<u>**Original Article**</u>

Bacterial contamination of Yemeni currency papers and their antibiotic resistance patterns

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ABSTRACT

This cross sectional study was carried out during 2020-2021 on Yemeni currency papers to determine the prevalence rate of bacterial contamination and associated risk factors. Anti-microbial sensitivity patterns of the common pathogens contaminated Yemeni currency papers were also investigated. A total of 324 currency papers were collected randomly from different areas of Dhamar city and examined. The samples were collected from different categories of community people such as health care workers, butcher's shops, vegetable vendors, restaurants waiters, public transport conductors, Berbers and Bankers. The currency papers were transferred to microbiology laboratory and tested for bacterial contamination using standard microbiological methods. Antibiotic resistance patterns of isolated bacteria were determined by disk diffusion method. The results revealed that, out of 324 samples examined, 315 samples were positive for bacterial contamination with overall prevalence rate as 97.22%. The results of bacterial culture revealed that, 14 bacterial species were identified. The species identified were *Escherichia coli* (*E. coli*) with the highest prevalence rate (23.81%) followed by *Citrobacter spp.* (18.73%), *Staphylococcus epidermidis* (14.6 %), *Klebsiella.* (9.52%), *Staphylococcus aureus* (8.57%), *Pseudomonas spp.* (6.03%), *Proteus spp.* (5.4%), *Shigella spp* (3.17%), *Streptococcus pyogens* (3.17%), *Salmonella spp* (2.86%), *Streptococcus pneumonia* (2.54%), *Enterobacter spp.* (0.95%) and *Enterococcus* (o.63%). There were significant differences (P<0.05) on the prevalence rates among isolates species. There were no association (P<0.05) between contamination rate of currency papers and source of samples. All isolated bacteria were resistant to Methicillin and Ceftazidime; whereas, sensitive to others antibiotics in particular Piperacillin/ Tazobactam. In conclusion, the Yemeni currency papers circulating among community people were contaminated with verity range of bacterial species and some of them multi-drug resistant to common antibiotics. The decontamination of currency paper with disinfections in the banks and markets is recommended to reduce the transmission of pathogens to human.

INTRODUCTION

Microorganisms are present almost everywhere in our surroundings. They may propagate via food, water, air and most importantly by fomites (Barolia et al., 2011; Ejaz et al., 2018; Abdul Kader and Al-Rawi, 2021). The environment plays an important role in transmission of microorganisms to humans and materials such as

currency papers/notes serve as vehicles for microbial transmission (Elumalai et al., 2012). Currency papers/notes are the most needed material by each and everybody in a civilized society to reach a socio-economical class of getting basic needs useful to human (Beattie, 2018). Currency note is widely exchanged for goods and services in the entire global economic environment, it has promoted trade in communities since its first introduction in China approximately 1,000AD (Ireland and Bernholz, 2003; Assayaghi et al., 2021).

People are aware about spread of pathogens through food, air, water and have taken enough steps to control it. However, Studies of the contamination of money with microbial agents are lacking in most countries of world. Shortage of information may contribute to the absence of public health policies regarding currency usage, handling, and circulation (Al-Ghamdi etal., 2011; Sucilathangam et al., 2016; Anuranjini et al., 2017).

Microbial contamination of currency paper could be occurred during the production process, storage, counting, and handling (Awodi and Nock, 2001; Thiruvengadam et al., 2014). Other, practices like wetting the fingers with saliva when counting the papers, scattering currency paper during the several events and ceremonies, keeping the papers in unclean pocket, dirty stockings, in time of animal slaughtering and food preparation (Ameh and Balogun, 1997; Ogo et al., 2004; Khalil et al., 2014). The contaminated papers go in circulation and spread contaminated microbes among the people. These ways of transmission are significance in public health community worldwide (Thiruvengadam et al., 2014). Currency papers play a significant role in transmission of infectious diseases such as trachoma, diphtheria, gastroenteritis, whooping cough, diarrhea and other diseases (Sharma and Dhanashree, 2011); and multidrug-resistant microorganisms (Gedik etal., 2013). Maintaining personal hygiene can help for reducing the presence of microbes on currency paper, thereby reducing the risk of transmission during currency exchange (Al-Hajj et al., 2024).

