International Journal of
Enteric Pathogens

Int J Enteric Pathog. 2017 November;5(4):106-110 Original Article

http://enterpathog.abzums.ac.ir

doi 10.15171/ijep.2017.25

Bacterial Contamination of Iranian Paper Currency and Their Antibiotic Resistance Patterns

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Published Online September 23, 2017

Keywords: Paper currency, Bacterial contamination, Antibiotic resistance

Abstract

Background: Paper banknotes would be a vector for transmission of pathogenic microorganisms through handling.

Objective: This study aimed to determine bacterial contamination of Iranian paper currencies in circulation and their antibiotic resistance patterns.

Materials and Methods: In this study, 337 currency notes of different value were collected from markets, shops, restaurants, bus stations and banks in Kashan, Iran during April 2015 to March 2016. The currency notes transferred to microbiology laboratory and were tested for bacterial contamination using standard microbiological methods. Antibiotic resistance patterns of isolated bacteria were determined by disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) standards. The results and data were analyzed using descriptive statistics.

Results: Of 337 currency notes, 262 (77.7%) were identified with bacterial contamination. Bacteria isolated from currency notes were as follows: *Bacillus* spp 113 (43.1%), coagulase-negative staphylococci 99 (37.7%), *Escherichia coli* 20 (7.6%), Enterococci species 14 (5.3%), *Staphylococcus aureus* 8 (3.1%), *Klebsiella* spp 4 (1.5%), *Shigella* species 2 (0.8%), *and Pseudomonas* species 2 (0.8%). The most and least contaminated currency notes were 50000 and 500 Rials, respectively. The highest resistance rates in gram-negative rods were against nalidixic acid, and ampicillin. However, the highest resistance rates in *S. aureus*, coagulase-negative staphylococci and Enterococci species were against ampicillin, erythromycin and tetracycline.

Conclusion: Our study revealed that the bacterial contamination among Iranian paper currency in circulation especially those obtained from certain sources including shops and bus stations is high and in most cases these bacterial isolates are antibiotic-resistant strains.

Received August 12, 2017; **Revised** September 13, 2017; **Accepted** September 16, 2017

Background

Paper currencies 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 notes are still commonly used all over the world for the purchase of services and materials.[1] Paper banknotes have a large surface area for bacterial attachment and would be a vector for transmission of potentially pathogenic microorganisms between population.[2] The risk of microbial transfer by paper currency is influenced by factors such as paper value and duration of usage.[3] It is documented that lower value paper notes commonly carry the higher number of bacterial contamination because they are circulated more than higher denomination notes especially among the people with special occupation including beggars, school children, butchers and so on.[4] Recent studies showed that currency papers were contaminated by nematodes, protozoa, fungi and specially bacteria.[5-7] Several studies in different countries showed high rates of microbial contamination among paper currencies in circulation.[8-10] For example, all currency papers from India, Ghana, Bangladesh and Iraq were found to carry pathogenic or potentially pathogenic bacteria.[9-13]

Moosavy et al showed that the most prevalent bacterial isolates from paper currencies were *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*.[14] In a study, it was revealed that coagulase-negative staphylococci, including *Staphylococcus saprophyticus*, *Staphylococcus epidermidis* and *Staphylococcus hominis* were the most

contaminated microorganisms isolated from Polish paper currencies.[15]

Till now, there are few studies on bacterial contamination of paper currencies in circulation and their antibiotic resistance pattern. This study aimed to determine bacterial contamination of Iranian paper currency in circulation and their antibiotic resistance patterns in Kashan, Iran.

Materials and Methods

Study Design

In this study, 337 currency notes of different value were collected from markets, shops, restaurants, bus stations and banks in Kashan, Iran during April 2015 to March 2016. The study samples were collected based on the level of usage by simple random sampling method as follows: the 13, 14, 21, 39, 46, 69, 71 and 64 pieces of 500; 1000; 2000; 5000; 10 000; 20 000; 50 000 and 100 000 Rials paper notes, respectively. Banknotes with different values which are currently in circulation (500; 1000; 2000; 5000; 10 000; 20 000; 50 000 and 100 000 Rials) were included in the study and notes that were withdrawn from circulation (100 and 200 Rials) were excluded.

