

## Isolate Bacterial Contamination From Iraqi Currencies Notes and Determination of Their Resistance to Antibiotic

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### ABSTRACT

**Background :** Paper currencies are widely exchanged, and because they are exchanged from one place to another and from one person to another, they can serve as ways of transmission of multi-resistant bacterial diseases.

**Objective :** The aim of the study is to isolate bacterial contamination in Iraqi currency and determine their antibiotic resistance in Mosul City.

**Materials and Methods :** The 30 samples of paper currency were collected from five local sources from the population of the community and examined using bacterial cultivation. The developing colonies on the selective culture media were identified using the gram stain and the biochemical tests. The bacterial isolates resistance for twelve standard antibiotics were tested according to clinical and laboratory institute guidelines.

**Results :** out of 30 samples of collected paper currencies, 27 (90%) paper currency were contaminated with bacteria, 16 paper currency containing different mixed culture isolates. 48 bacterial isolates ,37 (77%) Gram-negative bacteria and 11 (22.91 %) Gram-positive bacteria were identified belonging to Seven bacterial species, out of 48 bacterial species isolated, the *E. coli* represent (11) (36.67%); *Salmonella* (11) (36.67%); *Klebsiella* (7) (23.34%), *Coagulase Negative staphylococcus* (7) (23.34%), *Staph. aureus* (4) (13.34%), *Pseudomonas. aeruginosa* (4) (13.34%), and *Proteus. spp* (4) (13.34%). All the isolated strains revealed high resistance to several antibiotics such as Bacitracin, Methicillin, Cefixime, Erythromycin, Azithromycin, while all isolated showed susceptibility of (100%) to Impenime, moderate to sensitively to Amoxiclav and Nitrofurantoin, Trimethoprim-Sulfamethoxazole,

**Conclusion :** The Iraqi currency notes in circulation are contaminated with several bacteria species, the majority of which are resistant to commonly used antibiotics, posing risks and public health exposures to the community and individuals handling currency notes.

**Keywords :** Paper currencies , bacteria; antibiotic , culture , resistance , gram-negative , gram-positive .

### عزل التلوث البكتيري للعملة العراقية وتحديد مقاومتها للمضادات الحيوية

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#### الخلاصة

**الخلفية العلمية للبحث :** يتم تبادل العملات الورقية على نطاق واسع، ولأنها يتم تبادلها من مكان إلى آخر ومن شخص إلى آخر، فإنها يمكن أن تكون بمثابة طرق لانتقال الأمراض البكتيرية ذات المقاومة المتعددة للمضادات الحيوية .

**اهداف البحث :** تهدف الدراسة الى عزل الانواع البكتيرية المسببة للتلوث البكتيري بالعملة العراقية وتحديد مقاومتها للمضادات الحيوية في مدينة الموصل.

**المواد وطرق العمل :** تم جمع ثلاثون نموذجا من عملة عراقية ورقية مختلفة من خمسة مصادر محلية من المجتمع وتم زرعها على الاوساط الزرعية الانتخابية المختلفة وشخصت المستعمرات النامية باستخدام صبغة كرام والاختبارات الكيموحيوية ثم تم فحص حساسية العزلات تجاه مجموعة من المضادات الحيوية القياسية اعتمادا على ارشادات المختبرية السريرية التابعة لمؤسسة

CLSI

**النتائج :** من مجموع ثلاثون نموذجا من عملة ورقية كانت ٢٧ (٩٠%) منها ملوثة بالبكتيريا، و ١٦ عملة ورقية كانت ملوثة بعدة عزلات بكتيرية مختلفة. اذ تم عزل وتشخيص ٤٨ عزلة كانت تابعة لسبعة انواع بكتيرية مختلفة واحتلت بكتريا *Escherichia coli*، وبكتيريا *Salmonella* اعلى نسبة عزل بلغت (36.67%) (11) وعزلت بكتريا *klebsiella* و *pseudomonas aeruginosa* بكتريا *Coagulase Negative Staphylococcus* بنسبة (23.34%) (7)، في حين عزلت بكتريا *Proteus. Spp*, (13.34%) (4). *staphylococcus aureus* و مضادات حيوية (الببيراسيلين ، ميثيسيلين، أزيثروميسين ، سيفيكسيم ، ايريثرومايسين) و أظهرت العزلات حساسية متوسطة لـ (نايتروفيرانشن ، تريميثوبريم-سلفاميثوكسازول ، أموكسلاف) بينما كانت ذات حساسية بنسبة ١٠٠% لمضاد ايمبيينيم .

