enterobacter* spp. and *Proteus* spp. Table (2) shows how these bacterial isolates are spread out qualitatively.

Table (2): Qualitative distribution of bacterial isolates in raw rice samples.

Rice Variety	Detection Status	Gram-Positive Bacteria	Gram-Negative Bacteria
Kurdish	Present	*Bacillus cereus*, *Staphylococcus* spp.	*Enterobacter* spp., *Proteus* spp.
Mahmood	Absent	None	None
Local	Absent	None	None

3.2. Bacterial Growth in Cooked Rice under Different Storage Conditions

Rice cooked and stored at 5–7 °C had a far lower variety and number of microorganisms than rice maintained at room temperature (30 ± 2 °C). However, not all cases showed total prevention of bacterial growth; *Bacillus* spp. and *Staphylococcus* spp. were found in refrigerated Kurdish rice.

Conversely, no bacterial proliferation was seen in Mahmood and Local rice samples maintained under refrigeration. All types of rice had a wider range of bacterial species while they were at room temperature. Kurdish rice had the most different types of bacteria, such as *Bacillus* spp., *Streptococcus* spp., and *Staphylococcus* spp. Mahmood rice had *Staphylococcus* spp. and *Klebsiella* spp., while Local rice had *Streptococcus* spp. and *Staphylococcus* spp.

Overall, Gram-positive bacteria (*Bacillus* spp., *Streptococcus* spp., and *Staphylococcus* spp.) were the most prevalent across all conditions. Gram-negative bacteria were less common, with *Klebsiella* spp. detected only in Mahmood rice stored at room temperature.

Table (3): isolated bacteria from three kinds (Kurdish, Mahmood and Local rice) of cooked rice.

Rice Type	Room Temperature (30 ± 2 °C)	Refrigeration (5–7 °C)
Kurdish	*Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp.	*Bacillus* spp., *Staphylococcus* spp.
Mahmood	*Staphylococcus* spp., *Klebsiella* spp.	No growth
Local	*Streptococcus* spp., *Staphylococcus* spp.	No growth

3.3. Bacterial Proliferation after Reheating

Reheating was performed using a water bath method. Cooked rice samples were placed in sterile, sealed containers and immersed in a water bath maintained at 75–80 °C for 10 minutes. This protocol ensured that the internal temperature of each sample reached at least 75 °C, as verified using a calibrated thermometer. All samples were reheated under identical conditions to ensure consistency and reproducibility. Following reheating, samples were allowed to cool briefly under aseptic conditions prior to microbiological analysis.

The only cooked rice that showed bacterial growth after being refrigerated was local rice, which contained *Pneumonia*, *Staphylococcus*, and *Streptococcus* species. Reheated Kurdish and Mahmood rice showed no signs of progress. These findings demonstrate that bacterial survival and regeneration are reliant upon the rice type and the storage conditions. Gram-positive bacteria, particularly *Staphylococcus* and *Streptococcus*, had greater resilience following storage or reheating, but Gram-negative bacteria were undetectable.

Table (4): Bacterial proliferation in reheated cooked rice under various storage conditions.

Type of rice	Refrigeration (5–7 °C)	Room temperature (30 ± 2 °C)
Kurdish rice	No growth	*Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp.
Mahmood rice	No growth	*Streptococcus* spp.
Local rice	*Streptococcus* spp.	*Bacillus* spp., *Staphylococcus* spp.

3.4. Comparative Microbiological Assessment of the Three Rice Varieties

In general, how the three varieties of rice were stored affected how many germs they contained. Four species of bacteria were identified in the raw, uncooked Kurdish rice sample: *Bacillus cereus*, *Proteus* spp., *Enterobacter* spp., and *Staphylococcus* spp. Three species of bacteria were detected after cooking and remaining at room temperature: *Bacillus* spp., *Streptococcus* spp., and *Staphylococcus* spp. When the food was put in the fridge, only two types of bacteria could be found: *Bacillus* spp. and *Staphylococcus* spp. While cooked rice at room temperature promoted the growth of two bacterial species, *Staphylococcus* spp. and *Klebsiella* spp., while refrigeration prevented measurable bacterial growth, Mahmood rice did not show bacterial growth when it was raw and uncooked. Local rice also didn't have any bacteria in its raw form. When cooked rice was left out at room temperature, it had two types of bacteria: *Streptococcus* spp. and *Staphylococcus* spp. There was no growth in samples that had been refrigerated. Overall, the statistics show that Mahmood and Local varieties of raw rice usually have little or no bacterial growth. On the other hand, Kurdish rice has greater germs even before cooking. Refrigeration works to lower the amount of bacteria in all types of rice. As shown in Figure (1) and Table (5).