The quantity and quality of pathogens transfer by currency papers are influenced by many factors, for example; currency notes of lower denominations exhibit higher contamination level comparing to currency notes of higher denominations due to their more rapid turnover. Bacterial contaminations also depends upon the age of currency and the materials used in the production of the currency notes (Basavarajappa et al., 2005; Vriesekoop et al., 2010; Firoozeh et al., 2017; Ejaz et al., 2018).

Considerable investigations in different geographical regions of the world have been conducted on microbial contamination of currency papers in circulation and reported variation ratse of currency contamination (Thiruvengadam et al., 2014; Elsharief et al., 2018; Gedam et al., 2018; Usman et al., 2021; Ilyasu et al., 2021; Elleboudy et al., 2021; Cozorici et al., 2022); and in some governorates of Yemen (Hanash et al., 2016; Assayaghi et al., 2021; Al-Hajj et al., 2024). The bacterial species isolated and identified of above studies were *Escherichia coli, Salmonella spp.*, *Enterocci spp.*, *Klebsiella spp., Shigella spp., Mycobacterium tuberculosis, Vibrio cholera, Bacillus spp., Staphylococcus spp., Pseudomonas spp.,* and *Corynebacterium spp., Aerobacter* spp, *Mycobacterium tuberculosis* and *Streptococcus faecalis* (Kawo et al., 2009; Moosavy et al., 2013; Akond et al., 2015; Boidya et al., 2015; Hanash et al., 2015; Mbata et al., 2016; Firoozeh et al., 2017; 2018; Gedam et al., 2018; Obajuluwa et al., 2023). However, there are dearth of information on microbial contamination of Yemeni currency papers at Dhamar city. Therefore, the aim of the present study was to investigate the bacterial contaminants on majority denominations of Ryial notes in circulation at Dhamar city and their antibiotic resistance patterns.

MATERIALS AND METHODS
Study area
This study was conducted at Dhamar city, Dhamar governorate during 2020-2021. Geographically, Dhamar is located at 14°.58'N latitude, 44° 43'E longitude and at an elevation of 2415.09 meters (7923.52 feet) above sea level, Dhamār has a Mid-latitude desert climate. The city's yearly temperature is 20.97ºC (69.75ºF) and it is -4.56% lower than Yemen's averages. Dhamār typically receives about 109.11 millimeters (4.3 inches) of precipitation and has 149.45 rainy days (40.95% of the time) annually (Anonymous, 2024).

Study Samples
The study samples of this study were Yemeni currency papers with various denominations. The samples were

collected from different categories people of community working in hospitals, butchers` shops, vegetable vendors, restaurants, public transport, barber shops and banks at Dhamar city.

Size of sample

The sample size was calculated according keys given by Allan et al. (2018) considering 30% expected prevalence and 95% confidence interval with a 5% desired absolute precision using the following formula: $N = (Z)^2 P(1-P)/d^2$, where, (p)expected prevalence and (Z) 95% confidence interval (Z= 1.96) and (d) a 5% desired absolute precision. $N = 1.96^2 Pexp (1-Pexp)/D^2$: *Where, Pexp = expected prevalence; d= absolute precision; n=sample size*. A total of 323 currency papers were investigated in this study.

