Global Advanced Research Journal of Microbiology (ISSN: 2315-5116) Vol. 4(3) pp. 027-030, March, 2015
Available online http://garj.org/garjm/index.htm


*Full Length Research Paper*

# Occurrence of ESBL, MRSA and VRE pathogens in contaminated banknotes in Makkah, Saudi Arabia

***Omar B. Ahmed and Bassam H. Mashat**

Department of Environmental and Health Research, The Custodian of the Two Holy Mosques Institute for Hajj and Umrah, Umm Al-Qura University, Makkah, Saudi Arabia.

Accepted 03 March, 2015

**Paper currency has an important role in the transmission of pathogenic microorganisms and presents a moderate risk to public health. Multidrug-resistant (MDR) bacterial strains are increasingly being reported. The objective of this study was determination of microbial contaminants and resistant pathogenic strains mainly ESBL, MRSA and VRE of banknotes in circulation in holy Makkah city, Saudi Arabia. A total of 50 samples of Saudi banknotes were studied for isolation of contaminating bacteria, ESBL production, MRSA and VRE using standard laboratory procedures. Ninety two percent of the sample banknotes were found to be contaminated by bacteria or fungi. A total of 67 bacterial isolates were obtained. Three fungal species were isolated, two *Candida albicans* and one *Aspergillus* sp. The highest number of isolates were coagulase negative Staphylococci (23.9%) followed by *E. coli* (19.4%) while *Salmonella* and *Proteus* species were the least isolate (1.5%). ESBL producing bacteria were found to be 13.4% while MRSA and VRE were found to be 10.4% and 4.5% respectively. The circulating currency could serve as a vehicle for transmission of drug resistant pathogens or potential organisms. Disinfection of the currencies in banks and proper hand hygiene and overall hygiene are recommended.**

**Keywords:** ESBL, MRSA, VRE, Banknotes, Currency, Contaminated Money.

## INTRODUCTION

Contaminated banknote is any currency that has been exposed to some biological or chemical contaminant. Banknotes serve as an ideal breeding ground for microorganisms. Contaminated banknotes or coins could be a public health risk as it is handled by everyone (Kuria et al., 2009; Lamichhane et al., 2009). Contamination may occur from the general public in community, hospitals and food outlets or during use, storage or production. During handling, contamination from the skin, anal region, wounds, nasal secretions and aerosols are main sources of transferring microorganisms to banknotes during handling (Kuria et al., 2009). Therefore, banknotes has an important role in the transmission of pathogenic microorganisms and presents a risk to public health. The biological contamination could be virus, fungal spores or bacteria. The microorganisms known to be transferred by circulating banknotes in community are *S. epidemidis*, *Streptococci*, *Pseudomonas*. *aeruginosa* and *klebsiellaaeregenes*, (Ahmed. et al., 2010). The main microorganisms that are known to be transferred by circulating banknote recovered from hospitals are *S.*

*Corresponding Author's Email: abuaglah1@hotmail.com

*aureus salmonella*, *E. coli* (Angelakis et al., 2014; Feglo et al., 2010) and that recovered from food outlets is *S. aureus*. It is noted that there is an increasing multidrug-resistant (MDR) bacterial strains where the bacteria demonstrate resistance to more than one class of antibiotic (Kaur., 2012). Few studies had focused on antibiotic resistance among bacteria recovered from banknotes (Gabrie et al., 2013). Contaminated banknotes and coins contribute to the transmission of multi-drug resistant microorganisms in the community such as MRSA and vancomycin-resistant enter ococci (VRE) (Gedik et al., 2013; Tolba et al., 2007). It is also noted that antimicrobial resistance such extended-spectrum beta lactamases (ESBL) producing *E. coli* and *Klebsiella* spp has steadily been increasing (Gedik et al., 2010). Today, disinfectants used for human hygiene purposes make an essential component of human health care approach as they aim to remove and destroy harmful and undesirable microorganisms. The objective of this study is to determine contamination and resistant pathogenic strains mainly MRSA, ESBL and VRE of Saudi banknotes in circulation within holy Makkah, Saudi Arabia.