**الاستنتاجات :** إن العملات الورقية العراقية المتداولة في مدينة الموصل كانت ملوثة بأنواع بكتيرية مختلفة التي أغلبها كانت مقاومة لأغلب المضادات الحيوية مما يشكل مخاطر للصحة العامة للأفراد الذين يتداولون هذه العملات .

**الكلمات المفتاحية :** عملة نقدية ورقية؛ بكتيريا؛ مضاد حيوي؛ زراعة بكتيرية؛ مقاومة؛ سلبية الجرام؛ موجبة الجرام.

## INTRODUCTION

People exchange money on a daily basis in almost all communities, so circulating paper currencies may serve as a potential vehicle for the transmission of bacterial infection and multidrug-resistant pathogens, particularly in immunocompromised individuals<sup>1</sup>. Paper money can become contaminated during transactions, handling, storage, and contact with dirty surfaces. Unhygienic practices, such as wetting fingers with saliva prior to actually counting money, can introduce a variety of bacteria into the notes<sup>2</sup>. These modes of transmission have a significant impact on public health in developing countries, where the incidence of bacterial infection is on the rise<sup>3</sup>. Despite the fact that each location has its own endemic bacteria, members of the *Enterobacteriaceae*, *Bacillus spp.*, *Staphylococcus spp.*, *Micrococcus spp.*, and *Corynebacterium spp.* have been identified as common contaminants isolated from paper currency in various countries<sup>4</sup>. In addition, bacteria from the genera *Vibrio* and *Pseudomonas* have been isolated from paper currency in developing countries<sup>4</sup>. Furthermore, data has accumulated indicating that paper currency has been contaminated by antibiotic-resistant bacteria<sup>5</sup>. The widespread presence of these bacterial species may result in outbreaks of infections, resulting in high morbidity and mortality with significant economic consequences<sup>6</sup>.

There are several sources of currency note contamination, including production, handling, usage, storage, or the atmosphere<sup>7</sup> and food<sup>8,9,10</sup>. Currency notes have been found to have the highest concentrations of *Escherichia coli* and *Coagulase-negative Staphylococci*<sup>11</sup>. Acid-fast bacilli have also been isolated from circulating currency notes<sup>8</sup>. Because of their higher turnover, lower denomination currency notes have higher contamination levels than higher denomination currency notes<sup>12</sup>. Bacterial contamination is also affected by the age of the currency and the

material used in its production<sup>13</sup>. Currency, for example, plays an important role in the indirect transmission of infections such as trachoma, diphtheria, gastroenteritis, whooping cough, and diarrhea<sup>14</sup>. The contaminated money plays an important role in the transmission of multidrug-resistant microorganisms like Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococci (VRE). The multidrug-resistant nosocomial pathogen may survive on hospital surfaces for an extended period of time and may be transmitted to others via currency notes<sup>15</sup>.

Only a few studies from Iraq-Mosul highlighted the importance of microbial contamination of currency notes in the community while ignoring the possibility of multi-drug resistant pathogen transmission in hospitals. The purpose of the study was to report on the bacterial contamination and antibiotic resistance profile of circulating Iraqi currency notes collected from hospital and community sources in Mosul, Iraq.

## MATERIAL AND METHODS

### Sampling:

In the current study, 30 samples of Iraqi paper currency were collected in Mosul City based on the level of treatment, each sample contain three papers. Five community populations provided currency notes (markets, butchers, restaurants, fish sellers, and hospitals). The currency values were as follows: 6 of the 250 ID, 6 of the 500 ID, 6 of the 1000 ID, 6 of the 5000 ID, and 6 of the 10000 ID. In addition to fresh control before being handled by bankers, five mint brand-new notes were taken from the bank and collected at random to serve as controls. Each piece of paper money was immediately collected into a clean plastic container and delivered to the College of Science, Department of Biology, Microbiology Laboratory.

