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Assessment of the Bacteriological Safety of Ready-to-Eat Nuts and Seeds at Retail Markets in Benghazi City

Masiugah A Alhadaad [1*], Salha F Ben Jweiref [2], Ayah R Shembesh [3]

[1]University of Benghazi, Faculty of Public Health, Department of Health Administration, Benghazi, Libya
[2]University of Benghazi, Faculty of Science, Department of Botany, Benghazi, Libya
[3] Infection control, Department of Pediatrics, Benghazi medical center, Benghazi, Libya

*Corresponding author: E-mail address: masiugha.alhaddad@uob.edu.ly

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ABSTRACT

Nuts and seeds have historically held a significant place in the dietary practices of numerous cultures and civilizations, owing to their high energy and nutritional value. Their composition is characterized by elevated levels of proteins, fats, and minerals, coupled with minimal water content; consequently, they exhibit heightened susceptibility to microbial infestation, particularly by bacteria. This study aims to investigate the microbiological contamination of ready-to-eat nuts and seeds at retail markets in Benghazi. The research included 100 samples of different nuts and seeds distributed as follows: almond 14 samples, cashew 14 samples, hazelnut14 samples, pistachio 14 samples, walnut 14 samples, peanut14 samples, pumpkin seeds 16. had been collected from four traditional markets, seven supermarkets selling unpackaged nuts, and eight companies selling packaged nuts and were selected for sampling during the period from January to March 2024. The result of this study showed that after bacterial identification, 90 bacteria grew and 10 bacteria did not grow. The highest percentage of bacteria was isolated from pumpkin seeds (17%), followed by almond (16%), cashew, pistachio, and walnut (14%), peanut (13%); the lowest percentage was hazelnut (12%).Nine genes of bacteria were found based on Gram stain, biochemical test and RENDER automated system; the distribution of bacteria in nuts and seeds was the highest found were *E.cloacae* (23%) followed by *K.pneumoniae* (20%), *S.aureus* (17%), *Bacillus cereus* (13%), *E. coli* (11%), *Bacillus pumilus* (8%), *C. freundii,* and *Bacillu subtilis* with prevalence (3%), and *A. baumannii* (2%).conclusion. This study reveals a high prevalence of bacterial contamination, with 90% of ready-to-eat nuts and seeds sold in Benghazi markets being affected, with pumpkin seeds showing the highest rate of isolation.

1. INTRODUCTION

Nuts are rich in polyunsaturated fatty acids, dietary fiber, and micronutrients such as vitamin E, magnesium, and selenium (Eneroth et al., 2017). The food industry incorporates nuts into its offerings, as they are deemed a healthful, convenient, and nutrient-rich snack option. Hence, they constitute an essential component in the advancement of the bakery and confectionery sectors. (Pluta et al., 2021). Nut eating is often linked to a decrease in risk factors for chronic illnesses.

Due to their fatty acid profiles, vegetable proteins, fibers, vitamins, minerals, carotenoids, and phytosterols with potential antioxidant action, nuts have been shown in numerous studies to be healthy foods despite their high calorie content (Souza et al., 2017(. Nuts and seeds have higher contents of proteins, fats, and minerals, and have a low water content; therefore, they are highly vulnerable to microbial attack, especially bacterial attack (Bhat and Siruguri, 2003). The nuts account for 7% of the total foodborne outbreaks, resulting in serious concerns from the food industry and local communities. Especially for tree nuts, such as almonds, walnuts, and pistachios, some outbreaks and recalls have been reported caused by bacterial pathogens (Zhang and Wang, 2017). Nuts and seeds prevent the growth of the majority of foodborne pathogens on their surfaces. Dry foods have been reported in the literature to support the long-term survival of bacterial foodborne pathogens (Escherichia coli O157:H7 and Listeria monocytogenes) on a variety of nut types. (Brar and Danyluk, 2018). (Ellahi et al., 2019) studied that Staphylococcus aureus, Salmonella enterica, Escherichia coli (E. coli), and Listeria monocytogenes are considered important foodborne pathogens and were isolated from nuts. Pathogenic bacteria (Bacillus mycoides, Escherichia coli, Staphylococcus aureus, and Bacillus cereus) were isolated from different nut products (Aldayel, 2020). Considering the fact that nut consumption is very high in our country, we deemed it necessary to conduct this study to investigate microbial contamination of nuts and seeds in this region. The present study was conducted in Benghazi, Libya. It aimed to investigate the bacteriological contamination of ready-to-eat unpacked and packed nuts and seeds at retail markets in Benghazi, isolate and identify the bacteria associated with these products using morphological and biochemical methods, and study the sensitivity of isolated bacteria to different antibiotics.

