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IDENTIFICATION AND CHARACTERIZATION OF PATHOGENIC BACTERIA ISOLATED FROM LOCAL AND IMPORTED RICE

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Abstract

Rice is an important and essential food for the consumer, whether for the world or in Iraq because it contains many important mineral and essential nutrients, microbial or chemical contamination leads to a decrease in the amount of rice production, which in turn affects the economy of the producing country. A total of 48 samples of imported and local rice were collected from the local markets of the city of Baghdad from April to May 2019 which divided into several groups and examined in the Microbiology Laboratory/Market Research and Consumer Protection Center/ University of Baghdad. This research was conducted depending on the detection of hygienic quality, the highest percentage of the total number of bacteria were (16x105 CFU/ml) was observed in the sample (Pakistan), and total coliform bacteria were (3x103 CFU/ml) while the highest contamination with Staphylococci was (7x105) CFU/ ml, the highest total number of fungi was (11x103) CFU/ ml, and pathogenic bacteria in addition to heavy metal contamination. The pathogenic bacteria of Bacillus cereus and Listeria monocytogenes was recorded positive in many samples while negative for salmonella spp. When determination of nutritional values, the total percentages of protein, fats, fibers and carbohydrates was arranged between (5.4 to 6.7) %, (0.4 to 0.5) %, (1.3 to 2.7)%, and (78.6 to 82.5)% respectively, the contamination results with heavy metal elements with arsenic, mercury and cadmium was arranged between (0.0228 to 1.8308) mg/kg, (0.7984 to 0.0408) mg/ kg, and (0.7984 to 0.0408) mg/kg respectively.
Keyword: Rice, Microbial contamination, Heavy metal contamination, Nutritional value

Introduction

Rice is the most important cereal even of wheat in terms of direct benefit to humans all over the world for a long time, as it is considered a staple and basic crop of food security in the world (Orthoefer, 2005).

It is the main source of calories for almost half of the world's population, and rice is considered a major staple in all diets as a basic source of carbohydrates and also a good source of many water-soluble vitamins. Among the mineral elements found in rice are calcium, copper, iodine, iron, magnesium, manganese, phosphorous, potassium, sodium, silicon and zinc, as rice provides the human body with thiamine, which plays a role Necessary in the metabolism of carbohydrates and fats, where its deficiency causes symptoms such as loss of appetite, nausea, vomiting, and impaired heart function. It also provides the body with vitamin B3 (niacin), which helps supply energy from fat, protein, and carbohydrates (Ryan, 2011).

Rice is divided into three types based on the size of the seeds, which are long-grain, medium-grain, and short-grain rice. Long-grain rice is the most popular around the world. The whole grains remain unripe, smooth, and separate when cooked, but if they are cooked more than necessary or are greatly stirred during cooking, they become sticky, while rice grains are short-grain; their content of starch is more and becomes sticky when cooked (Roy *et al*., 2008) (U.S, 2011).

In 1971 the incidence of food poisoning was recognized in cooked rice, after careful examination, it was observed that rice was contaminated with *Bacillus cereus* that gives rise to two types of toxins that are spread through food, the emetic and diarrheal syndromes. Keeping the rice at temperatures below the temperature of heating then storing it the refrigerator before reheating, this practice leads to the spread of the *B. cereus* which contains spores that is resistant to the boiling (Anderson, 2004).

As a result of changing the nutritional behavior of the consumer and his resort to fast food, this led to a decrease in the consumption of agricultural crops, including rice consumption. this led to an increase in food stored for long periods in RPC, the storage period may last from 4 to 5 months, especially in the autumn and winter periods, as well as storing rice in markets and homes for long periods and may lead to non-consumption of rice for periods that last from 3 to 4 years (European Commission, 2006)As a result of this long period of storage, many changes will appear on the chemical, physical and biological properties, where rice loses its quality as a result of contamination with microorganisms such as bacteria and fungi, which produce various types of toxins that are harmful to animals and humans as well as to plants(Heinemann *et al*., 2005) (Deepa *et al*., 2008).