## MATERIAL AND METHODS

A total of 50 samples of Saudi banknotes were studied from February to May 2014. The banknotes were obtained from banks, supermarkets, restaurants, cafeterias, butcheries, traders and buyers in open-air markets, and filling-stations in and around the holy Makkah city. Fifty banknotes each of SR1, SR5, SR10, SR50 and SR100 denominations were collected using a septic sampling method. The SR500 denomination was excluded. Samples were randomly obtained and banknotes were placed in a sterile polyethylene bag. During banknotes sampling, each individual was requested to drop banknotes into a sterile plastic bag. The bag was sealed and the individual was given a replacement banknote, then all the collected samples were taken to the laboratory. All banknotes were in good physical condition. Each banknote was placed in 10-mL of nutrient broth and shaken for 5–10 min on and subsequently incubated at 37°C for 24 h. For isolation of bacteria, a sterile, cotton-tipped swab was introduced in the incubated nutrient broth and was then inoculated onto Blood and Mac Conkey agar plates and incubated for 24 h at 37°C. Bacterial species were identified using standard laboratory procedures. Isolates were further confirmed using the API 20E test strips following the manufacturer's instructions (Biomérieux, France). For identification of fungi, a loopful of incubated nutrient broth was inoculated onto Sabouraud dextrose agar plates and incubated at 37°C for 48–72 h. Identification of fungal isolates was based on growth characteristics and the lacto-phenol cotton blue reaction. The microbial susceptibility to antimicrobial agents was performed on Muller Hinton agar (oxoid) by modifying Kirby Bauer disc diffusion method according to the CLSI (CLSI., 2008). Gram negative bacilli were tested against 30µg each of the third generation cephalosporins, ceftazidime, cefotaxime and ceftriaxone. The inoculated plates were incubated for 16-18 hours at 37°C. Isolates found resistant or with decreased susceptibility (Intermediate) to any one of the third generation cephalosporins antibiotics were selected for the presence of ESBLs. Gram positive cocci were tested against vancomycin (30 µg), oxacillin (1 µg). Oxacillin disc was put on a Mueller Hinton Agar (MHA) (oxoid) plate supplemented with 4% NaCl. The inoculated plates were incubated for 16-18 hours at 37°C. Isolates found resistant or with decreased susceptibility (Intermediate) to oxacillin or vancomycin were suspected as methicillin or vancomycin resistant respectively. Suspected isolates of *Gram negative bacteria were tested for ESBL.* The test was performed as described by Jarlier et al (1988). The isolates were inoculated on MH-agar plates. Discs containing respectively ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30µg) and aztreonam (30 µg) disks were placed 20 mm (center to center) away from a disc containing a 20 µg amoxicillin/10 µg clavulanic acid disk before overnight incubation at 37°C. ESBL production was considered positive when the clavulanate mediated enhancement of the activity of an indicator drug produced a keyhole effect and regarded as a phenotypic confirmation of the presence of ESBL. For diagnosing MRSA, suspected isolates of Gram *positive cocci* were processed by culture in Muller-Hinton agar plates containing 4% NaCl and 6 µg/ml of oxacillin. A swab which was dipped in a suspension of the isolate and then was deposited as a spot on the agar surface and it was incubated at 35°C for 24 h. Any growth after 24 hr was interpreted as oxacillin resistance (Pramodhini et al., 2011). Suspected isolates of *Enterococcus* were screened for vancomycin resistance. The concentration of vancomycin in vancomycin screening agar was 6µg/ml. A swab which was dipped in a suspension of the isolate and then was deposited as a spot on the agar surface and it was incubated at 35°C for 24 h. Any growth after 24 hr was interpreted as vancomycin resistance.

## RESULTS

Forty-six (92%) of the sampled banknotes were found to be contaminated by bacteria or fungi. Most of the banknotes had more than one microbial contaminant. A total of 67 bacterial isolates were obtained from the 50 samples analyzed. Three fungal species were isolated, two *Candida albicans* and one *Aspergillus* sp. The highest number of isolates were coagulase negative Staphylococci (23.9%) followed by *E. coli* (19.4%) while *Salmonella* and *Proteus* species were the least isolate (1.5%) (Table1). ESBL producing bacteria were found to

