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Bacterial and Fungal Contamination of Saudi Arabian Paper Currency and Cell Phones

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ABSTRACT

Paper currency is used in exchange for goods and services and so the circulation of paper currency from one individual to another potentially spreads microorganisms. If pathogenic bacteria contaminate these currencies, the rate of infection and death rate from these infectious agents will continue to rise. This study was conducted to survey the bacterial and fungal contamination of paper money and cellular phones samples in Riyadh, Saudi Arabia in May 2010. Each bill and phone was prepared using standard procedures. Of the 390 currency notes, 282 (72.3%) were contaminated with bacteria which included *Aspergillus niger*, *Aspergillus flavus*, *Candida* spp., *Penicillium* spp. and *Rhizopus* spp. and bacteria which included *Enterobacter cloacae*, *Klebsiella ozaenae*, *Cedecea davisae*, *Yersinia pseudotuberculosis*, *Acinetobacter iwoffii*, *Staphylococcus warneri* and *Enterobacter agglomerans*. All isolated bacterial species were sensitive to ciprofloxacin, gentamicin, ticarcillin, tobramycin and trimethoprim-sulfamethoxazole. The use of commercial disinfectants was only effective against *Aspergillus niger* and *Rhizopus* spp. Cellular phones were contaminated with *Micrococcus* and *Staphylococcus* species and no fungal species were isolated from the sampled cellular phones. Prevention is the hallmark of reducing morbidity and mortality. An efficient health awareness campaign program should be fully implemented to inform the public of the hazards of contaminated paper currencies and even mobile phones.

Key words: Microorganism, fungi, paper bills, mobile phones, Saudi Arabia, contamination

INTRODUCTION

Paper currency is used repeatedly in exchange for goods and services (Oyero and Emikpe, 2007). Due to this, the circulation of paper currency from one individual to another potentially spreads microorganisms. It is a very good vector for transmission of diseases (Wamae, 2009). If these currencies are contaminated by pathogenic bacteria, the rate of infection and death rate from these infectious agents will continue to rise (Pinner *et al*., 1996; Pope *et al*., 2002). Communicable diseases spread through contact with fomites and transfer through paper currencies is a very possible route (Pope *et al*., 2002). A review of the medical literature reveals few investigations involving the bacterial contamination of money in the United States.

A study conducted in Australia in 2010 among currencies from 10 different countries showed that the lower the index value of the money, the higher the typical bacterial content of the currency. They further showed that the age of the notes and the material that was used to produce the notes influence the number of bacterial contamination (Vriesekoop *et al*., 2010). Lower denomination notes harbor the greatest bulk of infectious agents since they are exchanged more than higher denomination notes (Uneke and Ogbu, 2007). Several studies have reported bacterial

contamination from 60% to as much as 96% on tested paper currencies. A study conducted in Egypt in 2005 showed bacterial counts above 5.0 cm^2 in 65% of tested bills (El-Dars and Hassan, 2005). Pope *et al.* (2002) isolated pathogenic or potentially pathogenic organisms from 94% of $1 bills and Basavarajappa *et al.* (2005) found 96 out of 100 currencies contaminated with bacteria, in particular K. *pneumoniae*, *E. coli*, *S. aureus*, *Pseudomonas species* and *S. typhi*, fungal and protozoa. Virulent genes of *Staphylococcus aureus* were isolated from paper currencies and these strains show resistance to the more common antibiotics (Kumar *et al.*, 2009). This study was conducted to determine the amount of contamination among Saudi Arabian currency notes and used mobile phones and perform an antimicrobial susceptibility testing on the isolated organisms.

MATERIALS AND METHODS

Samples of paper money from Saudi Arabia were collected during May 2010. The 200 pieces 1 riyal paper bills, 50 pieces of 5 riyal paper bills, 40 pieces of 10 riyal bills and 20 pieces of 20, 50, 100, 200 and 500 riyal bills each. Each bill was washed in 500 mL of sterilized distilled water and soaked in the distilled water solution for 3 h and then 1 mL was taken from each specimen and placed in Potato Dextrose Agar (PDA) or Blood Agar Plates (BAP). Three replicas were made from each plate. The bills were torn into small pieces and placed directly on the PDA or BAP plate. The bills were swabbed with sterilized cotton Q-tips previously wetted with sterilized distilled water. The swabs were then used to streak the PDA or BAP.

Thirty samples of used mobile phones were collected. The cell phones were swabbed with sterilized cotton Q-tips previously wetted with sterilized distilled water. The swabs were then used to streak the PDA or BAP plates. Three replicas were made of each plate.