Study design and sitting

This cross sectional study was carried out during years of 2020-2021 in laboratory, Department of Microbiology, Faculty of Medical Science, AlHikma, Dhamar, Yemen. A total of 323 Yemeni currency papers of various denominations (100, 250,500 and 1000 Ryials) were collected from different community people of the city, namely, health care workers, butchers, vegetable vendors, restaurants waiters, public transport conductors, Berbers and Bankers; whereas, the currency papers with denomination of 50 and 200 Ryials were excluded from study due to their less circulation in study areas. To collect the paper currency, the individuals were requested to drop currency papers into a sterile polythene bag and labelled accordingly; Papers/notes were not touched by the researcher using bare hands at any stage. The individuals were given a replacement note equivalent to the denomination they had deposited in the sampling bag. The bag was sealed and immediately transported to the laboratory for analysis (Sucilathangam et al., 2016; Usman et al. (2021).

Isolation and Identification of bacteria isolates

In laboratory, isolation of bacterial species contaminated the currency paper was performed according the technique described by Cheesbrough (2000); Thiruvengadam et al. (2014) and Firoozeh et al., (2017). Briefly, a sterile cotton-tipped swab moistened with sterile physiological saline (0.85% NaCl) was used to swab both sides of the currency

paper. The swabs were directly inoculated on MacConkey agar and blood agar. The inoculated media were incubated aerobically at 35- 37ºC for 24h and then examined for bacterial growth.

Identification of bacteria isolates was based on morphology characteristics, Gram reaction as well as biochemical techniques such as the Indole, Coagulase, Oxidase, Urease, Catalase test and Triple sugar iron tests (sugar fermentation and gas production) in pure culture according to protocols described previously by Cheesbrough (2000) and Leboffe and Pierce, 2011).

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed according to keys given by Clinical and Laboratory Standards Institute (CLSI, 2014) and Ali et al. (2015). In brief, a standard bacterial suspension was prepared in sterile Mueller-Hinton broth with 8 hrs.' incubation at 37°C till its turbidity exceeds the standard McFarland tube No. 0.5. Then dipped sterile cotton swab into standard bacterial suspension and swab was used to streak entire surface of Mueller-Hinton agar by rotating clock wise and anti-clock. Then antibiotic disks were placed on the surface of inoculated plates by gentle pressing with sterile forceps to make sure the contact of disk with media plate surface. The plates were then incubated for overnight incubation at 37°C. Results for sensitivity were noted next day by measuring zone of inhibition with standard scale and interpreted the results according to guidelines given by CLSI (2014). The testes antibiotics were Cefuroxime (CXM), Piperacillin/Tazobactam (PTZ), Methicillin (MET), Amoxicillin (AX), Ampicillin (AS), Ceftazidime (CAZ), Penicillin G (P_{10}), Doxycycline (DOX) and Vancomycin (VA).

Data analysis

The collected raw data were organized and arranged using the Microsoft Excel spread sheet computer programme and analyzed using SPSS version 20 statistical software's. Chi-square tests was applied to test the statistical association exists among the variables, prevalence rate of bacterial infections. All results were considered statistically significant when the *P*-value <0.05.

RESULTS

In this study, 323 Yemeni currency papers were collected and screened for bacterial contamination, 315 papers were found positive for one or more bacterial species with overall prevalence rate as 97.22%, furthermore, the results of bacterial culture revealed that, many species of bacteria were identified with variety of percentages, these include: *Escherichia coli* (23.49%) followed by *Citrobacter spp* (20.95%), *Staphylococcus epidermidis.* (12.70 %), *Klebsiella spp.* (9.52 %), *Staphylococcus aureus* (8.89%), *P. aeruginosa.* (5.71%), *Proteus spp.* (5.40%), *Shigella spp.* (3.17%), *Streptococcus pyogens* (3.17%), *Salmonella spp.* (2.86%), *Streptococcus pneumonia* (2.54%), *Enterobacter spp.* (0.63%) and *Enterococcus spp.* (0.63%) as illustrated in Fig. 1. Significant differences (P<0.05) were observed among prevalence rates of different bacteria isolates.

The impact of month variation (season), currency denomination and currency papers sources on distribution of bacterial contamination on the currency papers were depicted in Table 1.