### Bacterial Isolation and Identification:

Each piece of paper money was immersed separately in bottles containing 10 ml of sterilized buffered peptone water for 2 minutes while the bottle was strongly shaken. The currency was taken down, and the remaining peptone water solution was inoculated onto nutrient broth and *MacConkey* broth, followed by a 24-hour incubation period at 37°C. To isolate gram-negative bacteria, the *MacConkey* broth-incubated sample was then cultured onto *MacConkey* agar, *Salmonella-Shigella* agar, and incubated at 37°C for 24 hours. According to the selective and differentiating characteristics of the culture media and on the gram stain, the nutrient broth incubated sample was cultured onto nutrient agar, mannitol salt agar, and then incubated at 37°C for 24 hours for the isolation of Staphylococci, gram-positive Bacilli, and *Pseudomonas* spp. The sub-culturing of separated colonies produced pure cultures. Similar processes occurred in control samples. Growing bacterial isolates were identified using common techniques, which can be summarized as follows: For identification of Gram-negative

bacteria, morphological features and Gram stain are performed. Growth patterns on *Salmonella-Shigella* agar and *MacConkey* agar. Gram-positive bacteria have morphological characteristics and the Gram stain. Mannitol salt agar and nutrient agar growth patterns<sup>16,17</sup>.

### Antibiotic Susceptibility Tests:

All of the isolated bacterial strains had their antibiotic susceptibility tested using Mueller-Hinton agar standard media and a commercially available antibiotic sensitivity disc (Oxoid, England) using a modified Kirby-Bauer method in accordance with CLSI guidelines. The tables of Clinical and Laboratory Standards Institute for interpretative zone diameters were used to measure the inhibition zone standards for antimicrobial susceptibility<sup>7</sup>. Amoxiclav (5 mg), Cefixime (30 mg), Azithromycin (10 mg), Trimethoprim-Sulfamethoxazole (30 mg), Erythromycin (10 mg), Gentamicin (15 mg), bacitracin (15 µg), methicillin (30 µg), Ciprofloxacin (5 mg), Nalidixic acid (20 mg), and Amikacin (30 mg), as well as Imipenem (5 mg), were the antibiotics used<sup>18</sup>.

## RESULTS AND DISCUSSION

The bacterial contamination of a total of thirty Iraqi paper currencies was examined; the contamination rate was ninety percent (90%), and the other 10% were negative for culturing, and fifty-three percent of them contained multiple bacterial isolates. Based on the morphological characteristics of bacterial colonies in selective and different primary culture media as well as the properties of Gram staining, Fig. 1 demonstrated that seven different bacterial species were presumptively identified.



A- *Staphylococcus aureus*



B- *Salmonella* spp



C- Coagulase negative staphylococcus



D- Eschereshia.Col



E- Klebssella spp



F- Pseudomonase spp



G- Proteus spp

Fig. 1. Isolated bacterial types from Iraqi currency paper on various selective media.

Out of 48 bacterial species isolated, the *E. coli* represent (11) (36.67%); *Salmonella* (11) (36.67%); *Klebsiella* (7) (23.34%), *Coagulase Negative staphylococcus* (7) (23.34%), *Staph. aureus* (4) (13.34%), *Pseudomonas. Aeroginosa* (4) (13.34%), and *Proteus. spp* (4) (13.34%). In total, 48 bacterial species representing 37 (77.09 percent) Gram-negative bacteria and 11 (22.91 percent) Gram-positive bacteria were found in all samples that were only slightly contaminated. These bacterial species identified by biochemical tests as reported in the materials and methods as shown in the Table 1.

Table 1. The biochemical tests for identification of isolates.