Isolation and identification of microorganisms: Bacteria was isolated and identified via the Analytical Profile Index (API) system. From the source material, samples were taken and plated on to prepared BAP in duplicates for isolation of microorganism. Plates were incubated for 24 to 48 h at 37°C. Once colonies were grown on BAP, they were observed for mixed cultures. Mixed cultures were separated, isolated and replated into different sets of BAP to obtain pure cultures. These were incubated for another 24 to 48 h at 37°C. After pure cultures were obtained, colonies were observed for size, texture, color and hemolytic reactions. Colonies were gram stained and individual cells observed under the microscope. The bacterial species were identified using these isolated colonies. The pathogenic or potential organisms were isolated and further identification of enteric organisms was done using the API 20E system (Analytical Profile Index, BioMerieux, Durham, NC, USA). Colonies from the BAP were harvested and mixed with 0.5 mL McFarland standard until turbidity of the solution and a bacterial suspension was obtained. Using a sterile pipette, the bacterial suspension was inoculated to rehydrate each of the wells making sure that the end of the pipette touched the end of the cupule, allowing capillary action to draw the fluid into the well as the bulb was slowly squeezed. This eliminated the possibility of bubble formation in the wells. Inoculation of specific test wells was done according to manufacturer's instructions. The strips were incubated for 18 to 24 h at 37°C. Test results were logged to an API 20E chart to determine the bacterial code which was compared to the API 20E Codebook for accurate identification of the organism.

Many other additional tests were done for further identification of the microorganism: API 20 Staph, API 20 Strep, API 20 Anaerobes and other morphological, biochemical and physiological tests. The API system was then used with the additional tests to collect the necessary data for the exact identification of the microorganisms.

Fungi were identified using the dilution plate method. Three types of media were used: (1) Glucose-Czapeck's agar medium in which glucose (10 gm L^{-1}) replaced sucrose and potato dextrose agar medium, chloramphenicol (20 μg mL^{-1}) and Rose Bengal (30 ppm) were added to suppress bacterial growth; (2) Sabouraud's agar and (3) Rose Bengal. Identification was performed by cultural and morphological characteristics. The fungal isolates were identified whenever possible in the original Petri-dish culture. When this was not possible, fungi were subcultured and stored for later identification.

This experiment also tested the effect of three types of commercial disinfectants on growth of fungi isolated from bills. The products used were antiseptic liquid, hand gel sanitizer and liquid soap sanitary brought from local stores in Riyadh, Saudi Arabia. Fifty milliliters of each commercial disinfectant was added to one liter of sterilized distilled water. From the mixture, 1 mL was taken from each specimen and placed in a central hole of a PDA plate. One milliliter of hand gel sanitizer was directly placed into a central hole made in plates that had been inoculated with the fungi. Three replicas were made from each plate. The plates were incubated for 48-72 h at 28°C (Dulger *et al*., 2004).

RESULTS

Of the 390 currency notes on which fungal and bacterial analysis was conducted, 282 (72.3%) were contaminated with bacteria and fungi. Fungi isolated from the currency notes included *Aspergillus niger*, *Aspergillus flavus*, *Candida* spp., *Penicillium* spp. and *Rhizopus* spp. *Aspergillus niger* was the most common isolate and was present in both old and new contaminated currency notes, followed by *Aspergillus niger* which was isolated in old 1 and 5 riyals, new 1 riyals and 50 and 100 Saudi riyals (Table 1). Bacteria isolated from paper currencies included *Enterobacter cloacae*, *Klebsiella ozaenae*, *Cedecea davisae*, *Yersinia pseudotuberculosis*, *Acinetobacter iwoffii*, *Staphylococcus warneri* and *Enterobacter agglomerans* (Table 2).

Contamination was related to the denomination of currency. Contamination was most prevalent among the old and new 1 Saudi riyal notes (37.2%), old and new 5 Saudi riyal notes (31.5%), 10 Saudi riyal notes (12.0%), 50 Saudi riyal notes (10.4%) and 100 Saudi riyal notes (8.9%). None of the 500 Saudi riyal notes had any bacterial and/or fungal contamination (Table 1).

Bacterial susceptibility analysis on the paper currencies that revealed bacterial species showed that all the isolated bacterial species were sensitive to Ciprofloxacin, Gentamicin and Trimethoprim-Sulfamethoxazole. Amikacin, Cefepime, Meropenem and Tobramycin were found to inhibit growth of all isolated bacteria except for *Staphylococcus warneri* which was susceptible to a combination of Amoxycillin and Clavulanic acid, Cefutaxime, Ciprofloxacin and Gentamicin and Trimethoprin-Sulfamothoxazole (Table 2).

