Full Length Research Paper

Bacteriological Analysis and Antibiogram of Pakistani Paper Currency Notes in Circulation in Karachi, Sindh, Pakistan

Razim Ali1*, Sayed Zaheer Abbas2, Zulkifal Hussain3, Khalil Hussain3, Amir Hayat2, Adnan Khan1

1University of Karachi, Faculty of Science, Department of Microbiology, Karachi 75270, Pakistan
2University of Sindh, Faculty of Natural Sciences, Microbiology/ IARSCS, Jamshoro 76080, Pakistan
3Kohat University of Science and Technology, Biological Sciences, Department of Microbiology, Kohat 26000, Pakistan
*Corresponding Author: razimalikhan@gmail.com

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Abstract. Paper currency notes are used as a medium for exchange of goods and other economic services in daily transaction. These notes serve like vector in transmission of pathogenic microorganisms and antimicrobial resistance while passing through many hands and contaminated surfaces. The study was carried out from May to September 2014 on 167 samples of Pakistani paper currency (PKR) of different denominations RS 10, RS 20, RS 50, RS 100 and RS 500. These notes were collected in Karachi city, Pakistan from artisan and non-artisan groups and analyzed for total bacterial flora, pathogenic bacteria and antimicrobial activity of pathogenic isolates. In all, 537 bacterial isolates were detected of 10 different species, from which Staphylococcus aureus were highly rated 53.1%, Enterobacters 46.5%, Bacillus cereus 33.7%, E. coli 31.7%, Klebsiella 24%, Pseudomonas aeruginosa 13%, Salmonella 9.6%, Streptococcus pyogenes 6.8%, Vibrio cholera 6.6% and Shigella species 6%. Isolates were also checked for antimicrobial resistance against Amoxicillin, Erythromycin, Gentamicin, Penicillin, Tetracycline and Streptomycin. Staphylococcus aureus and Streptococcus pyogenes were found resistant to all drugs used in the study. From our study, it was concluded that Pakistani currency notes represent a vehicle for pathogenic microbes and have significant role in public health infectious diseases and bacterial resistance.

Keywords: Antibiogram, Contamination, Paper currency, Staphylococcus aureus.

1. INTRODUCTION

Money is used as a pricing medium from decades for exchange of goods, services and in economic payments activities in worldwide. In everyday transaction, currency is handled by different categories of individuals with varying hygienic and health standards. Money is also stored under varying personal and environmental conditions (Shama and Sumbali, 2014). In Pakistan, paper currency is mostly damaged by squeezing, stapling, paper taping and writing on papers currency notes. People with unhygienic conditions will definitely contaminate notes while keeping it in their pockets, socks, shoes, under the carpets to introduce microbes to the currency notes. Other ways to contaminate notes like wetting of fingers with saliva, use of contaminated lubricants during counting or handling notes with food contaminated hands are the sources for bacteria to increase not only the chances for microbial introduction but may also increase the risk of infection from contaminated individuals. Further, infectious materials can also be transferred via notes from droplets during coughing, sneezing and touching with previously contaminated hands or placement on dirty surfaces (Shama and Sumbali, 2014, Saeed and Rasheed, 2011).

Currency notes pass through many hands and especially lower denomination notes receiving most handling due to their frequent use. These paper notes are contaminated by different microorganisms and provide large surface area for microbial breeding and act as vectors for infectious microbes. Previous studies on currency notes reported high rates of microorganisms during circulation especially normal skin flora (Staphylococcus aureus) and Gram positive bacteria (Bacillus species) (Saeed and Rasheed, 2011, Mailafia et al., 2013, Hakim et al., 2014, Khan et al., 2013). Currency notes are the common source for the transmission of pathogens among humans and environment. In daily life transactions, bacterial contaminants on notes are faced by different individual hands and these microbes are introduced on hands that may become a source for infection(s). Bacteria may be transferred to humans either by hand-to-hand contact, or via contaminated inanimate
objects like fomites and food items. In developing countries, these routes of transmission are of great importance in public health where the frequency of individual infection is a general indication of local hygiene and environmental sanitation status (Hakim et al., 2014, Yakubu et al., 2014, Ahmed et al., 2010).

Collected data of last 20 years on currency notes showed that these pathogenic microorganisms can survive, transfer and have the potential to cause serious food borne and other health issues. Currency notes could serve as a vehicle for microorganisms and considered to be the reservoir for enteric pathogens (Hakim et al., 2014). If these notes are contaminated with pathogenic microbes, then it is probably spread and will enter the antibiotic era to become resistive against anti-bacterial agents, in this way capability of drugs become low during recovery of infection(s) (Ahmed et al., 2010). The present study was conducted to evaluate the extent of bacterial contamination on Pakistani paper currency notes collected from different categories of people in Karachi city.