In addition to the presence of microorganisms in rice crops, there is another type of contamination that contributes to increasing the proportion of poisoning in humans and animals, which is pollution with mineral elements (Sperottoa *et al.,* 2012), where the percentage of heavy mineral elements such as arsenic, cadmium, mercury and other toxic elements that are caused by soil pollution with high levels of elements accumulate Mineral, which may be the result of the contamination of polluted irrigation water or by wind, where rice grains are possible to be administrate by these metals and these elements aggregate inside the plant parts. Because of the openness of the Iraqi market to many international origins and because of the import of a lot of foodstuffs, especially rice, therefore this study aims to isolate and diagnose pathogenic bacteria in local rice and compare it with imported rice in addition to estimating the proportions of nutritional values and heavy elements of some kinds that available in the local Iraqi markets.

Materials and Methods

Samples Collection

Samples of imported and local rice were collected from the local markets of the city of Baghdad during April to May 2019 and the total samples were 48 samples and by the four duplicate of each sample. These samples were divided into several groups as shown in Table (1). The quality of the rice was examined microbially and chemically. The Collecting samples were transporting with sterile and sealed bags to the Microbial Examination Laboratory and the chemical laboratory of the Center for Market Research and Consumer Protection/ University of Baghdad.

Table 1: Groups of local and imported rice and the country that collected from it

Samples No	Type	Country
G1	Imported	Thailand
G2	Imported	Brazil
G3	Imported	Vietnam
G4	Imported	Philippines
G5	Imported	Pakistan
G6	Imported	Japan
G7	Imported	India
G8	Imported	India
G9	Imported	Egypt
G10	Locale	Iraq
G11	Locale	Iraq
G12	Locale	Iraq

Microbiological examinations

The microbiological examinations of study samples were carried out depending on the examining the hygienic quality which representing in assessment of total number of bacteria, total coliform bacteria with *Staphylococci* in addition to total number of fungi, with detection and isolation of *Salmonella sp.* , *B. cereus* and *Listeria monocytogenes* as specific pathogenic bacteria samples were divided into several groups and a quantitative and qualitative assessment of bacteria, fungi, and molds was performed using the appropriate agricultural media for each examination in the Microbiology Laboratory / Market Research and Consumer Protection Center / University of Baghdad. The potato acids agar was prepared in addition to the blood and MacConkey agar in addition to using the selective culture media for each type of bacteria. peptone water (225 ml) was used for homogenized of (25) gram of rice samples then cultivated on selective agar media with streaking and pour plate technique, For fungi quantification, the Petri dishes were incubated at 25 °C for 72hours while for bacteria quantification at 35 °C for 48 hours, the positive isolates have been confirmed with (API 20E) (Silva and Silveira, 2001)

Determination of nutritional values of rice samples

The nutritional values of the collected rice sample were determined with chemical analyses using Microkjeldah device to an estimation of protein and using Soxhlet to determine of fat, while by using the incineration furnace method for estimation of ash fiber, moisture and carbohydrates were also determined (AOAC, 2005).

Estimate of heavy metals

The rice samples used in this study were prepared by placing ground rice samples in polyethylene bags, where they were ground into a fine powder by the mill after washing the samples with distilled water and preparing them for the digestion process by taking 1 g of rice, placing it in the digestion flask, 20 ml of the acid mixture (650 ml of HNO_3, 80 ml of perchloric acid and 20 ml of HCL) was added, a clear solution is obtained and the product was diluted to 500 mL which used to estimate the heavy metals (arsenic, mercury and cadmium) with atomic absorption spectrometer (Magamage *et al*., 2017)

Statistical analysis:

The SAS statistical program was used to explain the results collected. Significant differences were correlated with the potential for less significant difference (LSD) ($P < 0.05$), as demonstrated by (SAS, 2012).

Results and Discussion

Two types of microbial tests were performed, which are qualitative and quantitative tests. The following table (2, 3) shows the most important types of bacteria and fungi that were isolated and diagnosed with different rice samples and the total number of each bacteria and fungi.