Table (5): Distribution of Bacterial Species in Raw and Cooked Rice under Various Storage Conditions.

Rice Type	Raw (Uncooked) Rice	Cooked Rice – Room Temperature	Cooked Rice – Refrigerated
Kurdish rice	*Staphylococcus* spp., *Bacillus cereus*, *Proteus* spp., *Enterobacter* spp.	*Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp.	*Bacillus* spp., *Staphylococcus* spp.
Mahmood rice	No growth	*Staphylococcus* spp., *Klebsiella* spp.	No growth
Local rice	No growth	*Streptococcus* spp., *Staphylococcus* spp.	No growth

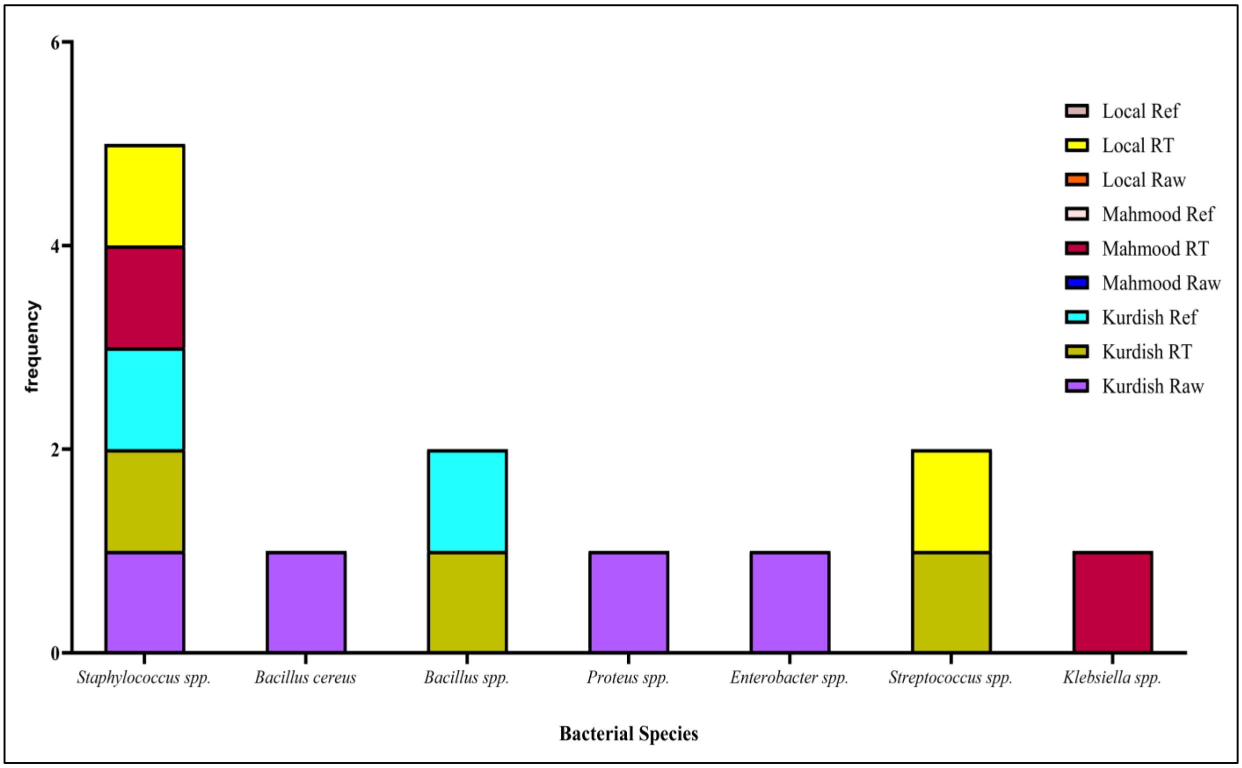

Figure (1): Distribution of bacterial species in Kurdish, Mahmood, and Local rice under different storage conditions.

