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Bacterial Contamination of Libyan Banknotes in Circulation in Hun City

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التلوث البكتيري للعملات الورقية الليبية المتداولة في مدينة هون - ليبيا

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Abstract:
Paper banknotes are contaminated with pathogenic microorganisms such as bacteria and fungi, as well as viruses. They can act as environmental vectors for diseases caused by these pathogens to many who handle them. This study aimed to isolate and identify bacteria from Libyan banknotes circulating in Hun city. 42 samples of different banknote denominations (1 dinar, 5 dinars, and 10 dinars) were randomly collected from various locations in the city. These samples were compared using the SPSS statistical software and ANOVA. Significant differences were found between the different banknotes studied. The results showed that 100% of the studied samples were contaminated with bacteria. Gram-negative bacteria were the most prevalent, representing 53% of the total bacterial isolates, while Gram-positive bacteria were less common, representing 47% of the total isolates. The most prevalent Gram-negative bacteria were Escherichia coli (31.6%) and Staphylococcus spp. (25%). The 1-dinar note showed the highest bacterial contamination rate at 38.2%, while the 5-dinar note showed 35% contamination. The 10-dinar note had the lowest contamination rate at 26%.

Keywords: Gram-negative and Gram-positive bacteria, banknotes, contamination, Gram stain.

الملخص:
تتلوث العملات النقدية الورقية بالاحياء المجهرية الممرضة، كالبكتيريا والفطريات، بالإضافة إلى الفيروسات. حيث يمكن أن تُشكل هذه العملات النقدية الورقية ناقلاً بيئياً للأمراض التي تسببها هذه الكائنات الممرضة للعديد ممن يتعاملون معها. من هنا جاءت هه الدراسة التي تهدف إلى عزل وتحديد البكتيريا من العملات الورقية الليبية المتداولة في مدينة هون. حيث جُمعت 42 عينة بطريقة عشوائية من فئات العملات النقدية الورقية المختلفة (فئة 1 دينار، 5 دينار، و10 دينار) من أماكن مختلفة بالمدينة. وتمت المقارنة بين هذه العينات باستخدام برنامج SPSS الإحصائي، وكذلك برنامج تحليل التباين ANOVA. حيث وُجد أن هناك فروقاً معنوية بين العملات الورقية النقدية المختلفة التي تمت دراستها. حيث أظهرت النتائج تلوث العينات التي تمت دراستها بنسبة 100% بالبكتيريا. وكانت البكتيريا السالبة لصبغة الجرام هي السائدة والأكثر انتشاراً، بنسبة 53% من إجمالي العزلات البكتيرية، بينما كانت البكتيريا الموجبة لصبغة الجرام الأقل شيوعاً، حيث مثلت 47% من إجمالي العزلات البكتيرية. حيث كانت أكثر أنواع البكتيريا السالبة لصبغة الجرام انتشاراً هي Escherichia coli بنسبة 31.6%، وبكتيريا Staphylococcus بنسبة 25%. حيث سجّلت فئة 1 دينار أعلى معدل تلوث بكتيري

بنسبة 38.2%، بينما سجلت فئة 5 دينار نسبة تلوث بلغت 35%. أما فئة 10 دينار فسجلت أدنى معدل تلوث بنسبة 26%.

الكلمات المفتاحية: البكتيريا السالبة والموجبة لصبغة الجرام، العملات الورقية النقدية، التلوث، صبغة الجرام، هون.

Introduction:

Bacteria are among the most widespread living organisms in nature. They are found in soil, air, and water; in certain foods; on the surface of the human body and within the intestines; and they also appear in animal secretions as well as in a variety of aquatic, terrestrial, and extreme environments. It can be said that bacteria thrive in environments that provide them with moisture, suitable temperatures, nutrients, and other factors necessary for their growth.

The widespread dissemination of bacteria is due to their small size, rapid division, diverse sources of nutrition, and the wide range of environments in which they can live (Abdullah Al-Issa 2004-2005). Paper currency, especially lower denominations, is used for all transactions in daily life, for exchanging goods and services worldwide. Due to their frequent use and circulation, these banknotes become contaminated with pathogenic bacteria (Umeh et al., 2007). During handling and circulation, banknotes pass through the hands of many people from all walks of life and age groups, including children. These banknotes, particularly older paper currency and smaller denominations, which are more frequently handled because they are dealt with more often than coins (which are less likely to transmit contamination), become contaminated.