Isolation and Identification of Bacteria

The currency notes transferred to Microbiology Laboratory at the Kashan University School of Medicine in sterile condition. Each note was swabbed with sterilized cotton swab which was wetted with sterilized distilled water. These swabs were plated on blood agar medium (Merck, Germany) and MacConkey agar medium (Merck, Germany) for isolation of gram-positive and gram-negative bacteria. After incubation for 24 to 48 hours at 37°C, each bacterial colony was re-cultured on different series of blood agar and MacConkey agar culture media to obtain pure cultures. Each colony in pure culture was stained with gram method and bacterial isolates in pure culture were tested and identification of bacterial genus and species was done using standard microbiological and biochemical methods.

Briefly, each colony in pure culture was sub-cultured on selective media including mannitol salt agar for *Staphylococcus* spp., eosin methylene blue agar (EMB) for *E. coli*, MacConkey agar for coliforms, Cetrimide agar for *Pseudomonas aeruginosa,* Bacillus agar for *Bacillus* spp and Salmonella-Shigella (SS) agar for isolation of *Salmonella* spp. and *Shigella* spp. Biochemical tests including coagulase, novobiocin, triple sugar iron (TSI) agar, oxidase, catalase, oxidative/fermentative (OF), urease, motility, indole, citrate, methyl red (MR) and voges-prosakuer (VP) tests were used for identification of species (Merck, Germany).

Antimicrobial susceptibility patterns of bacterial isolates were determined using disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[16] The tested antibiotics were as follow: ampicillin, ciprofloxacin, gentamicin, nalidixic acid, amoxicillin, aztreonam, cefoxitin, vancomycin, tetracycline and erythromycin (Mast Company, UK). The standard strain of *E. coli* ATCC 25922 was used for quality control in susceptibility testing. Interpretation of results of antibiogram as susceptible, intermediate or resistant was done according to the criteria recommended by the CLSI guideline.

Statistical Analysis

For statistical analyses, SPSS version 16.0 (SPSS, Inc.) was used. Fisher exact and chi-square tests were used to study the statistical relationship between the results. *P*-value of less than 0.05 was considered statistically significant.

Results

Of total 337 currency notes, 262 (77.7%) were contaminated with bacteria (Table 1). Bacteria isolated from banknotes were included *Bacillus* spp, coagulase-negative staphylococci, *E. coli*, enterococci species, *S. aureus*, *Klebsiella* spp, *Shigella* species, and *Pseudomonas* species. *Bacillus* spp was the most common bacterial isolates from 113 currency notes of all denomination which were studied (Table 2). There were no significant correlations between bacterial growth, bacterial genera or species and denomination of currency notes ($P > 0.05$). Although the highest contamination was detected in 50 000 Rials paper notes and the 500 Rials paper notes had the lowest contamination (Table 1). There were relatively higher carriage rates of *E. coli* and enterococci species among banknotes isolated from shops and markets whereas the isolation of *Staphylococcus* species in paper notes collected from bus stations were higher. The highest resistance rates in gram-negative rods were against nalidixicacid and ampicillin. Also most resistance rates in *S. aureus*, coagulase-negative staphylococci and enterococci species were against ampicillin, and erythromycin (Table 3).