Results from testing the effect of three types of commercial disinfectants on the growth of fungi isolated from bills showed that the antiseptic liquid and the sterilized liquid soap were able to

Table 1: Fungi isolated from Saudi currency notes

Fungal isolates	Old 1 SR	New 1 SR	Old 5 SR	New 5 SR	Old 10 SR	New 10 SR	50 SR	100 SR	500 SR
Aspergillus niger	+	+	+	+	+	+	+	+	NG
Aspergillus flavus	+	+	+				+	+	NG
Candida spp.	+	+	+	+		+	+		NG
Penicillium spp.		+		+					NG
Rhizopus spp.					+				NG

N: SR-: Saudi riyal; NG-: No growth; +: Indicates presence

Table 2: Bacteria isolated from Saudi currency notes and results of antibiotic susceptibility testing

		Antibiotics tested																					
Currency	Bacterial isolates	Am	Ao	Ap	Ce	Cf	Cg	Cn	Cp	Cu	Cx	Cz	Eb	Es	Ga	Ge	Im	Me	Ni	Pi	Ti	To	Tr
1 SR	*Enterobacter cloacae*	+				+	+	+	+	+	+	+			+	+	+	+		+	+	+	+
5 SR	*Klebsiella ozaenae*	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+		+	+	+	+
10 SR	*Cedecea davisae*	+	+	+	+			+		+	+	+			+	+	+	+		+	+	+	+
	Yersinia pseudotuberculosis	+	+	+	+			+				+			+	+		+		+	+	+	+
20 SR	*Acinetobacter iwoffii*	+			+	+			+	+		+				+		+			+	+	+
100 SR	*Staphylococcus warneri*		+				+					+				+							+
	Enterobacter agglomerans	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+		+	+	+	+
200 SR	*Acinetobacter iwoffii*	+				+	+			+		+				+		+			+	+	+

+: Indicates sensitivity to the antibiotic (Am: Amikacin, Ao: Amoxiclav, Ap: Ampicillin, Ce: Cefazolin, Cf: Cefepime, Cg: Cefotaxime, Cn: Cefotetan, Cp: Ceftazidime, Cu: Ceftriaxone, Cx: Cefuroxime, Cz: Ciprofloxacin, Eb: ESBL-a, Es: ESBL-b, Ga: Gatifloxacin, Ge: Gentamicin, Im: Imipenem, Me: Meropenem, Ni: Nitrofurantoin, Pi: Piperacillin, Tic: Ticarcillin, To: Tobramycin, Tr: Trim. sulfa)

Table 3: Fungi isolated from Saudi currency notes and results of testing with sanitizer gels and liquids

Fungal isolates	Antiseptic liquid	Hand gel sanitizer	Liquid soap (sterilized)
Aspergillus niger	+	-	+
Aspergillus flavus	-	-	-
Penicillium spp.	-	-	-
Rhizopus spp.	+	-	-

N: (+): With effect, (-) No effect

Table 4: Bacteria isolated from Saudi currency notes and results of antibiotic susceptibility testing

		Antibiotics tested															
Sample phones	Bacterial isolates	Ao	Ap	Ce	Cu	Cz	Cl	Er	Ge	Ni	No	Ox	Ri	Sy	Te	Tr	Va
	Staphylococcus warneri	+		+	+	+	+	+	+			+	+	+	+	+	+
Phone B	*Staphylococcus simulans*	+		+	+	+	+	+	+			+	+	+	+	+	+
Phone C	*Staphylococcus hominis*	+	+	+	+	+	+		+			+	+	+	+	+	+
Phone D	*Staphylococcus hominis*	+	+	+	+	+	+		+			+	+	+	+	+	+

N: +: Indicates sensitivity to the antibiotic (Ao: Amoxiclav; Ap: Ampicillin, Ce: Cefazolin, Cu: Ceftriaxone, Cz: Ciprofloxacin, Cl: Clindamycin; Er: Erythromycin, Ge: Gentamicin, Ni: Nitrofurantoin, No: Norfloxacin, Ox: Oxacillin, Ri: Rifampin, Sy: Synercid, Te: Tetracycline, Tr: Trim.Sulfa, Va: Vancomycin)

disinfect notes contaminated with *Aspergillus niger* and *Rhizopus* spp. but not *Aspergillus flavus* and *Penicillium* spp. The hand gel sanitizer showed no effect on the isolated fungal species (Table 3).

Bacteria isolated from sample cellular phones included *Micrococcus* spp., *Staphylococcus simulans*, *Staphylococcus warneri* and *Staphylococcus hominis*. When antibacterial susceptibility tests were performed on these bacterial isolates, *Micrococcus* spp. showed resistance to all antibiotics used. The three *Staphylococcal* species (*warneri*, *simulans* and *hominis*) showed sensitivity to most of the antibiotics, especially Co-amoxiclav, Cefazolin, Ceftriaxone, Ciprofloxacin, Clindamycin, Oxacillin, Rifampin, Tetracycline, Trimethoprim-Sulfamethoxazole and Vancomycin. Nitrofurantoin and Norfloxacin showed no effect on these bacterial species. No fungal species were isolated from the sampled cellular phones (Table 4).