### 2. MATERIALS AND METHODS

#### 2.1. Sample collection

The study was conducted from May to September 2014. A total of 167 paper currency samples of different denominations of 10, 20, 50, 100 and 500 PKR (Figure 2) were randomly collected in Pakistan Karachi city from different artisan groups of meat, fish, fruits, vegetables, bakers, hotels and non artisans were students, teachers, beggars, office workers, bus conductors and car drivers. Samples were collected directly into sterile bags and the individuals were given replacement notes. New minted currency notes were used as control samples obtained from commercial banks.

The sample bags were immediately sealed and transported to the Department of Microbiology laboratory, University of Karachi for analysis. Samples were analyzed on the same day as collected for bacterial analysis. These currency notes were graded as new, moderate, old and torn on the basis of physical appearance as shown in Table 1.

#### 2.2. Qualitative bacterial analysis

Analysis was performed according to the method of Ahmed et al. Bacterial suspensions were prepared by placing each denomination in a separate 250 ml screw caped bottle containing 100 ml of sterile nutrient broth. Then each bottle after gentle agitation was incubated for 6 – 8 hours at 37°C. Thereafter, the broth cultures were plated by pour plate method on selective and differential agars on overnight incubation at 37°C. Bacterial isolations were studied using special nutritional requirements were placed

<table>
<thead>
<tr>
<th>Denominations</th>
<th>New</th>
<th>Moderate</th>
<th>Old</th>
<th>Torn</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKR 10</td>
<td>18</td>
<td>20</td>
<td>16</td>
<td>8</td>
<td>62</td>
</tr>
<tr>
<td>PKR 20</td>
<td>13</td>
<td>17</td>
<td>13</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>PKR 50</td>
<td>9</td>
<td>11</td>
<td>7</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>PKR 100</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>PKR 500</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>56</td>
<td>41</td>
<td>21</td>
<td>167</td>
</tr>
</tbody>
</table>

#### Table 2: Percent prevalence of bacterial isolates per denomination of currency notes

<table>
<thead>
<tr>
<th>Denomination</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKR 10 (n=62)</td>
<td>50.8</td>
<td>38.5</td>
<td>85.4</td>
<td>48.9</td>
<td>31.9</td>
<td>16.8</td>
<td>18.7</td>
<td>12.9</td>
<td>14.8</td>
<td>12.9</td>
</tr>
<tr>
<td>PKR 20 (n=50)</td>
<td>39.5</td>
<td>32.2</td>
<td>68.2</td>
<td>44.4</td>
<td>29.8</td>
<td>15.3</td>
<td>18.0</td>
<td>10.6</td>
<td>9.4</td>
<td>13.8</td>
</tr>
<tr>
<td>PKR 50 (n=31)</td>
<td>52.7</td>
<td>37.9</td>
<td>56.3</td>
<td>37.9</td>
<td>29.2</td>
<td>9.8</td>
<td>12.0</td>
<td>9.8</td>
<td>9.8</td>
<td>3.6</td>
</tr>
<tr>
<td>PKR 100 (n=16)</td>
<td>39.6</td>
<td>33.3</td>
<td>31.5</td>
<td>37.5</td>
<td>29.2</td>
<td>6.3</td>
<td>16.7</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>PKR 500 (n=8)</td>
<td>50.0</td>
<td>16.7</td>
<td>24.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>


2.2. Qualitative bacterial analysis

Analysis was performed according to the method of Ahmed et al. Bacterial suspensions were prepared by placing each denomination in a separate 250 ml screw caped bottle containing 100 ml of sterile nutrient broth. Then each bottle after gentle agitation was incubated for 6 – 8 hours at 37°C. Thereafter, the broth cultures were plated by pour plate method on selective and differential agars on overnight incubation at 37°C. Bacterial isolations were studied using special nutritional requirements were placed
for enrichment in liquid broth. *Salmonella* was exposed to dual enrichment media before plating or streaking (Vriesekoop et al., 2010 and Jay et al., 1997). Colonies were identified on the basis of bacterial morphology, staining and biochemical features as described by Cheesbrough, 1984.

### 2.3. Determination of total bacterial load

Total bacterial load was determined by making serial dilutions in buffered peptone water from test suspension (Lakmini and Madhujith, 2012). From each dilution, starting with highest dilution (after agitation) 1 ml of test dilution was plated by pour plate method onto plate count agar (Oxoid, Hampshire England) in duplicates. The plates were then incubated for overnight 24 hours at 37°C. Colonies were enumerated as colony forming units and total bacterial load was calculated from each currency note.