Test	<i>E. coli</i>	<i>Klebsiella spp.</i>	<i>Salmonella</i>	<i>Proteus. spp</i>	<i>Pseudomonas. aeruginosa</i>
Gram stain	----	----	----	----	----
Morphology	Small rods	Rods	rods	rods	rods
Lactose fermenting	+	+	-	-	-
Indole	+	+	-	-	-
Citrate	-	+	+	+	+
Methyl red	+	+	-	-	+

The relationship between bacterial contamination and paper currency denomination revealed that samples with IDs of 250 and 500 had higher levels of mixed bacterial species contamination than samples with IDs of 1000, 5000, and 10,000 (Fig. 2). The results reveal that there were 19 different bacterial isolates, including 4 from the market, 11 from butchers, 7 each from fish sellers and restaurants, and 11 from currencies collected from hospitals (Fig 3). Additionally, the results showed that 22 bacterial isolates were taken from 250 ID paper money, 14 from 500 ID money, 7, 4, 1, from 1000ID, 5000ID, and 10000ID, respectively.

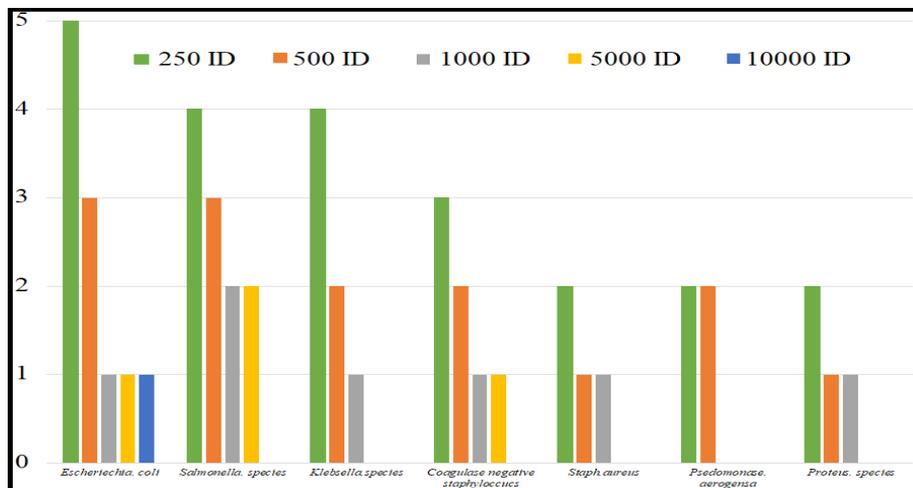


Fig. 2. Distribution of bacterial isolated according to the currency note denomination



Fig. 3. Distribution of bacterial isolated according to the type of community population

The results of this study showed that various bacteria were frequently found on currency notes. In contrast to earlier studies from Tanzania<sup>19</sup> and India<sup>20-22</sup>, in which almost all of the tested currency

notes were contaminated, the study discovered that 90% of the tested currency notes were contaminated with bacteria. The various bacterial species that were isolated for this study are almost

in agreement with those from studies conducted in Nigeria<sup>23,24</sup>, Bangladesh<sup>25</sup>, and Iraq<sup>26</sup>. A potential minefield for nosocomial infections is indicated by the fact that money contamination is linked to unhygienic behavior on the part of people, suggests significant fecal contamination of currency, and reflects poor local environmental sanitation<sup>26</sup>. These studies all determined that the most common isolates from the contaminated coins and money were gram-negative bacteria, and these results are consistent with our study.

As a result, it is impossible to completely rule out the possibility of spreading infections brought on by pathogenic organisms discovered at lower frequencies. The research results conflict with those of other studies, which found *Enterococcus* to be the most prevalent circulating currency contaminant<sup>27,28,29</sup>. Regional variations in bacterial patterns and local traditions may be to blame for the discrepancy in bacterial pattern.

Bacterial isolation was lesser in the higher currencies in this study, which may be related to their high paper quality and the fact that people save in large denominations at home or in banks, keeping them away from hand contamination for a while, and this is in keeping with other studies<sup>3,23,30,31,32</sup>.

Smaller unit currencies (250, 500) were found to be more contaminated than larger unit notes such as 10000 notes in the current study. Similar results of bacterial contamination in Iraqi currency were 100 percent and 88 percent, respectively, in other studies<sup>26,27</sup>. These results could be explained by the fact that smaller unit notes are most frequently handled and circulated among people from various occupations and walks of life. Therefore, as result, lower denomination notes may have higher levels of microbial contamination<sup>33,34</sup>.