Paper currency is typically made from a blend of 75% cotton and 25% linen (Brady & Kelly, 2002). Generally, banknotes, especially paper currency, become contaminated by droplets expelled during sneezing or coughing, or by contact with contaminated skin, wounds, or hands contaminated with saliva, nasal secretions, or feces. They also become contaminated by contact with contaminated materials and surfaces. This means they pick up pathogens from the environment during their long journey from manufacturing to storage (Ahmed et al., 2010).

Even the health aspects of banknotes are constantly and discreetly examined in laboratories. Due to concerns about carcinogens, for example, central banks have replaced the usual animal gelatin used as the surface layer of banknotes with synthetic products. This has extended the lifespan of banknotes but also increased their susceptibility to bacterial contamination (Klaus Bender, 2015). People often do not wash their hands after handling money, and many may actually contract several serious diseases transmitted through cash handling. Thus, banknotes are a latent risk factor that threatens public health. Some studies have proven the contamination of banknotes with illicit drugs such as cocaine and heroin (Jenkins, 2001; Lavins et al., 2004).

Since banknotes are among the most frequently used items among people, bacteria and viruses can easily be transmitted from person to person through them. Studies indicate that some viruses (such as SARS) can remain active on surfaces, including banknotes, for a certain period that may reach 24 hours or more sometimes. Recently, the People's Republic of China took precautionary measures by holding banknotes for 24 hours to reduce the likelihood of the virus being present on their surfaces when they are put back into circulation (Klaus Bender, 2015).

Aim of the study:

This study aims to isolate and identify bacteria from Libyan banknotes circulating in Hun city, and to determine their percentage and the significant differences between the different denominations of banknotes.

Materials and Methods:

Isolation and Identification of Bacteria:

42 samples were randomly collected from all different denominations of banknotes (1 dinar, 5-dinar, 10 dinar) circulating in Hun city, with 14 banknotes of each type, from different sectors (vegetable shops, meat, food stores) during the period from January to July 2025. They were placed in sterile plastic bags, then placed in Tryptone soya medium and shaken for 10 minutes. Using sterile forceps under sterile conditions, the banknotes were picked up, and the tubes containing the bacterial suspension were placed in the incubator for 24 hours at a temperature of 37 °C. Afterwards, these tubes were removed from the incubator, and 1 ml of the suspension was taken. Then, isolation was performed using the Streak Plate Method (Quadrant Streaking Method) with injection needles, and the suspension was spread on Petri dishes containing Blood Agar and MacConkey Agar culture media. The dishes were then moved in a circular motion until the solution was completely distributed. Afterwards, these dishes were transferred to the incubator at 37 °C for 24 hours. Afterwards, the dishes were removed from the incubator, and the growing colonies were identified based on the apparent morphological characteristics of these colonies. Also, the counting of the growing bacterial colonies on the dishes was performed after their isolation using a bacterial counting device.

Diagnostic Tests:

Gram Staining:

Gram staining is one of the most important methods used to study bacteria and differentiate between their types. This method was introduced by the Danish scientist Christian Gram in 1884. In this method, the thin smear of spread bacteria on a glass slide, after being left to air dry and then fixed by heat, is immersed to crystal violet stain (as the basic stain), then to iodine solution (as a stabilizer), then to ethanol (as a decolorizing agent), and finally to safranin or dilute fuchsin (as the counter-stain).

Cells that retain the basic stain color (violet) after the application of the decolorizing agent are called Gram-positive. Cells that lose the basic stain and take on the counter-stain color (reddish) are called Gram-negative. Therefore, using the Gram staining method, bacteria can be divided into two groups: Gram-positive and Gram-negative (El-Sawy, 2005).