### 2.4. Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed according to Hakim et al., 2014. A standard bacterial suspension was prepared in sterile Mueller-Hinton broth with 8 hours 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 (Oxoid, Hampshire England) 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 National Committee for Clinical Laboratories, 2000 and Koneman et al., 1997.

![Fig. 1: Percent prevalence of bacterial isolates from Pakistani currency notes](image)

### Table 3: Average viable bacterial count of different denominations

<table>
<thead>
<tr>
<th>No.</th>
<th>Denomination PKR</th>
<th>Number Examined</th>
<th>Bacterial Counts CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>08</td>
<td>1.24 x 10^3</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>08</td>
<td>3.09 x 10^4</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>08</td>
<td>4.39 x 10^4</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>08</td>
<td>3.56 x 10^2</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>08</td>
<td>2.14 x 10^3</td>
</tr>
</tbody>
</table>

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Percent prevalence of bacterial isolates

This study showed Pakistani paper currency contamination by various bacterial pathogens and their burden on each particular denomination. All denominations were analyzed for 10 different bacterial species (Table 2). 537 bacterial strains were isolated from 167 samples and it was found that lower currency denominations PKR 10, PKR 20 and PKR 50 have greater contamination frequency than higher denominations PKR 100 and PKR 500. It has been observed that *Staphylococcus aureus* were found more frequent 53.1%, *Enterobacters* 46.5%, *Bacillus cereus* 33.7%, *E. coli* 31.7%, *Klebsiella* species 24%, *Pseudomonas aeruginosa* 13%, *Salmonella* species 9.6%, *Streptococcus pyogenes* 6.8%, *Vibrio cholera*
6.6% and *Shigella* species 6%. New minted notes were used as a control showed no bacterial count.

It was concluded from the results that smaller denominations (RS 10, 20, 50) were highly contaminated than larger denominations (RS 100 and 500) that was accordance to the previous findings (Saeed and Rasheed, 2011, Mailafia et al., 2013, Hakim et al., 2014, Khan et al., 2013 and Ahmed et al., 2010). High contamination of smaller units was due to frequent use in daily transaction and damaged notes were held with sticky paper tapes that are considered to be dangerous (Yakubu et al., 2014).

In present study we observed *Staphylococcus aureus*, *Enterobacter* species and *Bacillus cereus* were found most frequent 53.1%, 46.5% and 33.7% respectively, which are nearer to those as obtained in previous studies (Mailafia et al., 2013, Hakim et al., 2014 and Khan et al., 2013). *S. aureus* species are normally present on body skin and can be transmitted by hand contact and respiring aerosols or droplets, water, wounds and abrasions in the skin. High prevalence of *S. aureus* has clinical significance in human pathogenicity and has the potential to cause urinary tract infections (UTIs) and conjunctivitis (Saeed and Rasheed, 2011). *Bacillus species* are endospore formers present in soil and environment that can cause food borne gastrointestinal (GIT) infections (Kotiranta et al., 2000). Money can be contaminated by *Bacillus species* due to its handling by dirty hands and placement on dirty surfaces. *E. coli* was observed 31.7% which agree with that of Yakubu et al., 2014 who worked on Naira notes in Nigeria. Prevalence of *Pseudomonas aeruginosa*, *Salmonella* and *Klebsiella sp*. were similar to those of previously calculated (Khan et al., 2013, Hakim et al., 2014, Yakubu et al., 2014 and Awe et al., 2010). *Vibrio cholera*, *Streptococcus pyogenes* and *Shigella species* were matching to those obtained previously by Ahmed et al., 2010 and Feglo, 2010.

![Fig. 2: Pakistani paper currency notes](image)

### 3.2. Total viable count

The average total viable bacterial counts of isolates from denominations were $1.24 \times 10^5$ CFU/ml (highest) for PKR 10, $3.09 \times 10^4$ for PKR 20, $4.39 \times 10^5$ for PKR 50, $3.56 \times 10^5$ for PKR 100 and $2.14 \times 10^2$ CFU/ml (lowest) for PKR 500 (Table 3).

### 3.3. Antibiotic susceptibility

Bacterial isolates were checked against six antimicrobial agents (Figure 3). *Staphylococcus aureus* and *Streptococcus pyogenes* showed resistant to all drugs used in the study that are closely agree with those stated by Mailafia et al., 2013 and Awe et al., 2010. We also observed highest susceptibility rate for Gentamicin against *Escherichia coli* (6.3%), *Pseudomonas aeruginosa* (6.3%), *Vibrio cholera* (14.3%) and *Shigella species* (16.7%), which are likely follow the previous findings (Mailafia et al., 2013, Awe et al., 2010, Jafer et al., 2015 and Akond et al., 2015).