4. Discussion

The present study was carried out to determine whether there are differences in spoilage among different rice varieties in Kurdistan. It was found that the total bacterial population, as well as Gram-positive and Gram-negative bacterial populations, differed significantly among the three rice varieties tested. According to the data analysis, the bacterial strains isolated included *Bacillus cereus*, *Staphylococcus* spp., *Klebsiella* spp., *Enterobacter* spp., and *Proteus* spp. [13]. This is supported by previous study which showed that the isolated bacteria from rice were *Staphylococcus*, *Bacillus cereus*, *proteus*. However, rice spoilage is almost always associated with Gram positive bacteria, but according to Table (2) in raw rice two types of gram-negative bacteria were isolated (*Enterobacter* and *proteus*). As well as the good quality of local rice can be determined by the characteristic of cooking quality [18]. Multiple research studies indicate that Gram-positive spore-forming bacteria, especially Bacillus cereus, can endure rice cooking due to their endospores' resistance to thermal processing, subsequently germinating during storage under optimal conditions [19, 20]. A systematic review established that cereals, including rice, have one of the highest prevalence rates of *B. cereus* contamination across global food categories, frequently above 40% incidence in certain datasets, highlighting rice as a significant vector for this disease [21]. Gram-positive spore-forming bacteria are the principal cause of rice deterioration because their endospores may survive cooking and then grow during storage. Their thick peptidoglycan cell wall also makes them more resistant to stress from the environment, which lets them live in foods with minimal moisture, like rice.

The only Gram-negative bacteria found in cooked rice samples were *Klebsiella* spp. This may be explained by time in cooked rice the Gram positive bacteria population increased and decreased in Gram negative bacteria population with time can be, because of the competition from Gram positive bacteria. Also it is supported by previous study that Gram positive bacteria spoil rice to a greater degree than did gram negative [22]. Prior investigations demonstrate that Gram-negative bacteria, such as Enterobacter and Klebsiella, are predominantly associated with post-harvest contamination and insufficient sanitation rather than their resilience during cooking. The detection of Klebsiella spp. in cooked rice signifies post-processing contamination, since it has been recorded in cooked and ready-to-eat foods, including rice-based products [23]. Gram-negative bacteria such as

Enterobacter, Klebsiella, and Proteus are incapable of producing endospores. They possess smaller peptidoglycan layers and an outer membrane that is more susceptible to damage from heat, dehydration, and oxidative stress. Frying significantly reduces their likelihood of survival, and they exhibit diminished competitiveness when stored post-cooking. This explains the absence or scarcity of Gram-negative bacteria in cooked rice samples relative to raw rice.

This current study can be concluded that keeping rice in room temperature is more susceptible for bacterial growth than storing in refrigerator. As shown in Table (3) As it is clarified that there are *bacillus*, *streptococcus*, *staphylococcus*, *streptococcus* and *klebsiella* as gram negative bacteria, they are all these types of bacteria isolated on cooked rice stored in room temperature, while few bacterial colony isolated on these types of rice stored in refrigerator [24]. In a similar vein, a new study conducted in Basrah found that storing cooked rice at room temperature greatly raised the number of Bacillus cereus [25]. However, this finding corresponds with a recent study demonstrating that cooked rice stored at room temperature promotes fast microbial growth, whereas refrigeration significantly inhibits bacterial proliferation and extends safe storage time [26]. Refrigeration slows down enzymes and stops spores from germinating, which lowers the number of microbes.

Moreover, to determine the period of acceptability of these three cooked rice after reheating, both samples kept in room temperature and refrigerator have been reheated. In the result as shown in Table (4) it has been clarified that there was just one kind of bacteria which could grow on local rice kept in refrigerator, while there were (*bacillus*, *streptococcus*, and *staphylococcus*) bacteria on samples kept in room temperature [11]. Because the room temperature is optimum temperature for most bacteria and for these types of bacteria isolated [27]. Furthermore, keeping cooked rice in room temperature led more microbes to grow because of cooking rice is a gelatinization and it is easily affected by microorganisms which is ferment rice starch under room temperature. This is supported by previous study that keeping rice in a room temperature is more susceptible for microbial growth [28]. Although, to ensure maximum hygienic quality the cooked rice should be kept for a maximum period of 20 hours at room temperature and 38 hours in a refrigerator before reheating and consumption [11].