Biochemical Tests:

Oxidase Test:

- **Use:** For Gram-negative bacteria to detect the presence of the cytochrome oxidase enzyme, which converts the colorless oxidase reagent into a dark purple compound.
- **Procedure:** Moisten the test paper with the oxidase reagent and then spread the Gram-negative bacterial colony onto it using a wooden stick.
- **Result:** The test is positive if the colony turns a dark purple color on the paper within a few seconds. The test is negative if there is no color change or a very slow color change occurs (within minutes).

Catalase Tests:

- **Use:** This test detects the presence of the catalase enzyme in Gram-positive cocci, which breaks down hydrogen peroxide and releases oxygen (positive for Staphylococcus).
- **Procedure:**

1. Using a sterile, refrigerated loop, take a small portion of the bacterial colony from the Petri dish and spread it onto a glass slide.
2. Put a drop of hydrogen peroxide onto a glass slide.
3. Carefully invert the slide onto the slide and move it up and down. Observe the rapid formation of bubbles, which indicates the release of oxygen from the hydrogen peroxide. Observe the rapid formation of bubbles, which indicates the release of oxygen from the hydrogen peroxide.

- **Result:** The test is positive if bubbles are produced rapidly on the slide. If no bubbles form or their formation is slow, the test is negative.

Urease Test:

- **Use:** To detect certain types of bacteria that possess the urease enzyme, which breaks down amino bonds.
- **Procedure:** Cultivate the bacteria densely on an inclined surface and incubate at 37°C for 4 hours (positive results may not appear until after 4 days).
- **Result:** A positive result is indicated by a change in the medium from yellow to a dark color, signifying a shift towards alkalinity.

Statistical Analysis:

Using the Excel statistical program to perform the statistical operation, as well as using the ANOVA one-way variance program to determine if there were significant differences between the number of bacteria in the paper currency under study at a significance level of 0.05.

Table (1): Distribution and percentages of bacteria isolated from different banknote denominations:

Type of isolated bacteria	Different banknote denominations			Total
	1 LD denomination	5 LD denomination	10 LD denomination	
Staphylococcus Spp. Count %within	8 (34,87)	2 (9,5)	5 (31,25)	15 (25)
Streptococcus count %within	2 (8,70)	1 (4,8)	0 (0,0)	3 (5)
Bacillus cereus count %within	3 (13,0)	6 (28,6)	1 (6,25)	10 (16,67)
Serratia count %within	0 (0,0)	2 (9,5)	1 (6,25)	3 (5)
Escherichia coli count %within	5 (21,74)	7 (33,3)	7 (34,75)	19 (31,67)
Klebsiella pneumonia Count %within	4 (17,39)	2 (9,5)	1 (6,25)	7 (11,67)
Proteus spp count %within	1 (4,3)	0 (0,0)	0 (0,0)	1 (1,67)
Enterobacter aerogenes count %within	0 (0,00)	1 (4,8)	1 (6,25)	2 (3,33)
Total Count %within	23 100	21 100	16 100	60 100

Table (2): Distribution and percentages of bacteria isolated from different isolation sites:

Type of isolated bacteria		Isolation sites			Total
		Food	Meat	Vegetables	
Staphylococcus spp	count	5	3	7	15
%within		(33,33)	(16,67)	(25,92)	(25)
Streptococcus	count	0	1	2	3
%within		(0,0)	(5,56)	(7,40)	(5)
Bacillus cereus	count	4	4	2	10
%within		(26,67)	(22,22)	(7,40)	(16,67)
Serratia	count	0	2	1	3
%within		(0,0)	(11,11)	(3,70)	(5)
Escherichia coli	count	4	6	9	19
%within		(26,67)	(33,33)	(33,33)	(31,67)
Klebsiella pneumonia	Count	1	1	5	7
%within		(6,67)	(5,56)	(18,51)	(11,67)
Proteus spp	count	0	0	1	1
%within		(0,0)	(0,0)	(3,70)	(1,67)
Enterobacter aerogenes	count	1	1	0	2
%within		(26,67)	(5,56)	(0,0)	(3,33)
Total	Count	15	18	27	60
%within		100	100		100,0

Results:

Bacteria isolated from different banknote denominations:

The study of bacterial contamination of some denominations of Libyan banknotes in circulation in Hun City, which consisted of 42 bacterial isolates, yielded results showing that all samples studied were 100% contaminated with bacteria, and there were significant differences between the results obtained for the various denominations of Libyan banknotes.