As shown, the highest contamination rate was recorded in month of February (24.44%); whereas, the lower in month of May (10.16%). The higher contamination rate was recorded in currency papers of 100-denomination; whereas, the lower rate in currency papers of 250-denomination. The highest contamination rate was recorded in health care workers' category working at Dhamar General Hospital (16.2%); whereas, the lower rate (12.70%) in workers working at Groceries. Statistically, significant differences (P<0.05) were observed between contamination rate and month variation; while, none with denomination and source of papers variables.

The antibiotics analysis of sensitivity and resistance patterns of the isolated bacteria showed increasing resistance of isolates to the methicillin and ceftazidime antibiotics; whereas, susceptibility to other antibiotic drugs with particular to Piperacillin / Tazobactam as depicted in Table 2.
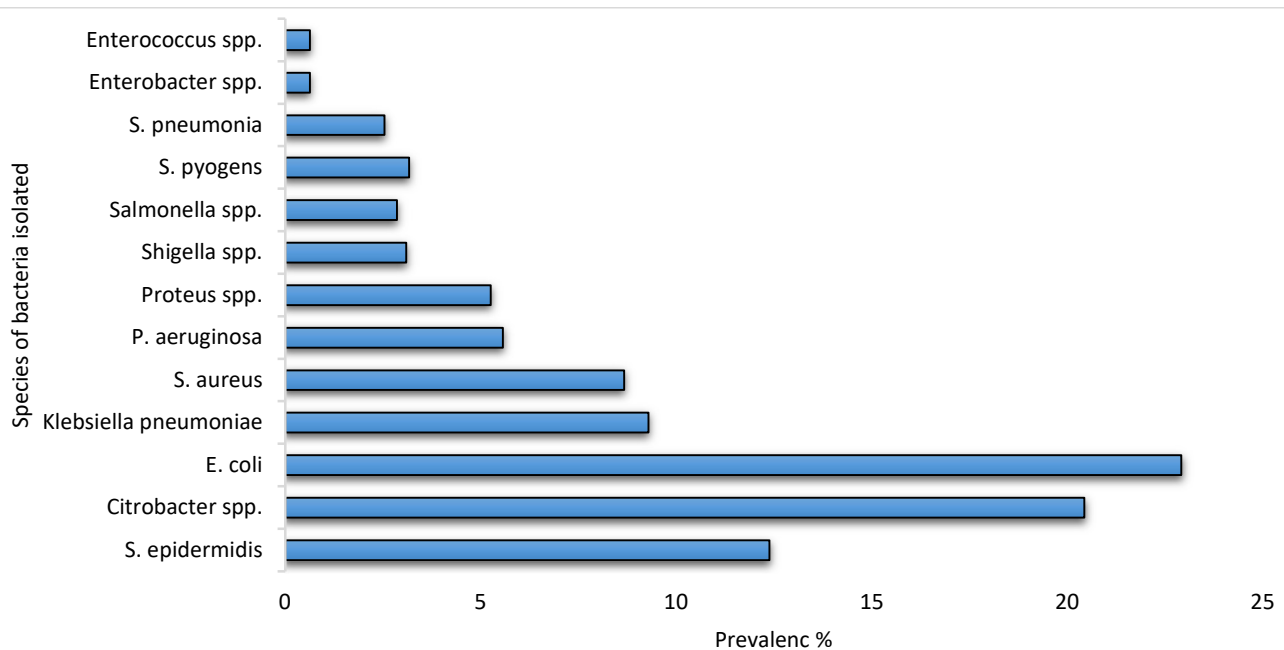

Fig. 1. Bacterial species isolated from contaminated Yemeni currency Papers at Dhamar city

Table 1. The impact of month variation, currency denomination and currency`s source on distribution of bacterial contamination on Yemeni currency (n=315)