Discussion

Currency notes are frequently handled by various people with different hygienic level, are also stored in multiple

Table 1. Bacterial Contamination of Paper Currencies With Different Denomination

Paper Denomination (Rials)	Growth Bacteria		Total
	No No. (%)	Yes No. (%)	
500	6 (46.2)	7 (53.8)	13
1000	5 (35.7)	9 (64.3)	14
2000	8 (38.1)	13 (61.9)	21
5000	10 (25.6)	29 (74.4)	39
10 000	9 (19.6)	37 (80.4)	46
20 000	9 (13.0)	60 (87.0)	69
50 000	5 (7.0)	66 (93.0)	71
100 000	23 (35.9)	41 (64.1)	64
Total paper currencies	75 (23.3)	262 (77.7)	337

Table 2. Bacterial Isolates From Paper Currencies With Different Denomination

Contaminated Bacteria	Currency Denomination Rials; No. (%)								
	500	1000	2000	5000	10 000	20 000	50 000	100 000	Total
Bacillus spp.	1 (14.3)	4 (44.4)	6 (46.2)	16 (55.2)	16 (43.2)	30 (50)	25 (37.9)	15 (36.6)	113 (43.1)
Coagulase-negative staphylococci	5 (71.4)	2 (22.2)	4 (30.8)	10 (34.4)	15 (40.5)	19 (31.6)	27 (40.9)	17 (41.5)	99 (37.8)
Staphylococcus aureus	0 (0.0)	2 (22.2)	2 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	4 (6.1)	0 (0.0)	8 (3.1)
Enterococci species	1 (14.3)	0 (0.0)	1 (7.7)	0 (0.0)	1 (2.7)	4 (6.7)	5 (7.6)	2 (4.9)	14 (5.3)
Escherichia coli	0 (0.0)	1 (11.1)	0 (0.0)	2 (6.9)	4 (10.8)	7 (11.7)	2 (3.0)	4 (9.8)	20 (7.6)
Klebsiella spp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.7)	0 (0.0)	2 (3.0)	1 (2.4)	4 (1.5)
Pseudomonas species	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	1 (2.4)	2 (0.8)
Shigella species	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)	2 (0.8)

Table 3. Antibiotic Resistance Patterns in Bacteria Isolated From Paper Currencies

Bacterial isolates of currencies	Antibiotics Tested Resistance (%)									
	AMP	AMC	GEN	NA	ATM	FOX	CIP	VA	E	T
Bacillus spp	62.8	35.1	14.7	-*	-	44.1	9.7	23.4	41.3.	48.1
coagulase-negative staphylococci	62.7	27.1	25.3	49.5	-	15.1	3.4	20.2	60.7	45.4
Staphylococcus aureus	75.0	62.5	50.0	50.0	-	25.0	0.0	25.0	50.0	50.0
Enterococci species	78.6	71.4	42.9	54.1	-	64.3	14.3	42.9	85.7	42.9
Escherichia coli	75.0	60.0	40.0	90.0	90.0	60.0	20.0	-	65.0	40.0
Klebsiella spp	100.0	50.0	50.0	75.0	25.0	75.0	25.0	-	75.0	75.0
Pseudomonas species	100.0	100.0	50.0	100.0	100.0	100.0	50.0	-	100.0	50.0
Shigella species	100.0	50.0	0.0	50.0	50.0	50.0	0.0	-	50.0	50.0

[a] Were not tested.
Abbreviations: AMP, ampicillin; AMC, amoxicillin; GEN, gentamicin; NA, nalidixic acid; ATM, aztreonam; FOX, cefoxitin; CIP, ciprofloxacin; VA, vancomycin; E, erythromycin; T, tetracycline.

environments, and therefore are considered as an important tool for transmission of pathogens within a population.