2. METHOD

2.1 Study design, setting, and Sample collection

A descriptive cross-sectional study was conducted from January to March 2024 at retail markets in Benghazi City, Libya. A total of 100 samples representing different types of nuts and seeds were collected from retail markets and subjected to microbiological examination for the detection and identification of bacterial contaminants.

Table 1. Distribution of the samples according to the type of nuts and seeds

Nuts	No of unpacked samples	No of packed samples		Percentage
		bagged	canned	
Almond	7	5	2	14%
Cashew	7	5	2	14%
Hazelnut	7	5	2	14%
Pistachio	7	5	2	14%
Walnut	7	5	2	14%
Peanut	7	5	2	14%
Pumpkin seeds	8	4	4	16%
Total	**100**			**100%**

2.2. Microbiological analysis and identification

The microbiological analyses were done following the methodologies described by the International Organization for Standardization (ISO) cited in (Silva *et al.,* 2022). Ten grams of each sample were homogenized separately with 90 mL of sterile peptone solution 0.1% and then stomached for 1 min. The containers containing the samples were incubated at 35 °C /48h. The samples were cultured on blood agar and MacConkey agar plates using a standard, sterile, calibrated loop, and then incubated at 37°C for 24-48 hours. After that Bacteria growth was examined (Brar *et al.,* 2018). Isolates were identified by colony morphology, Gram staining, and conventional biochemical testing, including catalase, coagulase, oxidase, urease, and citrate tests, as appropriate, and susceptibility analysis.

2.3 Antimicrobial susceptibility

Antimicrobial susceptibility was assessed by the agar disk-diffusion method on Mueller-Hinton agar. The antibiotic panel included Gentamicin, Cefotaxime, Amikacin, Ciprofloxacin, Cefepime, Ertapenem, Cefoxitin, Amoxicillin Imipenem, Levofloxacin, Meropenem, Chloramphenicol, Aztreonam, and Erythromycin. Following incubation, the diameter of the inhibition zones surrounding each disc was precisely measured in millimeters according to Clinical Laboratory Standards Institute Guideline (CLSI, 2023). Minimum inhibitory concentrations (MIC) were estimated according to the company's instructions. Final detection and confirmation were done using the automated render machine system.

2.4 Statistical analysis

Calculating the proportion of bacterial and fungal occurrences. The analysis was conducted using SPSS (Statistical Package for the Social Sciences). Data were summarized using counts and percentages.

3. ETHICAL APPROVAL

Ethical approval was not required for this study because it involved only the microbiological examination of commercially available ready-to-eat nuts and seeds purchased from retail markets in Benghazi City. The study did not involve human participants, animals, human tissue, or personal data

4. RESULT

4.1 Distribution of contamination according to Gram stain

This study showed that 90%) out of 100 were positive for bacteria; Gram-negative bacteria accounted for the highest proportion at 62%, followed by Gram-positive bacteria at 28%, and no growth at 10%.

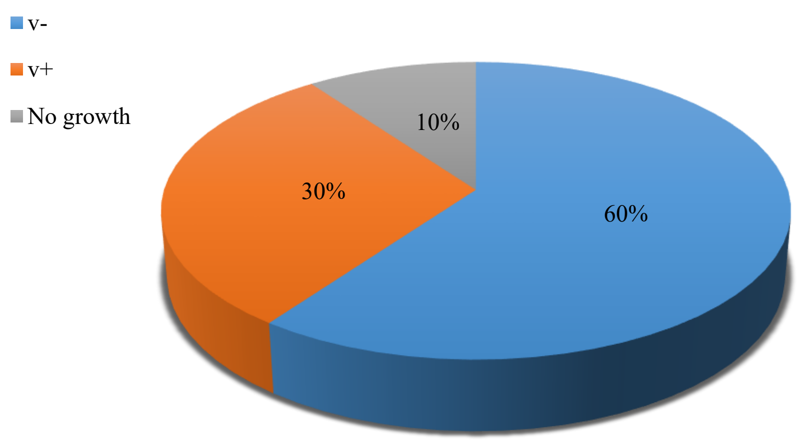

Figure (1): Distribution of bacterial contamination according to Gram stain

4.2 Percent of Bacteria isolated from all nuts and seeds

The highest prevalence of bacteria isolated from all nuts was *Enterobacter cloacae*, at 23% according to the data presented in Table 2, followed by *Klebsiella pneumoniae,* 20%, and *Staphylococcus aureus,* 17%. *Bacillus cereus* is also prevalent at 13%. Other bacteria, such as *Escherichia coli,* account for 11%, while *Bacillus pumilus* is present at 8%. Lesser prevalence is observed for bacteria *Citrobacter freundii* 3%) and *Bacillus subtilis* 3%. The lowest percentage is observed for *Acinetobacter baumannii,* 2%.