Table 2: The total bacterial and fungal that count in different rice samples

Samples No	Total number of bacteria (CFU / ml)	Total Coliform bacteria	*Staphylococcus aureus*.	Total Yeast and mold
G1	$14x10^5$	$3x10^3$	$7x10^5$	$6 x10^2$
G2	$3x10^2$	-	$6x10^2$	$2x10^1$
G3	$6x10^3$	$2x10^1$	$4x10^3$	$4x10^2$
G4	$15x10^5$	$2x10^3$	$11x10^4$	$6x10^3$
G5	$16x10^5$	$4x10^3$	$9x10^4$	$11x10^3$
G6	$4x10^1$	-	$3x10^1$	$1x10^1$
G7	$4x10^2$	$1x10^1$	-	-
G8	$5 x10^3$	$3x10^2$	$2x10^3$	$3x10^1$
G9	$4x10^3$	$8x10^2$	$7x10^2$	$5x10^2$
G10	$11 x10^2$	$1x10^1$	$5x10^2$	-
G11	-	-	-	-
G12	-	-	-	-

Table 3: Detection of pathogenic bacteria in different rice samples

Samples No	*Salmonella* sp.	*B. cereus*	*Listeria monocytogenes*
G1	Not detected	+	+
G2	Not detected	+	Not detected
G3	Not detected	+	Not detected
G4	Not detected	+	+
G5	Not detected	+	+
G6	Not detected	-	Not detected
G7	Not detected	+	Not detected
G8	Not detected	+	Not detected
G9	Not detected	+	Not detected
G10	Not detected	**-**	Not detected
G11	Not detected	**-**	Not detected
G12	Not detected	**-**	Not detected

Serial dilutions were prepared from rice samples and cultivated on different cultural media, which are selected according to the type of bacteria and according to the type of examination. Tests included detection of microbial content and detection of the amount of contamination in rice samples in addition to isolation and diagnosis of pathogenic bacteria, the highest rate of dilution was reached to 10^5 and after the incubated for a period of 24 hours at 37°C, the results were varied and different between the rice types.

In the total count of bacteria, the highest percentage were ($16x10^5$, $15x10^5$, $14x10^5$ CFU/ml) was observed in samples (Pakistan, Philippines, Thailand) consecutively, while the lowest contamination rate for the samples was ($4x10^1$, $3x10^2$, $4x10^2$ CFU/ml) was seen in samples (Japan, Brazil, India) in succession, A similar high number of bacteria has also been reported by (Oranusi *et al.,* 2013). As for the Iraqi rice samples, bacterial contamination was not observed, and the reason is due to the differences that were observed in contamination may be due to packing or storing operations for a long period in the stores, also the high total count of bacteria due to the length of the transport period from the producing country and the length of its arrival to Iraq where pollution comes from transport and packing operations in addition to the lack of cleanliness of the workforce that transports these samples (Brasil, 2006)

The reason for the absence of bacterial contamination in Iraqi rice samples is due to the reason for the rapid arrival of rice from farms producing rice to the consumer without storing them for long periods in stores in addition to the good treatment of this product

MacConkey agar and Mannitol Salt Agar were used for detection the contamination of rice samples with coliform bacteria and *staph. aureus* bacteria, the high contamination was found with (Thailand, Philippines and Pakistan) rice samples in which the total coliform bacteria was ($3x10^3$, $2x10^3$ and $4x10^3$) CFU/ ml respectively while the low contamination with coliform was found in (Vietnam, India and Iraq) in which the total coliform bacteria was ($2x10^1$, $1x10^1$ and $1x10^1$) CFU/ ml respectively. A coliform bacterium is a fecal origin and has been involved in many foodborne diseases (Eni, *et al.,* 2010). (Thailand, Philippines and Pakistan) samples were found highly contaminated with *staph. aureus* bacteria and the high total *Staph. aureus* was ($7x10^5$, $11x10^4$ and $9x10^4$) CFU/ ml. However, the lowest percentage of contamination was found in the samples (Brazil, Japan and Iraq) where the total number of *staph. aureus* bacteria was ($6x10^2$, $3x10^1$ and 5x102) CFU/ ml respectively, (Taulo *et al.,* 2008) reported in his study about the contamination with these organisms are related to the poor hygiene. The reason for the presence of these numbers of coliform bacteria and *staph* bacteria in rice samples indicates the poor environmental conditions in which rice was treated from labor hygiene or pollution as a result of not using adequate thermal treatment of rice