This study showed that the isolated bacteria belong to 8 bacterial genera. It was found that the percentage of Gram-negative bacteria is predominant, accounting for 53% of the total bacterial isolates. Specifically, the isolates were comprised of Serratia at 5%, Escherichia coli at 31.6%, Klebsiella pneumonia at 11.67%, Proteus at 1.67%, and Enterobacter Spp. at 3.3%. While the Gram-positive bacteria were the least common, accounting for 47% of the total isolates, specifically: Staphylococcus Spp. at 25%, Streptococcus pneumonia at 5%, and Bacillus cereus at 16.67%.

It was found that the 1-dinar denomination recorded the highest bacterial contamination at 38.2%. The most present genera were Staphylococcus Spp. at 34.78%, Escherichia coli at 21.7%, followed by Klebsiella pneumonia at 17.3%, followed respectively by Bacillus cereus at 13.0%, Streptococcus pneumonia at 8.69%, and the least present was Proteus at 4.34%. The bacterial genera Enterobacter Spp. and Serratia were not present in this denomination.

As for the 5-dinar currency denomination, the results showed that bacterial contamination in it was 35%, with the highest percentage being Escherichia coli at 33.33%, followed by Bacillus cereus at 28.57%, followed respectively by the bacterial genera Staphylococcus Spp., Serratia and Klebsiella pneumonia at equal percentages of 9.52%, while the bacterial genus Proteus spp. was not present in this denomination.

As for the 10-dinar denomination, it was the least contaminated paper currency denomination at 26%, with the highest contamination rate recorded for Escherichia coli at 43.75%, followed by Staphylococcus Spp .at 31.25%, and the bacterial genera Bacillus cereus, Serratia, Klebsiella pneumonia, and Enterobacter Spp. at an equal rate of 6.25%. As for Streptococcus pneumonia and Proteus, they were not found in this denomination.

The results of bacterial species isolation showed bacterial contamination in all isolation sites at 100%. Vegetable shops recorded the highest presence of bacterial contamination at a rate of 45%, followed by meat shops (butchers) at a rate of 30%. The lowest presence of bacterial contamination was observed in bacteria isolated from food shops at a rate of 25%. There are no significant differences between the isolation sites.

The results of bacterial isolates from vegetable shops showed that Escherichia coli bacteria recorded the highest presence with a presence rate of 33.3%, followed by Staphylococcus Spp. bacteria with a presence rate of 25.9%, followed by Klebsiella pneumonia bacteria with a presence rate of 18.5%, followed by Streptococcus pneumonia, Bacillus cereus bacteria with a presence rate of 7.4%, followed by Serratia, Proteus Spp. bacteria with a presence rate of 3.7%. In contrast, the bacterial genus Enterobacter Spp. was not present in these locations of isolates.

While the results obtained from bacteria isolated from meat shops (butchers) show that Escherichia coli was the most prevalent bacterial species, with 6 isolates, representing 33.3%. Bacillus cereus bacteria were recorded with 4 isolates, representing 22.2%. The results showed that Staphylococcus Spp. bacteria were represented by 3 isolates, accounting for 16.7%, while Serratia bacteria were present in 2 isolates, representing 11.1% of the total. This was followed by Klebsiella pneumoniae and Enterobacter Spp. bacteria were each represented by only one isolate, accounting for 5.6%, while Proteus Spp. bacteria were not recorded in these shops.

The study of bacteria isolated from food shops with the least bacterial contamination found that Staphylococcus Spp. was the most contaminated bacterial genus, with 5 bacterial isolates, and was present at a rate of 33.3%. This was followed by Bacillus cereus and Escherichia coli, with 4 isolates each at a rate of 26.6%. Finally, Klebsiella pneumonia and Enterobacter Spp. were found in only one isolate at a rate of 67.6%, while Proteus Spp. and Serratia were not detected in these shops.