Table 2. Percentage of bacteria isolated from all nuts and seeds

Bacteria	Percentage
A.baumannii	2 %
Bacillus ceres	13%
Bacillus pumilus	8 %
Bacillus subtilis	3 %
C.freundii	3 %
E.cloacae	23%
E. coli	11%
K.pneumoniae	20 %
S. aureus	17%

4.3 Percentage of the total contamination of bacteria among all nuts and seeds

Pumpkin seeds have the highest percentage at 17%, according to the data presented in Table 3, followed by almonds at 16%. Cashews, pistachios, and walnuts each hold an equal share of 14%. Peanuts come next at 13%, while hazelnuts have the lowest percentage at 12%. This distribution highlights a relatively balanced presence of various nuts, with pumpkin seeds and almonds being the most prominent.

Table 3: Percentage of total contamination of bacteria among all nuts and seeds

Nuts	Percentage
Almond	16 %
Cashew	14 %
Hazelnut	12 %
Pistachio	13 %
Walnut	14 %
Peanut	14 %
Pumpkin	16 %

4.4 Distribution of bacteria among packed and unpacked nuts and seeds

Escherichia coli was found in several nut types in almonds and walnuts. The genus *Bacillus*, including *B. cereus, B. pumilus, and B. subtilis*, was also widely distributed in peanuts and pumpkin seeds. *Klebsiella pneumoniae*, *Citrobacter freundii,* and *Acinetobacter baumannii* were detected at lower frequencies. *Staphylococcus aureus* was recovered from almonds and pumpkin seeds. Unpacked samples consistently showed higher bacterial prevalence than packed ones, as shown in Table 4.

Table 4. Distribution of bacteria among packed and unpacked nuts and seeds

Nuts / Microorganism	Almond		Cashew		Hazelnut		Pistachio		Walnut		Peanut		Pumpkin seeds	
	Un P	P	Un P	P	Un P	P	Un P	P	Un P	P	Un P	P	Un P	P
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
A.baumannii					1.1			1.1						
Bacillus cereus			1.1		2.2								5.5	4.4
Bacillus pumilus					2.2		1.1				2.2	2.2		
Bacillus subtilis				3.3										
C. freundii							1.1		2.2					
E. cloacae	2.2	4.4			2.2	2.2	3.3		2.2		2.2	2.2	2.2	
Escherichia coli	3.3		2.2				1.1			4.4				
K. pneumonia	1.1		2.2	3.3		2.2	1.1	4.4		1.1	2.2	2.2		
S. aureus	1.1	3.3	2.2						3.3	1.1	1.1		1.1	2.2

- Un p = unpacked P = packed

4.5 Antibiotic susceptibility among Gram-negative bacteria

Most of the isolates show high susceptibility (S) to key broad-spectrum antibiotics such as Imipenem, Meropenem, and Levofloxacin, indicating their continued effectiveness in treatment. Notably, A. baumannii is fully susceptible to all tested antibiotics. However, E. cloacae also shows resistance to several beta-lactam antibiotics, including Cefotaxime, Cefepime, and Amoxicillin, as shown in Table 5.

Table 5 Antibiotic susceptibility among Gram-negative bacteria

Bacteria / antibiotic	*A.baumannii*	*C.freundii*	*E. coli*	*E.cloacae*	*K. pneumoniae*
Gentamicin	S	S	S	S	S
Cefotaxime	S	S	S	R	S
Amikacin	S	S	S	S	S
Ciprofloxacin	S	S	S	I	S
Cefepime	S	S	I	R	S
Ertapenem	I	S	S	I	S
Cefoxitin	I	I	R	I	S
Amoxicillin	I	I	S	R	S
Imipenem	S	S	S	S	S
Levofloxacin	S	S	S	S	S
Meropenem	S	S	S	S	S
Chloramphenicol	I	S	S	S	S
Aztreonam	I	S	R	R	S
Erythromycin	I	I	R	I	S

4.6 Antibiotic susceptibility among Gram-positive bacteria

The results show variable susceptibility across the bacterial species tested. Most of the Bacillus species demonstrate good sensitivity to meropenem, amikacin, and Ciprofloxacin. However, some intermediate responses (I) were observed, particularly in B. pumilus and B. subtilis. Staphylococcus aureus shows strong susceptibility to a majority of tested antibiotics, including Gentamicin, Amikacin, Ciprofloxacin, Levofloxacin, Erythromycin, and Meropenem. However, resistance is noted to Amoxicillin, and some degree of intermediate susceptibility is observed in others. This pattern raises concerns regarding limited treatment options and may require further testing or the use of alternative antibiotics, as shown in Table 6.