Variable	Categories	No. of samples contaminated	Prevalence %	P value
Months	Jan	70	22.22	0.03
	Feb	77	24.44	
	Mar	70	22.22	
	Apr	66	20.95	
	May	32	10.16	
Currency denominations	1000 RY	78	24.76	0.452
	500 RY	75	23.81	
	250 RY	71	22.54	
	100 RY	91	28.89	
Source of currency paper	Restaurants	42	13.33	0.066
	Transports	46	14.60	
	Banks	46	14.60	
	Hospitals	51	16.19	
	Butchers` shops	46	14.60	
	Barber shops	44	13.97	
	Groceries	40	12.70	

Table 2. Antimicrobial susceptibility pattern of bacterial isolates contaminated currency Papers

Isolates	N	CXM			PTZ	MET			AX			AS			CAZ			P10			DOX			AV		
		S	M	R	S	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R	S	M	R
S. epidermidis	5	4	0	1	5	0	1	4	2	2	1	3	1	1	0	1	4	3	0	2	1	1	3	4	1	0
Citrobacter spp.	4	0	3	1	4	0	0	4	1	3	0	1	3	0	0	1	3	0	1	3	2	2	0	3	1	0
E. coli	3	1	2	0	3	0	0	3	2	1	0	3	0	0	0	0	3	2	1	0	1	2	0	3	0	0
Klebsiella spp.	2	2	0	0	2	0	0	2	1	1	0	1	1	0	0	1	1	0	2	0	1	1	0	2	0	0
S. aureus	4	3	1	0	4	0	0	4	3	0	1	4	0	0	2	0	2	3	1	0	1	2	1	2	2	0
Pseudomonas spp.	3	2	0	1	3	1	0	2	0	2	1	2	0	1	1	1	1	1	1	1	2	0	1	2	1	0
Proteus spp.	2	0	1	1	2	0	0	2	2	0	0	1	0	1	0	0	2	1	0	1	0	2	0	0	1	1
Shigella spp.	2	1	1	0	2	0	0	2	2	0	0	2	0	0	0	2	2	0	0	2	0	0	2	0	0	0
Salmonella spp.	4	2	2	0	4	0	0	4	3	1	0	4	0	0	0	4	2	2	0	3	1	0	2	0	2	2
S. pyogens	2	2	0	0	2	0	0	2	0	2	0	1	1	0	0	1	1	1	0	1	2	0	0	1	0	1
S. pnumonia	2	1	0	1	2	0	0	2	0	1	1	2	0	0	0	1	1	1	1	0	0	2	0	1	0	1
Enterobacter spp.	2	2	0	0	2	0	0	2	1	1	0	1	1	0	0	0	2	1	0	1	1	0	1	1	1	0

N= Number of Isolates, S=sensitive, M=Moderate, R= resistant, CXM= Cefuroxime, PTZ= Piperacillin / Tazobactam, MET= Methicillin, AX= Amoxicillin, AS= Ampicillin, CAZ= Ceftazidime, P10= Penicillin G, DOX= Doxycycline, VA= Vancomycine

DISCUSSION

Currency papers are widely exchanged for goods and services in countries worldwide. Currency Papers are exchanged by persons with different hygiene habits, and are often stored under improper hygienic conditions. Although credit cards have replaced cash exchange in some cases, currency papers are still commonly used all over the world for the purchase of services and materials (Firoozeh et al., 2017).

Microorganisms that are present in air, water, etc., are easily spreading from one person to another. The most common mechanism of the spread of pathogens is by fomites including currency papers (Barolia et al., 2011; Elsharief and Haider, 2018). The main aim of this study was to investigate bacterial species contaminated Yemeni currency papers and associated factors in circulation at Dhamar city, Yemen.

The results of present study revealed that the bacterial contamination rate of Yemen currency papers was 97.52%. These results are in agreement with findings of Gabriel et al. (2013)) and Ejaz et al. (2018), who reported the contamination rate as 97%, and lower than contamination rates previously reported by Feglo and Nkansah (2010) in Ghana (98.6%); Moosavy et al. (2013) in Iran (100%); Hanash et al. (2016) in Yemen (100%); Allan et al. (2018) in Uganda (100%); Elsharief and Haider (2018) in Libya (100%); Sunil et al. (2020) in India (100%); El-dars and Hassan (2020) in Egypt (100%); and higher than rates recorded by Saadabi et al.(2017) in Saudi Arabia (96.2%); Barua et al.(2019) in Bangladesh (95%). The differences between the contamination rates of current study and above studies could be attributed to hygienic practices and handling of currency, economic and social habits of people in different countries.