DISCUSSION

The results of this study confirmed that currency notes could serve as a vector for disease transmission of pathogenic microorganisms and fungal elements. We found that 72.3% of our tested currency notes were contaminated with bacteria and fungi. This is higher than that previously reported from Egypt (El-Dars and Hassan, 2005) but is lower than the Indian (Basavarajappa *et al*., 2005) and the US reports (Pope *et al*., 2002).

Bacterial agents found to contaminate our local currency notes included *Enterobacter cloacae*, *Klebsiella ozaenae*, *Cedecea davisae*, *Yersinia pseudotuberculosis*, *Acinetobacter iwoffii*, *Staphylococcus warneri* and *Enterobacter agglomerans*. *Enterobacter cloacae* can cause lower respiratory tract infections, skin and soft tissue infections, urinary tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis and ophthalmic infection. Of all Enterobacter infections, *E. cloacae* infection is associated with mortality rates of up to 87% (Rossi *et al*., 2006). *Klebsiella ozaenae* is considered only a colonizer of the nasopharynx or a putative cause of ozena (atrophic rhinitis) (Malik and Singh, 2010). However, recent reports show that it can be an invasive pathogen, especially in immunocompromised hosts and can cause pituitary abscess (Danilowicz *et al*., 2008). *Cedecea davisae* is reported to cause super-infection especially in immunocompromised patients. They are new members of Enterobacteriaceae which have inherent resistance to antibiotics (Abate *et al*., 2011; Mawardi *et al*., 2010; Batista *et al*., 2011). *Yesinia pseudotuberculosis* has been reportedly isolated from postaneurysmal prosthetic vascular infection, showing the potential affinity of this species to endovascular tissues (Loiez *et al*., 2010). Furthermore, *Yersinia pseudotuberculosis* is known to be resistant to killing by human neutrophils (Laws *et al*., 2011). *Acinetobacter iwoffii* can cause bacterial meningitis in adults with a high mortality rate (Laguna-del Estal *et al*., 2010). *Staphylococcus warneri* does not generally cause serious infections in human because it has a low virulence; however, it was discovered recently that *S. warneri* could cause a rare endocarditis in humans even without risk factors (Kini *et al*., 2010; Saeed and Ahmed, 2009). *Enterobacter agglomerans*, also known as *Pantoea agglomerans*, is a new cause of primary pneumonia and peritonitis (Kahveci *et al*., 2011). Fortunately, bacterial susceptibility analysis on the paper currencies that revealed bacterial species showed sensitivity to most of the antibiotics tested (Adeleke and Omafuvbe, 2011). In this regard, this serves as a guideline for management of patients who will harbor infections caused by these agents.

Present study also showed fungal elements (Sharma, 2011) including *Aspergillus niger*, *Aspergillus flavus, Candida* spp., *Penicillium* spp. and *Rhizopus* spp. *Aspergillus niger* is known to produce mycotoxin, specifically ochratoxin A. Though it is less likely to cause human disease, when many spores are inhaled, it can cause Aspergillosis (Schuster *et al*., 2002). *Aspergillus flavus*, on the other hand, is a more pathogenic species of Aspergillus than *A. niger*, since it produces aflatoxin which causes pulmonary infection and cancer when it becomes invasive (Ozhak-Baysan *et al*., 2010). Candida can cause serious endocarditis (Halawa *et al*., 2011; Madhavan *et al*., 2011), Penicillium can cause pneumonia (Ekenna *et al*., 2007) and Rhizopus can be an agent for zygomycosis and eye infections (Antoniadou, 2009).

Staphylococcus species isolated in our sample cellular phones are regarded as contaminants. However, these agents may cause severe infections in humans, especially those who are immunocompromised and are on chemotherapeutic agents. The implication of this study is that the majority of the people are exposed to pathogenic bacteria and fungal elements that can cause serious diseases (Tambekar *et al*., 2008).

In Saudi Arabia, the use of paper currency is widespread, from ordinary small stores to supermalls. A major past-time of people in Saudi Arabia is shopping followed by dining. Although most shopping centers have well-maintained restrooms where people can wash their hands after shopping and before dining, not all individuals practice hand washing. Furthermore, even use of sanitizing gels and liquids does not guarantee 100% elimination of these harmful pathogens, as shown in our result (Table 3) (Olasehinde *et al*., 2008). Therefore, prevention is still the hallmark of reducing morbidity and mortality. An efficient health awareness campaign program should be fully implemented to inform the public of the hazards of contaminated paper currencies and mobile phones.

CONCLUSION

Saudi currency notes and mobile phones are highly contaminated with enteric bacteria and fungi, most of which can cause disease in humans. An effective prevention campaign to reduce morbidity and mortality should be fully implemented to inform the public of the hazards of contaminated paper currencies and mobile phones, through proper hand washing procedures after handling money.

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