Table 6. Prevalence of antibiotic susceptibility among Gram-positive bacteria

Bacteria / antibiotic	*Bacillus cereus*	*Bacillus pumilus*	*Bacillus subtili*	*Staph. Aureus*
Gentamicin	R	I	I	S
Amikacin	S	I	I	S
Ciprofloxacin	S	I	S	S
Ertapenem	I	R	I	I
Cefoxitin	S	R	R	I
Amoxicillin	R	S	R	R
Imipenem	S	I	I	I
Levofloxacin	I	I	I	S
Chloramphenicol	I	I	I	S
Erythromycin	R	I	S	S
Meropenem	S	S	S	S

5. DISCUSSION

The research found that pumpkin seeds had the highest bacterial prevalence at 17%, followed by almonds at 16%, cashews, peanuts, walnuts, and pistachios at 14%, and hazelnuts at 13%. These findings are supported by the fact that Azizkhani *et al.* (2020) discovered that hazelnuts had less microbial contamination. Hazelnuts may be less vulnerable to contamination due to their lower moisture content, which promotes preservation and inhibits the growth of bacteria. Sejinyet *et al.* (1989) found that walnuts had the highest average microbial contamination, while cashews and pistachios had fewer microbes. Additionally, this study's results showed that canned and tightly sealed nuts exhibited no bacterial or fungal growth, whereas bagged nuts did. These findings align with previous research indicating that packaging methods significantly influence microbial growth in nuts. where Naeem *et al.* (2022) reported that storage in airtight containers effectively inhibits microbial contamination by limiting exposure to air and moisture. Porous or loosely sealed packaging increases the risk of fungal proliferation due to higher humidity and oxygen levels (Masamba, 2024). These findings highlight the critical role of packaging integrity in preserving the microbial safety of nuts. Cans or airtight containers seem to be a dependable way to prolong shelf life and stop spoiling, which is important for product quality.

The results of this study identified nine types of bacteria: *A.baumannii* (2%), *Bacillus cereus* (14%), *Bacillus pumilus* (8%), *Bacillus subtilis* (3%) *C.freundii* (3%) *E.cloacae* (22%), *E. coli* (11%), *K.pneumoniae* (22%), and *Staph. Aureus* (15%). The most prevalent type of bacteria among nuts and seeds are E. cloacae and K.pneumonia at a rate of 22% For each. This wide range is in line with E. cloacae's pervasiveness in the environment, which includes soil, water, and plant matter. Its existence may be an indicator of general unhygienic conditions or fecal contamination (Rainey *et al* 2015). Unpacked and packed pumpkin seeds were contaminated with Bacillus cereus, Bacillus licheniformis, Enterobacter cloacae, and Staphylococcus aureus, with prevalence of 7.7%, 2.2%, 4.4%, and 3.3%, respectively. Omoya *et al.* (2024) reported similar bacterial contamination with Bacillus cereus (22.07%), while Naeem *et al.* (2022) .

recorded Enterobacter spp. (25.9%), Staphylococcus spp. (33.7%), and Bacillus spp. (8.3%). One important foodborne pathogen that can produce heat-stable enterotoxins is S. aureus. Because it can cause staphylococcal food poisoning, its presence in nuts, particularly at higher levels, poses a major risk to food safety (Lelieveld & Motarjemi 2013).

6. CONCLUSION

Overall, various types of nuts and seeds were found to be contaminated with bacteria. Hazelnuts had the lowest levels of contamination, while pumpkin seeds and almonds had the highest bacterial prevalence. *Bacillus species, Escherichia coli, Enterobacter cloacae, Klebsiella,* and *Staphylococcus aureus* were the most frequently isolated bacteria in all samples. Nut species differed in their microbial prevalence, which was probably impacted by their moisture content, chemical makeup, and storage conditions. Microbial safety depended heavily on the integrity of the packaging. While bagged or loosely packed nuts showed considerable contamination, canned and tightly sealed nuts showed no microbial growth. This confirms earlier research showing that airtight packaging successfully prevents microbial growth by reducing exposure to moisture and oxygen. Strict hygiene procedures, appropriate roasting, and airtight packaging are crucial to preserving the safety and quality of ready-to-eat nuts because seasoning and post-processing procedures can reintroduce microbial contaminants.

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