**Table 1. Type and frequency of bacteria isolated from banknotes currency**

| Species | No (%) | ESBL (No) | MRSA (No) | VRE (No) |
|---|---|---|---|---|
| Coagulase negative *staphylococ* | 16 (23.9%) | - | - | - |
| *E. coli* | 13 (19.4%) | 7 | - | - |
| *S. aureus* | 10 (15%) | - | 7 | - |
| *Bacillus sp* | 8 (12%) | - | - | - |
| *Enterococci species* | 7 (10.4%) | - | - | 3 |
| *Klebsiellasp* | 5 (7.5%) | 2 | - | - |
| *α-hemolytic Streptococci* | 4 (6%) | | | |
| *Shigella sp* | 2 (3%) | - | - | - |
| *Salmonella sp* | 1 (1.5%) | - | - | - |
| *Proteus sp* | 1 (1.5%) | - | - | - |
| Total | 67 (100%) | 9 (13.4%) | 7 (1o.4%) | 3 (4.5%) |

be 13.4% while MRSA and VRE were found to be 10.4%and 4.5% respectively.

## DISCUSSION

The present study show the extent and the level of contamination of Saudi Arabia's banknotes with pathogenic microorganisms. Other findings have shown that viable pathogenic organisms (viruses, bacteria, and fungi) can be isolated on the surfaces of banknotes and coin currency (Kuria et al., 2009; Lamichhane et al., 2009 ) (1, 2). Forty-six (92%) of the sampled banknotes analyzed were contaminated by bacteria or fungi. A total of 67 bacterial isolates were obtained from the 50 samples analyzed. Most of the sample banknotes analyzed were contaminated by bacteria, some had more than one microbial contaminant. That was near to Igumbor et al (2007) who found 96% of the sample banknotes were contaminated by bacteria or fungi. In the present study, 10 different bacterial species and three fungal species were isolated. The SR500 denomination was excluded because low denomination notes were more likely to be contaminated than higher denomination notes. This may be due to socio-economic factors (Uneke and Ogbu 2007). Other researchers have detected similar or near contamination levels (Hosen et al., 2006; Singh et al., 2002; Tswana et al., 2000; Bosch and Steyn 1997; Ugobor 1998). The degree of contamination and type of isolates on the money vary depending on the area, season, environmental conditions, age of money, local community flora, the general hygiene level of the population and personal hygienic practices. Coagulase Negative Staphylococci and *E. coli* species were the highest isolates while *Salmonella* and *Proteus* species were the least isolated. Although coagulase negative Staphylococci typically reside on healthy human skin and mucus membranes and rarely cause disease, they have been increasingly recognized to cause clinically significant infections (Rupp and Archer 1994). *E. coli* was the second most common isolated bacterium. This situation may be attributed to the possibility that some people disregard hand wash after using toilets. Isolates which are resistant to commonly used antibiotics represents risks and public health hazards to the community and individuals handling currency notes (Alemu 2014). In the present study, ESBL producing bacteria were found to be 13.4% while MRSA and VRE were found to be 10.4% and 4.5% respectively.. There are several investigations confirm that antibiotic resistant bacteria contaminate banknotes and might play an important role in the transmission of pathogenic microorganisms as well as in the spread of drug-resistant organisms. Banknotes collected from meat sellers in market places of Tanga city of Tanzania shows that 28.125% *S.aureus* isolates were MRSA (Neel 2012). Some studies detected 100% of *S.aureus* isolates were multidrug resistance (Neel 2013). Gedik et al. (2013) investigated survival of MRSA, VRE and ESBL-producing *E. coli* on banknotes from various countries. They found that the survival rate of bacteria was highest in the Romanian currency while other currencies enabled the survival of ESBL and VRE with variable survival rates (Gedik et al., 2013). It could be concluded that, the circulating currency could serve as a vehicle for transmission of drug resistant pathogens or potential organisms. Disinfection of the currencies in banks and proper hand hygiene and overall hygiene are recommended.

**REFERENCES**


Ahmed S, Parveen S, Nasreen T, Feroza B (2010). Evaluation of the Microbial Contamination of Bangladesh Paper Currency Notes (Taka) in Circulation. Advances in Biological Research. 4 (5): 266-271.

Alemu A (2014). Microbial Contamination of Currency Notes and Coins in Circulation: A Potential Public Health Hazard." *Biomedicine and Biotechnology*. 2 (3): 46-53.

Angelakis E, Azhar E I, Bibi F, Yasir M, Al-Ghamdi A K, Ashshi A M, Elshemi A G, Raoult D (2014). Paper Money and Coins as Potential Vectors of Transmissible Disease. Future Microbiol. 9(2):249-261

Bosch AMT, Steyn PL (1997). Microorganisms of South African banknotes. *S. Afr. J. Food. Sci.Nutr.* 9 (1): 24-26.