Microorganisms can spread from any location where they are attached. Hand-in-hand Money transfers play an important role in the spread of diseases. The number of organisms transferring from paper currency or notes is determined by a number of factors, including the number of organisms present and their ability to survive in a dry environment<sup>5,17</sup>. The method of contact also matters, such as touching contaminated money, which can transfer organisms to the hand. Contamination may be caused primarily by dirty hands, food, and water. Many methods exist for reintroducing Coliform and *Staphylococcus aureus* into food. During vending operations, it was observed that the same hand alternately served and held food and money<sup>8,13</sup>.

Another risk factor for street food contamination is money handling<sup>33</sup>. These transmission routes are critical to the health of many populations in developing countries, where the frequency of

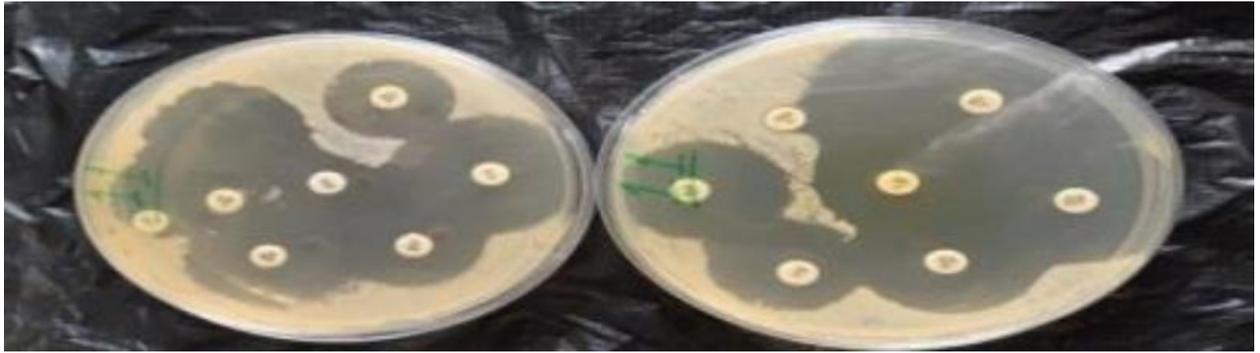
infection is a general indicator of local hygiene and environmental sanitation levels. To date, no outbreaks of foodborne or other illness have been associated to money infection. Evidence of pathogenic bacteria on currency, on the other hand, emphasizes the importance of strict adherence to hygienic practices among money handlers who also handle food and water.

This study found higher rate of *E. coli* and *Salmonella spp* isolation which is consistent with the findings of<sup>34</sup>. They collected currency notes and paper currency from various communities, revealing the bacteria profile of circulating paper currency and currency notes. Other studies were conducted to isolate various loads of microorganisms on currency notes and paper currency. Lower frequencies were detected with *Staph aureus*, *Proteus spp*, and *Pseudomonas aeruginosa* in both studies<sup>34</sup>.

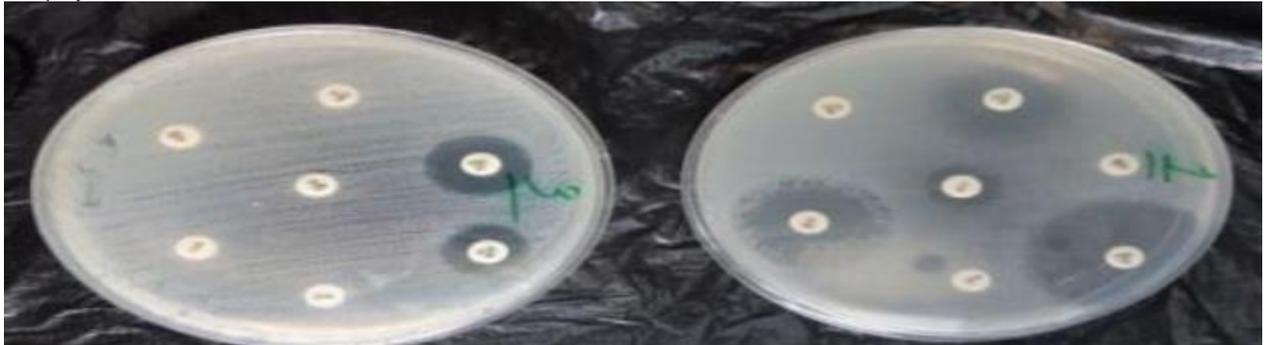
This study also found higher frequencies of *Escherichia coli* and *Salmonella spp* than previous studies<sup>19</sup> in Tanzania and<sup>26</sup> in Iraq. Also show higher *Staphylococcus* frequencies than the previous study by<sup>20</sup>. Furthermore, this study found lower frequencies of *Staph aureus*, *Pseudomonas aeruginosa*, and *Proteus spp*. than previous studies<sup>19</sup>.