Discussion:

Banknotes are among the most frequently used items in circulation, making them a suitable environment for the transmission of microbes, especially those capable of surviving for extended periods under varying environmental conditions. Banknotes are often made from cotton fibers, sometimes up to 100%, or from a blend of cotton and linen in ratios of 75% to 25%, or less. These porous organic materials allow bacteria to adhere to and multiply on their surfaces more readily than banknotes made from plastic polymers (Cozorici et al., 2022). For this reason, some countries, such as Australia and Canada, have sought to replace traditional paper money with polymer banknotes, as they are less susceptible to microbial contamination (Rafiei et al., 2023).

The results of this research showed that all samples of Libyan banknotes circulating in the city of Hun were 100% contaminated. These results are consistent with those obtained by Huda (2012), confirming that the circulation of money is an effective means of transmitting microbes between individuals. Using two types of bacterial media, 42 bacterial isolates were obtained by direct isolation, belonging to 8 different genera. Five of these are Gram-negative (53%), and three are Gram-positive (47%). These results indicate the predominance of Gram-negative bacteria, which is consistent with the findings of similar studies in Libya (Al-Asawi et al., 2019) and Houma et al. (2013), as well as in Jordan (Shehab, 1998). These studies confirmed that Gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, and Enterobacter spp. are the most prevalent on banknotes due to their association with fecal and aquatic sources of contamination.

In the present study, the results revealed that the one-dinar note was the most contaminated compared to higher denominations, at 38.2%, followed by the five-dinar note at 35%, and then the ten-dinar note at 26%. This demonstrates that the lower the denomination, the higher the contamination rate, a finding supported by this study. The higher contamination rate of the one-dinar note is attributed to its frequent circulation and exposure to higher levels of handling in markets and public transportation. Other studies have reported similar results, such as Al-Ghamdi et al. (2011) in Saudi Arabia and Butt Malik S (2015) in Pakistan, where smaller denominations were found to be the most contaminated due to their rapid circulation in daily transactions.

As for the isolated bacterial species, Escherichia coli was the most common, an enterobacterium indicative of direct fecal contamination. This is consistent with the findings of the study by Ahmed et al. (2010) in Bangladesh and the study by Hanash S. et al. (2015), which demonstrated the presence of E. coli in almost all samples of banknotes circulating in Taiz, Yemen. This widespread presence has been attributed to fecal contamination, stemming from the prevalence of sewage in the city, poor infrastructure and sanitation, and poor health practices or personal hygiene, such as washing hands after using the toilet or handling food.

Other Gram-negative bacteria, such as Klebsiella pneumoniae, Proteus spp., Enterobacter spp., and Serratia spp., were also isolated. These findings are similar to those of studies by Al-Asawi et al. (2019) in Misrata, Libya, and Huda (2012) in Iraq. These species share the characteristic of being commonly found in the digestive tract, soil, and wastewater, indicating that the sources of contamination in banknotes are multiple and include hands, unclean surfaces, and the general environment.

Conversely, the study revealed the presence of Gram-positive bacteria such as Staphylococcus spp., Streptococcus pneumoniae, and Bacillus cereus. Staphylococcus aureus is one of the most common bacteria found on human skin and in the nose, so it can easily be transferred to banknotes through direct contact after rubbing the nose, coughing, or sneezing. This finding is similar to that of Mohialdeen R. & Kirah A. (2024) and Butt A. Malik S. (2015), which showed that Staphylococcus species are among the most common bacteria contaminating banknotes because they are part of the normal flora of the human body. In this regard, the presence of Bacillus cereus spp., a spore-forming bacterium, is explained by its high resistance to environmental conditions and dryness, which allows it to survive on banknotes for extended periods (Zoë R. Barandongo et al., 2023).

Although the results regarding the types of bacteria detected are similar to many previous studies, both in Libya and abroad, the overall contamination rate in this study (100%) is relatively higher than that recorded in other studies, such as the study by Mohialdeen R. & Kirah A. (2024) in Tunisia (93%) and the study by Ofoedu CE et al. (2021) in Nigeria (81.7%). This difference may be attributed to variations in climatic conditions, levels of personal hygiene, handling methods, and the nature of the materials used in printing Libyan banknotes.

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