Bacteria species isolated from the Yemeni currency paper were *S. epidermidis*, *Citrobacter spp.*, *E. coli*, *Klebsiella pneumonia, S. aureus, P. aeruginosa, Proteus spp., Shigella spp, Salmonella spp., S. pyogenes, S. pneumonia, Enterobacter spp., Enterococcus spp.* with varied percentages. These results are partially or in complete accordance with findings of other researchers (Asikong et al., 2007; Oyero and Emikpe, 2007; Tagoe et al., 2009; Hiko et al., 2016; Saadabi et al., 2017; Dadgostar et al., 2017; Elsharief and Haider, 2018). Contrary or consistent

among the results of this study and findings of above workers may due to the environmental factors and size of sample. Furthermore, Sunil et al. (2020) suggested that, several behavioral practices in study site may contribute to currency contamination: keeping money underbody surfaces, improper washing of hands after using the toilet, wetting fingers with saliva when counting currency, coughing and sneezing on hands and handling currency, and placement or storage of money on dirty surfaces during transactions.

E. coli was found slightly predominant species compared to other species isolated; these results are in agreement with findings of Sunil et al. (2020) who assessed the microbial contamination of Indian currency papers in circulation and reported similar results. The predominant of *E. coli* species may due to the nature of this species and poor personal hygiene practices of currency handlers.

The impact of months (season), currency denominations and source of currency papers on distribution of bacterial contamination on currency papers also was investigated. The results displayed that, the higher contamination rate was recorded in month of February; whereas the lower in month of May. The higher contamination rate in February may be due that microclimate favor for survival and multiplication microorganisms in this month. All currency denominations papers investigated revealed that bacterial growth on lower denominations papers (100 YR) was more compared to higher denominations papers. These results are on line with previous studies conducted by many workers (Basavarajappa et al., 2005; Igumbor et al., 2007; Bhat et al., 2010; Anning et al., 2019; Yar , 2020). The reason behind that could be attributed to the lower denomination paper pass through more hands of people in diverse economic and social activities than the higher denomination papers which are often saved in banks, more respected and less frequently handled.

The currency papers obtained from hospitals and restaurants showed more bacterial contamination rate compared to groceries. These results are in agreement with findings of Ahmed et al. (2017). The relationship between contamination rate and source of currency papers may be attributed to variety of hygienic practices and handling of currency in different areas. Furthermore, hospitals are main sources for

nosocomial pathogens (Ejaz et al., 2018; Sunil et al., 2020).

Nowadays, antimicrobial resistance has become a burning issue throughout the world. Indiscriminate use of antibiotics has leads to treatment failure and augments health cost (Sharma and Dhanashree, 2011). Currency papers are usually contaminated with pathogenic microorganisms in circulation, of which most of them are resistant to commonly used antibiotics reported elsewhere world (Firoozeh et al., 2017). Transmission of these antibiotic resistance microorganisms from one individual to another through currency papers may cause serious public health hazards. In the current study, bacteria isolated from Yemeni currency paper were subjected to antimicrobial susceptibility test with nine commonly used antibiotics in markets; the results revealing that most of the antibiotic tested, namely, MET, CAZ and P_{I0} were non-effective against majority of bacterial isolates compared to Piperacillin / Tazobactam antibiotics tested. These results are partially in agreement with findings of Ali et al. (2015). The developed resistance of some isolates to tested antibiotics in our study may be attributed overuse and misuse of these medications, as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements as suggested by Gould and Bal (2013).