Tetracycline was susceptible to *Bacillus cereus* (12.5%) and *Shigella* (16.7%) only. *Salmonella* species were susceptible to Erythromycin (18.8%) and Streptomycin (6.3%) but resistant to Amoxicillin
(68.8%), Gentamicin (81.3%), Penicillin (81.3%) and tetracycline (87.5%), this result agree with those of Hakim et al., 2014 and Awe et al., 2010. It was also observed in the study that all isolates were resistant to Penicillin and Amoxicillin drugs (Hakim et al., 2014, Oluduro et al., 2014, Ayandele and Adeniyi, 2011, Awe et al., 2010, Mailafia et al., 2013 and Akond et al., 2015).

The study showed wide distribution of bacteria in environment that suggest the necessity of further investigation on Pakistani paper currency to analyze the source of pathogenic bacterial contamination. Further investigation on pathogenic fungi will add more information in this study.

![Fig. 3: Percent antibiotic resistance of bacterial isolates from Pakistani currency notes](image)

4. CONCLUSION

It was concluded from the current study that currency notes serve as a vehicle for pathogenic microorganisms. In the study, we found high frequency of *Staphylococcus aureus* (53.1%) and *Bacillus cereus* (33.7%) on paper currency notes that have the potential to cause urinary tract and gastrointestinal tract infections in humans. These currency notes pass through many hand contacts, dirty surfaces and other environmental sources that make it contaminated. It was also concluded that paper currency notes have significance role in bacterial resistance, as isolated bacterial species from notes were mostly resistive against antimicrobial drugs.

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Razim Ali is M.Phil student in Microbiology at University of Karachi (Pakistan). He received BS in Microbiology from Kohat University of Science and Technology, Pakistan (2009). Since graduation, he has been involved with commercial organizations (SGS Pakistan Private Limited and Ismail Industries Limited) in Pakistan, having more than 4 years professional experience in analytical microbiology. Email: razimalikhan@gmail.com

Sayed Zaheer Abbas is biology lecturer at The Country School & College Karachi (Pakistan); he is also teacher’s coordinator in biological sciences in college. He graduated from Kohat University of Science and Technology, with bachelor degree in Microbiology in 2011. After graduation he moved to industrial city Karachi where he worked for one year as a Food Microbiologist (2012-2013) in Ismail Industries Limited. He is currently studying M.Phil (Microbiology) from University of Sindh, (Pakistan), and a research scholar at Microbiology Department, University of Karachi. E-mail: zabbas1144@yahoo.com

Zulkifal Hussain is a senior Microbiologist at Ulker international (PTB Food company private limited, Pakistan), had completed his BS in Microbiology from Kohat University of Science and Technology (KUST), Pakistan. Since graduation he has been involved in analytical microbiology and medical clinical diagnostic laboratories nationally and internationally. He has two publications in molecular biology on viral genome extraction and detection in national and international journals. Throughout professional life he had established commercial microbiology and food testing microbiology laboratories. Email: zeejan_micro@yahoo.com

Khalil Hussain obtained his BS degree in Microbiology (2008) from Kohat University of Science and Technology (Pakistan). Currently he is working as a Senior Microbiologist in a reputable Pakistani pharmaceuticals manufacturing firm named as Shaigan Pharmaceuticals Private Limited located at Rawalpindi, Pakistan. He also served as a microbiologist in different national and multinational firms with more than 6 years of professional experience in the field of microbiology. Email: khalil.kust110@gmail.com

Amir Hayat is a PhD candidate in the field of analytical chemistry combined with food and agriculture at Institute of Advance Research Studies in Chemical Sciences (IARSCS) University of Sindh Jamshoro, Pakistan. He received his Master degree in Organic Chemistry from Federal Urdu University of Science, Arts and Technology Karachi, Pakistan in 2008. His current research is related to development of new analytical method on HPLC for the determination of different biogenic compounds and their accumulation during germination and fermentation in agriculture samples like Rice. He has published several scientific publications in well reputed international journals in the field of food and agricultural analytical chemistry. Email: hayat.amir1@gmail.com

Dr Adnan Khan serves as an Assistant professor of Microbiology at University of Karachi (Pakistan), the largest public sector university in Pakistan. He has been associated with this institute since 2005 and involved in teaching related to Immunology, antimicrobial chemotherapy, medical microbiology and virology. He obtained PhD in Microbiology in 2010 from University of Karachi. He has been involved to work with various international research groups in United States, Canada, China and Italy on several research projects. He has several scientific publications in international and national journals. Email: adnankh@uok.edu.pk