A total number of yeasts and molds were observed in imported rice samples, unlike the Iraqi rice samples in which the absence of these fungi was observed, as the highest percentage of samples containing the highest number of fungi was observed in (Thailand, Philippines and Pakistan) samples in which the percentage of fungi was ($6x10^2$, $6x10^3$ and $11x10^3$) CFU/ ml respectively, In contrast to (Brazil, Japan and India) samples that had the lowest fungi contamination rate, the percentages were ($2x10^1$, $1x10^1$ and $3x10^1$) CFU/ ml respectively. The reason for the high humidity in the warehouses where rice is stored and the high water activity is a major cause of rice contamination in fungi as well as storage for long periods and the length of transportation for the consuming country leads to an increase in the number of fungi contamination numbers (JAY, 2000).

For detection of pathogenic bacteria, the spore forming *Bacillus cereus* bacteria was found in the most samples and recorded as positive except in Iraqi's and Japan rice samples, a Processing steps of drying, peeling, polishing and using of pyocyanin of *Pseudomonas aeruginosa* will reduce the number of *B.cereus* in the end product (Sarrias *et al.,* 2002) (Qasim, 2019), while the (Thailand, Philippines and Pakistan) were recorded as positive for *listeria monocytogenes*. The reason for the contamination of the samples with pathogenic bacteria is that the rice is not exposed to the appropriate temperature during sterilization and the presence of spores that bear high temperatures even during the rice cooking process. The *salmonella* bacteria was not found in any sample and recorded as negative result.

Table (4) represents the results of rice samples with chemical analysis which included analyzing the percentage of moisture and ash in addition to examining the ratio of protein, carbohydrates and fats.

Table 4: Chemical analysis results of rice samples

Samples	Moisture %	Ash %	Protein %	Fat %	Fiber %	Carbohydrate %
G1	11.3	1.1	5.6	0.4	1.4	81.2
G2	9.8	1.1	6.3	0.5	2.6	78.6
G3	9.6	1.2	5.4	0.4	2.2	80.5
G4	11.4	1.0	6.2	0.4	1.8	81.5
G5	12.4	1.1	6.6	0.5	1.6	80.3
G6	8.1	1.7	6.4	0.4	1.3	81.4
G7	9.3	1.2	6.7	0.4	2.7	80.7
G8	9.5	1.1	5.8	0.5	2.5	78.9
G9	10.6	1.3	6.6	0.5	1.9	79.6
G10	6.4	1.7	6.4	0.4	1.6	82.5
G11	8.7	1.1	6.3	0.4	2.4	80.1
G12	8.6	1.2	6.2	0.4	2.3	78.7
LSD	**2.152 ***	**0.478 ***	**0.789 ***	**0.162 NS**	**0.461 ***	**4.759 NS**

The highest percentage of moisture was recorded significantly in samples (Thailand, Philippines and Pakistan), where the percentage of each sample was (11.3, 11.4 and 12.4) consecutively, but it was found in Iraqi rice samples that they contain the lowest percentage of moisture, where the lowest percentage of moisture (6.4)%.

Rapid damage to grains, especially rice, occurs when storage is carried out in wet storage conditions, and this damage leads to a qualitative loss of rice, such as a change in the appearance of rice and the inability to germinate again in addition to being infected with insects and molds, as well as quantitative losses of the produced crop, and the percentage of this damage can be reduced either Providing good stores for storing rice or a good thermal treatment that reduces humidity.

It was found that there is a convergence in the ash content between the samples, where the proportions ranged from (1.0 to 1.7) % and the highest ash content was in Iraqi and Japanese rice. When the ash content is high in the samples, the percentage of moisture is lower and they contain more minerals.