CLSI Wayne, USA:. Clinical and Laboratory Standards Institute (CLSI) (2008). Performance standards for antimicrobial susceptibility testing. 18th ed 2008.

Feglo P, Nkansah M (2010). Bacterial load on Ghanaian currency notes. African J. Microbiol., Res. 4(22):2375-2380.

Gabrie EMl, Coffey A, Mahony JMO (2013). Investigation into the prevalence, persistence and antibiotic resistance profiles of staphylococci isolated from euro currency. J. Applied Microbiol. 115: 565-571.

Gedik H, Voss TA, Voss A (2013). Money and transmission of bacteria. Antimicrob Resist Infect Control. 2:22.

Gedik H, Yahyaoğlu M, Yörük G, Fincancı M (2010). Extended-Spectrum Beta- Lactamase production Rates of *Klebsiella* spp and *Escherichia coli* strains isolated from infections and fecal samples of healthy people. Infectious Diseases in Clinical Practice . 18:104-106.

Hosen, JM, Sarif DI, Rahman MM, Azad MAK (2006). Contamination of Coliforms in Different Paper Currency Notes of Bangladesh. Pak. J. Bio. Sci.. 9: 868-870.

Igumbor EO, Obi C L, Bessong PO, Potgieter N, Mkasi TC (2007). Microbiological analysis of banknotes circulating in the Venda region of Limpopo province, South Africa. South African Journal of Science. 103: 365-366.

Jarlier V, Nicola MH, Fournier G, Philippon A (1988). Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis . 10:867–878.

Kaur T, Nafissi N, Wasfi O, Sheldon K, Wettig S, Slavcev R (2012). Immunocompatibility of bacteriophages as nanomedicines. J Nanotechnol

Kuria JK, Wahome RG, Jobalamin M, Kariuki SM (2009). Profile of bacteria and fungi on money coins. East African Med. Journal. 86 (4): 151-155.

Lamichhane J, Adhikary S, Guatam P, Maharjan, R (2009). Risk of Handling Paper Currency in Circulation Chances of Potential Bacterial Transmittance. Nepal Journal of Science and Technol. 10:161-166.

Neel R (2012). Multidrug Resistance of Isolates of Methicillin Resistant Staphylococcus Aureus (MRSA) in Paper Currency Notes From Meat Sellers in Tanga, Tanzania. *Int J Life Sc Bt and Pharm Res*. 1 (4): 9-13.

Neel R (2013). Multidrug Resistance of Isolates of Methicillin Resistant Staphylococcus Aureus (MRSA) in Paper Currency Notes From Restaurants and Hotels in Lusaka in Zambia. *International Journal of Pharmacy and Pharmaceutical Sciences*. 5 (1): 363-366.

Pramodhini S, Thenmozhivalli PR, Selvi R, Dillirani V, Vasumathi A, Agatha D (2011). Comparison of Various Phenotypic Methods and *mecA*Based PCR for the Detection of MRSA. *Journal of Clinical and Diagnostic Research*. 5 (7): 1359-1362.

Rupp ME, Archer GL (1994). Coagulase-negative staphylococci: pathogens associated with medical progress.Clin Infect Dis. 19 (2) : 231-43.

Singh DV, Thakur K, Kalpana, Goel A (2002). Microbiological surveillance of currency. Indian J Med Microbiol. 20:53

Tolba O, Loughrey A, Goldsmith CE, Millar BC, Rooney PJ, Moore JE (2007). Survival of epidemic strains of nosocomial- and community-acquired methicillin-resistant *Staphylococcus aureus* on coins. *Am. J. Infect. Control* . 35 (5): 342–346.

Tswana SA, Chihota VN, Moyo SR, Chigonde SC, Nzara MJ (2000). Contamination of Zimbabwean banknotes by microorganisms – a pilot study. *S. Afr. J. Epidemiol. Infect.* 15 (1), 14–18.

Ugobor OF (1998). Prevalence of microorganisms in Nigerian currencies. *J. Natl Appl. Sci.* 4: 18-22.

Uneke CJ, Ogbu O (2007). Potential for parasite and bacteria transmission by paper currency in Nigeria. J Environ Health. 69: 54-60