It can be concluded that currencies which collected from (hospitals, butter, and fish sellers) are frequently contaminated with various pathogenic and potentially pathogenic bacteria, and all people who handle currency are invariably exposed to those microorganisms. As a result, raising awareness about personal hygiene and good money handling practices, such as washing hands properly with soap and water after handling currency before eating and avoiding using saliva while counting money, is strongly advised as the main pillar to reduce the risk of infection. It is suggested that the public be further educated on the importance of hand washing in restaurants, homes, and after using the restroom, and it is anticipating that regular use of disinfectants during the COVID-19 pandemic will reduce pathogen contamination of money.

The isolated Pathogen was evaluated for susceptibility to twelve antibiotics, the resistance of bacterial isolate to antibiotics was commonly observed, and the majority of isolated strains revealed high resistance to several antibiotics such as Bacitracin, Methicillin, Cefixime, Erythromycin, Azithromycin, while all isolated showed susceptibility of (100%) to Imipenime, moderate to sensitively to Amoxiclav and Nitrofurantoin, Trimethoprim-sulfamethoxazole, and Piperacillin (Fig. 4).



A- *Staphylococcus aureus*



B- *Salmonella* spp



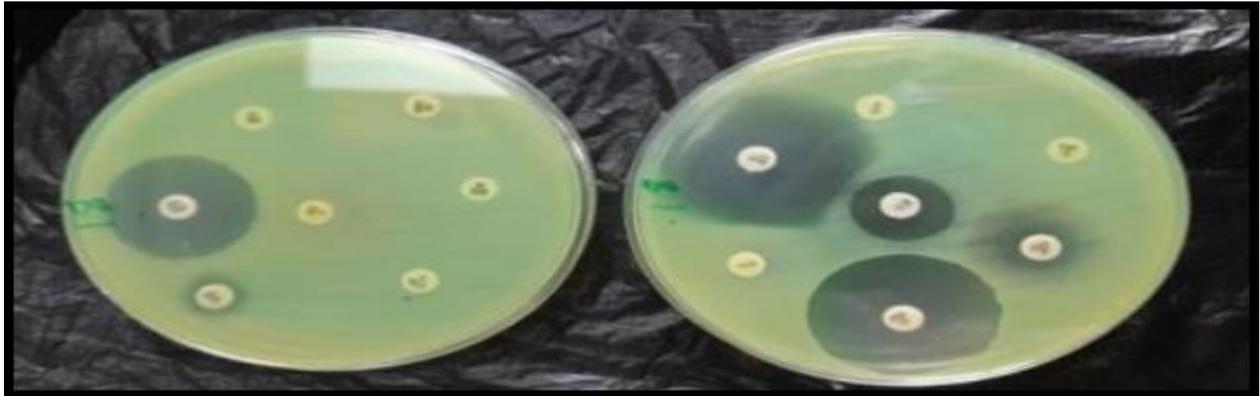
C- Coagulase negative staphylococcus



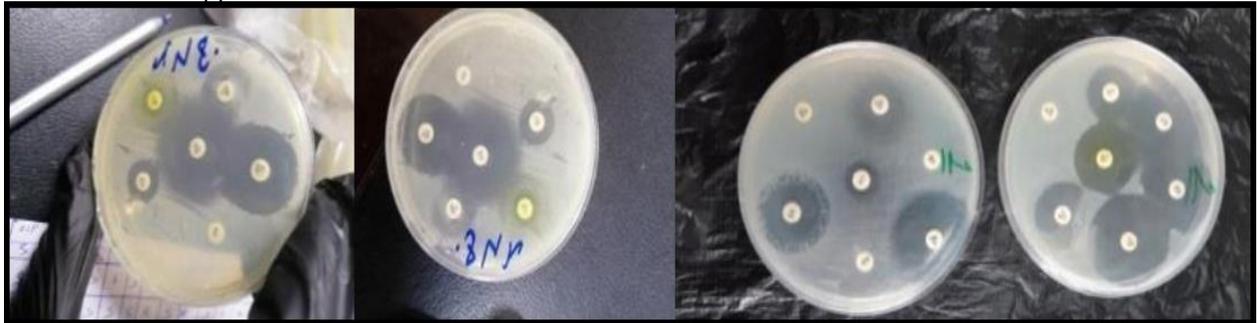
D- *Escherichia.Coli*



E- *Klebsella* spp



F -Pseudomonase spp



G- Proteus spp

Fig. 4. Antibiotic susceptibility of micro-organism isolated from currencies paper of Iraq.

An isolated *E. coli* showed complete resistance to Bacitracin and Methicillin while being completely sensitive to Imipenem, which also revealed varying degrees of resistance to several antibiotics (81.8 percent) of isolates were resistant to Cefixime (CFM).

*Salmonella* isolates showed (100%) resistance to bacitracin and methicillin, while (100%) isolates were sensitive to Impinem and also showed (90.9%), (81.8%), and (72.7%) resistance to Erythromycin, Azithromycin, and Cefixime, respectively.

A *klebsiella spp* isolate showed (100%) resistance to (ME, CFM), while (100%) were sensitive to Impinim (IMP). They also showed resistance to Ciprofloxacin, Amikacin, Nitrofurantion, Trimethoprim-Sulfamethoxazole and Azithromycin (cip, AK, PRL, F, SXT, and AZM) at (85.7%), (57.1%), (85.7%), (71.4%), and (57.1%), respectively.