Another discovery of the current study is that cooked rice had the highest bacterial contamination, contradicting a recent investigation in Sulaimani City that indicated greater contamination in raw rice compared to cooked rice [29]. Studies in Iraq regularly demonstrate that uncooked rice is polluted during agricultural and storage phases, mostly owing to soil, irrigation water, and environmental exposure. There are many numerous kinds of bacteria in raw rice, and the amount of contamination depends a lot on how it was handled and stored before cooking [29-31].Uncooked rice samples of Local Markets in Kurdistan are harboring less microbial contaminants as compared to cooked rice samples, indicating that, these are protected from contamination while subsequent handling, packaging, storage and transporting. In the other hand ,between these three rice verities Mahmood rice are used to be the most acceptable rice according these considerations we used for this study and it may be due to hygiene harvesting and storing. The result of this study showed that Local rice and Kurdish rice are contaminated in relatively higher level by two dominant storage bacteria *Bacillus* and *Streptococcus*. The disease outbreaks are associated with cooked rice consumption are also resultant from Gram positive Bacillus type bacteria, especially, *B. cereus* which is heat resistance bacteria which has ability of producing endospores [32]. Most of the bacteria grow on rice are normal flora of human but within accumulation, they will increase toxicity and with their opportunistic property become pathogens [22]. Uncooked and cooked rice samples were also examined for the presence of *E. coli*. All The data shows the absence of *E. coli* in all of the examined samples. *Coliforms* can influence food safety and preservation because these organisms are an indicator of fecal contamination and can carry water borne pathogens. Also it has been supported by other study that there were free from coliform contamination [14]. Uncooked rice samples showed less microbial variety than cooked rice samples. This suggests that contamination happens mostly during handling, cooking, cooling, and storage, and not from the grain itself. Mahmood rice had the best microbiological quality of the types evaluated. This may be because higher hygiene was used during harvesting, processing, and storage.

In the other hand there is other study which coli form has been isolated [33]. As well as, to protect from microbial contamination the cooled rice should be transferred immediately into a refrigerator and kept for a maximum period of 38 hours. It is also to ensure the original taste of cooked rice the refrigerated rice should be reheated by microwave oven for 1 minute before serving to the consumer [11]. Conclusion Rice is commonly used almost everywhere which is also is the principal food for more than two billion people. Rice production considerably needs more water than any other cereal crops. It can take up to 5000 liters of water to grow just 1 kg of rice [27]. As it is clear that watery environments are more desirable for microbial growth. Gram positive bacteria are responsible for cooked rice spoilage to a greater extent than Gram negative bacteria. Kurdish and Local rice samples of Local Market are harboring more microbial contaminants as compared to Mahmood rice samples, before cooking, after cooking and after reheating. It is indicating that these are protected from contamination during subsequent handling, packaging, storage and transporting. Keeping cooked rice in a room temperature increases susceptibility of bacterial contamination. So to protect rice from microbial contamination the cooked rice should be transferred immediately into a refrigerator and kept for a maximum period of 38 hours [18]. Further studies are recommended to evaluate the effect of eating reheated rice on health and related problems.

5. Conclusions

Rice is a commonly consumed staple meal that becomes particularly vulnerable to microbial contamination post-cooking. This study illustrates that bacterial contamination in rice is significantly affected by storage conditions and differs among rice varieties. Minimal contamination was detected in raw samples, however cooking and subsequent storage at room temperature significantly elevated bacterial presence and diversity. Kurdish rice had a consistently greater vulnerability to contamination than the Mahmood and Local types. Conversely, cooling significantly inhibited bacterial proliferation, resulting in negligible contamination in the majority of samples. These findings underscore the paramount significance of appropriate post-cooking management. Cooked rice must not remain at room temperature for prolonged durations and should be swiftly refrigerated to decrease the danger of bacterial growth and possible foodborne disease. Enforcing rigorous temperature regulation during storage is crucial for maintaining food safety.

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