CONCLUSION

It could be concluded from this study that, The Yemeni currency papers circulating among community people are contaminated with highly pathogenic and most of them resistant to common antibiotics used in markets. The pathogenic organisms represent risks and public health hazards to the community and individuals.

RECOMMENDATIONS

This study could be recommended that, the decontamination of currency papers by ultraviolet light or formalin vapors at the banks and markets could help to reduce the transmission of the pathogen organism to human. It is also suggested that people should keep strict adherence to hygienic practices before handling food and water after contact with currency papers.

Assessment of bacterial resistance to common antibiotics should be performed regularly.

AUTHORS CONTRIBUTIONS
IRMS AlShaibani and HM Hatem, proposed, wrote protocol and final version of study. Authors, Akram AlHaj and Hisham AlMahdi, analyzed, interpretation of data. Authors, E A AlObbasi, AA, AlAmeen, EH AlSoffy, SH, AlSheni, BA Nahilah, A Al AlSanabany, carried out the collection, processing of specimen and antimicrobial susceptibility tests.

CONFLICTS OF INTEREST
The Authors have not declared any conflict of interests.

DATA AVAILABILITY
The data are available within text of the article.

ETHICS APPROVAL
Not applicable.

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التلوث البكتيري لأوراق العملة اليمنية وأنماط مقاومتها للمضادات الحيوية

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الملخص

أجريت هذه الدراسة العرضية القطاع خلال الفترة 2020-2021م على أوراق العملة اليمنية، لمعرفة معدل انتشار التلوث البكتيري وعوامل الخطورة المرتبطة بها. كما تم اجراء اختبار أنماط الحساسية المضادة الحيوية للميكروبات الشائعة الملوثة لأوراق العملة اليمنية. تم جمع 324 عينة ورقية من العملة اليمنية من الفئات المختلفة بشكل عشوائي من أماكن مختلفة من مدينة ذمار. واجريت لكل عينة ورقية الزراعة الميكروبيولوجية والاختبارات البيوكيميائية. أظهرت النتائج أنه من إجمالي 324 عينة تم فحصها وجد أن 315 (97.22٪) كانت موجبة وملوثة بنوع او اكثر من أنواع االبكتيريا. كشفت النتائج المزرعية ان تم التعرف على 14 أنواع من البكتيريا وهي: الإشريكية القولونية *E. coli* وسجلت بأعلى نسبة (23.81٪)، تليها *Citrobacter spp* (18.73%)، (*S. epidermidis* (14.6% *Klebsiella* (9.52%) ، *S. aureus* (8.57%)، *Pseudomonas spp* (6.03%) *Proteus spp* (5.4%))، *Shigella spp* (3.17%)، *Streptococcus pyogens* (3.17%) *Salmonella spp* (2.86%) *Streptococcus pneumonia* (2.54%) *Enterobacter spp* (0.95%), *Enterococcus* spp.(o.63% خيرا *Enterococcus* spp. لوحظت فروق معنوية ذات دلالة احصائية (P< 0.05) بين معدلات انتشار الأنواع البكتيرية المعزولة. لم يلاحظ اي ارتباط معنوي (P<0.05) بين تلوث أوراق العملة اليمنية والعوامل الديموغرافية. أظهر تحليل المضادات الحيوية لأنماط الحساسية والمقاومة لانواع البكتيريا المعزولة في هذه الدراسة ان هناك مقاومة متزايدة Methicillin and Ceftazidime. بينما أظهرت كل من Piperacillin Tazobactam حساسية تجاه جميع البكتيريا بشكل عام. خلصت هذه الدراسة ان هناك عدة أنواع من البكتيريا تلوث العملة الورقية اليمنية في منطقة الدراسة. يوصى بتعقيم العملة اليمنية في البنوك بالمعقمات الخاصة لتقليل فرص اصابة الانسان بالأنواع المعزولة من البكتيريا وغيرها.

الكلمات المفتاحية: مقاومة المضادات الحيوية، التلوث البكتيري، العملة الورقية اليمنية