A *Proteus spp* isolates demonstrated (100%) resistance to Cefixim, bacitracin, Trimethoprim-Sulfamethoxazole and Erythromycin (CFM, B, SXT, and E), (100%) susceptibility to Ciprofloxacin and Impinim (CIP, and IPM), (75%) resistance to Methicillin and Azithromycin (ME, AZM), and (50%) resistance to Amoxiclav, Nitrofurantion and Piperacillin (AMC, F, PRL).

A *Pseudomonas aerogenosa* isolates demonstrated (100%) resistance to Methicillin,

Erythromycin, bacitracin and Cefexim (ME, E, B, CFM), (100%) susceptibility to Impenem (IPM), (75%) resistance to Azithromycin, Amikacin (AZM, AK), and (50%) resistance to Ciprofloxacin, (cip, SXT, AMC).

*Staph. aureus* isolates showed (100%) susceptibility to Ciprofloxacin and Imipenem (CIP, IPM), (100%) resistance to bacitracin and erythromycin (B, E), (75%) resistance to Cefixim and Methicillin (CFM, ME), (50%) resistance to Piperacillin, Trimethoprim-Sulfamethoxazole, Nitrofurantion, Amoxiclav, (PRL, F, SXT, AMC), and (25%) resistance to Amikacin (AK).

This study revealed that many multidrug-resistant strains of different isolates were prevalent in Iraqi currencies, emphasizing the importance of the notes for public health. Isolates of various bacterial species recorded high rates of resistance, as shown in table (3). This outcome is consistent with<sup>14,16-18</sup>.

The presence of multidrug-resistant strains poses a significant challenge to human survival. The detected high antibiotic resistance could be attributed to antibiotic abuse, indicating that the majority of the population sampled purchases antibiotics without medical instruction and uses them for the wrong diseases and infections<sup>14</sup>.

## CONCLUSIONS

The outcomes from the current study indicate that Iraqi currency notes in circulation are contaminated with several bacteria, the majority of which are resistant to commonly used antibiotics, posing risks and public health exposures to the community and individuals handling currency notes. People should raise their personal health awareness by repeatedly washing their hands after handling currency notes, preventing babies from handling currency notes, avoiding the use of saliva when calculating currency notes, and refraining from putting money in their mouths, stabbing currency notes in brassieres, and sharpening off corners of banknotes.

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