There is also a large convergence percentage in the percentages of protein, fats, fibers and carbohydrates, as the protein percentages ranged between (5.4 to 6.7) %, the percentage of fats ranges from (0.4 to 0.5) %, the fiber ratio between (1.3 to 2.7) %, and the percentage of carbohydrates ranging from (78.6 to 82.5) %.

These results were close to the results obtained by (Ambreen *et al*., 2006) which found that the percentage of protein in the rice samples examined is (6.7) %, and unlike the results of (Xheng and Lan, 2007), which found that the protein ratio in the samples is (8.7) % which is higher than the ratio obtained in this study

Table (5) It shows the concentrations of heavy metals (arsenic, mercury, and cadmium) of rice samples, where the results revealed a statistically significant differences at the average level of $P < 0.05$ between the concentrations of heavy metals in the rice samples.

Table 5: Heavy metal concentration of rice samples

Samples	As conc. (mg/kg)	Hg conc. (mg/kg)	Cd conc. (mg/kg)
G1	1.8308	0.1591	0.1983
G2	0.0966	0.1171	0.0647
G3	0.3889	0.6825	0.0871
G4	1.5446	0.6640	0.1011
G5	1.4323	0.3674	0.1408
G6	0.5862	0.1891	0.0916
G7	1.0364	0.7984	0.1695
G8	0.0822	0.7981	0.1956
G9	0.2027	0.3071	0.1130
G10	0.0228	0.0408	0.1362
G11	0.2352	0.3052	0.0145
G12	0.2911	0.1381	0.0157
LSD	0.358 *	0.182 *	0.0355 *

The fact that the food contains a percentage of heavy metals in its contents is considered dangerous and unsafe for the consumer so the samples of this study were examined and ratios of heavy metals were obtained for the samples selected in this study

When examining rice samples with arsenic element, different and different ratios were found between the samples, where the ratios ranged between (0.0228 to 1.8308) mg/kg in rice samples (Thailand) and (Iraq), respectively, and these ratios are higher than the natural ratios accepted by (FAO/WHO, 2015) (0.2) ml/ kg and the results of our study were compared along with the results of previous studies of (Lu *et al*., 2010) and (Zhang *et al*., 2013), found that the concentration of arsenic was at (1.153) and (1.922) mg/ kg respectively, Which shows that there is an increase in the proportion of arsenic in samples. But in some other studies such as (Zeng *et al*., 2015) it was found that there is a ratio of arsenic sample in rice samples (0.062) mg/kg , which is a small percentage and is safe for the consumer.

While the examining rice samples with mercury, there was a difference in the ratios between the samples, where the highest percentage of mercury was (0.7984) mg/ kg in a sample (India) while the lowest it was (0.0408) mg/ kg in a sample (Iraq), and it is also higher than the normal proportions of World Health Organization and the Food and Agriculture Organization (FAO/ WHO, 2015) which should not exceed of 0.1 mg / kg, (Simon and Kimanya, 2016) notes a varying levels of mercury element which ranging from 0.231 and 0.726 mg/ kg , when watering the rice with wastewater, the mercury ratios in the samples will be was (0.922) mg / kg and these results were obtained by (Kibria *et al.,* 2012),

The concentration of cadmium in rice samples ranged between (0.1983 and 0.0145) mg/ kg in samples (Thailand and Iraq) in succession. These results came close to the results permitted by (FAO/ WHO, 2015) because the permissible limit is (0.2) mg/ kg. Some studies that have been proven by (Zeng *et al.,* 2015) and (Singh *et al.,* 2011) indicate that the percentage of cadmium in rice samples in (China) and (India) was (0.089) mg/ kg and (0.012) mg / kg in succession, but the ratio of this element in the sample (China) was (0.48) mg / kg and that (*Luo et al.,* 2012) indicated in his study.

By examining the rice samples, it was found that there are high levels of pollution with elemental mercury and arsenic in the imported and local samples (Iraqi). As these ratios were higher than the permitted rates, It can be attributed the cause of the contamination of Iraqi rice may be due to soil contamination, especially in central and southern Iraq, and the cause of contamination may be the result of residues of war remnants which remained for several years in the soil, which led to the contamination of soil and agricultural products for this region.

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