The results of our study showed that 77.7% of our studied currency notes were contaminated with bacteria. This finding is almost in agreement with the report of Saudi Arabia by Alwakeel and Nasser.[17] However, higher levels of contamination were reported by Basavarajappa et al[4] in India and Pope et al[18] in the United States. *Bacillus* spp was the most common bacterial isolates in our contaminated currency notes. In accordance with our findings in other reports, the environmental bacteria and gram-positive normal flora especially *Bacillus* spp have been introduced as common contaminant of paper currency.[12] *Bacillus* species have been found to cause significant infections in people with certain risk factors, while they scarcely cause infection in healthy people. The second most common bacterial isolate in current study was coagulase-negative staphylococci. In a survey, coagulase-negative staphylococci were reported as the most common contaminant of one-dollar bills which are widely used in the United States.[18] The high frequency of contamination with coagulase-negative staphylococci among currency notes is logical because this bacterium is the main normal flora of human skin. However, coagulase-negative staphylococci are not considered as true pathogens, but immunocompromised patients are at risk of severe infection with them. The other isolates included in the current study were food-borne pathogens or causes of serious nosocomial infections. Studies on the persistence of pathogens on paper currency showed that currency notes are considered as a potential cause of food-borne diseases.[19] There are evidences of isolation of food-borne pathogens including *Salmonella* spp, *E. coli* and *S. aureus* from the banknotes of different countries.[1] The investigators have reported that many gram-positive and gram-negative bacteria such as enterococci spp, *S. aureus*, *E. coli*, *Klebsiella* spp, *P. aeruginosa*, and *Serratia marcescens* have the potential to survive on surfaces for several months.[20] Among the bacterial isolates with more pathogenicity, we identified *S. aureus* which can cause wide range of infections in both hospitals

and community.[21] The isolation of *S. aureus* as one of the bacterial isolates from paper currency in different countries has been reported.[12,14,18,21] Considering the fact that *S. aureus* present in human nose and skin, isolation of this bacteria in paper currency is acceptable where the hygienic practices could be in low level. One result with particular importance in the present study was the isolation of *Shigella* species from the contaminated currency notes, showing fecal contamination. The results of a study on 1280 banknotes in 10 different countries showed that the currency papers with lower index value have higher microbial contamination.[1] In other studies also shown that currency notes with lower denomination carry more microbial agents due to more exchange among people with different occupations.[7,21] In an investigation in Iran, the 50 000 Rials paper notes had the lowest bacterial contamination.[14] In our study, unlike the other studies, the highest contamination was seen in 50 000 Rials paper notes whereas the 500 Rials paper notes had the lowest contamination. The difference between our results and other reports is due to that in our country currency notes with lower denomination like 500 Rials are not used regullary and the most exchanged paper notes are 50 000 Rials. Also small sample size could be the reason, as in our study only 13 pieces of 500 Rials paper notes were evaluated.

The person to person transmission of antimicrobial resistant bacteria via paper currency could be of great concern. Our antibiotic susceptibility test results revealed that there is resistance to clinically important antibiotics such as vancomycin in enterococci species, *S. aureus* and coagulase-negative staphylococci which could be a threat to cause vancomycin resistant enterococci (VRE) and vancomycin resistant *S. aureus* (VRSA) infections especially among immunocompromised persons. Although more accurate tests such as determination of MICs values are needed for identification of vancomycin resistant strains. In a survey in Saudi Arabia, it has been shown that all bacterial species were susceptible to gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole, and in agreement with our results, resistance was seen against ampicillin in *Enterobacter cloacae*, *Acinetobacter iwoffii* and *Staphylococcus warneri*.[17]

Finally, the present study revealed that the bacterial contamination among Iranian paper currency in circulation especially those obtained from certain sources including shops and bus stations is high and in most cases, these bacterial isolates are antibiotic resistant strains. Although here the clinical importance of bacterial contamination of currency notes is not investigated, transmissions of these bacteria especially antimicrobial resistant strains to immunosuppressed people may lead to serious infections, and they are notable.

Authors' Contributions

FF: the study design, management and supervision; ED, HA, SMSS, MMM and AS: sampling, processing and performing the conventional and procedures; MZ: provided advice, read and arranged the final manuscript.

Ethical Approval

This study was carried out according to the ethical framework for research at the Kashan University of Medical Sciences and was approved by the Ethical Committees of Kashan University of Medical Sciences.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Financial Support

The current study was financially supported by Deputy for research and technology affairs of Kashan University of Medical Sciences (grant No. 93109).

Acknowledgments

We are thankful to Mr. Mirzaei and Mr. Hadad for their technical assistance during the course of this study.

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