Sudan Journal of Medical Sciences
Volume 13, Issue no. 3, DOI 10.18502/sjms.v13i3.2955
Production and Hosting by Knowledge E

Research Article

Identification of *Proteus mirabilis* on Banknotes Using 16s rRNA Gene in Khartoum State

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Abstract

Background: The presence of pathogenic bacteria in circulated currency was recorded as a public health hazard. In this study, all examined Sudanese banknotes (100%) were found to be contaminated by gram-negative bacteria. *Proteus mirabilis* were recovered from 10 examined notes (22.2%, *f* = 10), *E. coli* (13.3%, *f* = 6) and *Klebsiella spp.* (8.9%, *f* = 4) were also identified. Only the most resistant *P. mirabilis* isolate was identified using culture-based and 16S rRNA gene sequencing techniques. **Methods:** *Proteus* isolates were identified phenotypically and tested for their susceptibility to 16 of commonly used antibiotics, then most resistant isolate was confirmed genotypically via 16S rRNA gene amplification and sequencing. Bioinformatics analysis using BLAST for sequence similarity search, Clustal W program for multiple sequence alignment, MEGA7 software for phylogenetic analysis. Tree was constructed to show the evolutionary relationships of the obtained sequence with similar sequences in the databases using. **Results:** The obtained sequence was found to be 100% identical to *P. mirabilis* 16S rRNA gene using BLAST. The phylogenetic tree was constructed to show the evolutionary relationships of the obtained sequence with similar sequences in the databases using MEGA7 software, and the closest strain was found to be *P. mirabilis* strain from India (EU411047). **Conclusion:** This study has shown that some currency notes circulated at Khartoum transportation are carriers of antimicrobial-resistant *P. mirabilis* that could be potential source for their transmission in public.

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Received 2 August 2018
Accepted 15 September 2018
Published 24 September 2018

Production and Hosting by Knowledge E

Editor-in-Chief:
Prof. Mohammad A. M. Ibnouf

OPEN ACCESS

Keywords: *Proteus mirabilis*, banknotes, 16S rRNA, Khartoum State

1. Introduction

Proteus mirabilis causes 90% of Proteus infections [1]. It has been implicated in meningitis, empyema, osteomyelitis, and gastroenteritis. Also, it frequently causes health care-associated infections of the urinary tract, surgical wounds and lower respiratory tract [2]. Identification of bacteria is frequently performed by isolation of the organisms and study of their phenotypic characteristics, including gram staining, morphology, culture requirements, and biochemical reactions [3]. However, these traditional techniques have some disadvantages. Firstly, they are time-consuming and laborious. Secondly, a variability of culture due to different environmental conditions may lead to ambiguous results. Thirdly, a pure culture is required to undertake identification making the identification of fastidious and unculturable bacteria difficult and sometimes impossible [4]. Moreover, phenotypic systems cannot account for the variable characteristics observed among members of the same species, resulting in poor precision upon repeated testing [5]. Construction of phylogeny tree based on phenotypic methods may be more difficult because it needs a comparison of a large set of independent co-varying characters, and it is difficult to perform cladistic analyses based on it [6]. In addition to the fact that phenotype represents a very small part of each organism's genome [7].

16S rRNA gene is highly conserved within species and among species of the same genus, and hence, can be used as an alternative technique for identification of bacteria to the species level [8, 9].

Specific properties of the 16S rRNA gene include its ubiquitous distribution, mosaic structure [10], and relative stability that qualify it to be used in the taxonomic assignment and phylogenetic relationship determination [11]. The genotypic bacterial identification begins with nucleotide sequence analysis of the PCR product of specific gene/s followed by a comparison of these sequences with known sequences stored in a database [12, 13]. The 16S rRNA gene is suitable for identification since its size (1500 bp) is large enough for bioinformatics purposes [14]. Direct sequencing of 16S rRNA genes from environmental samples has become a standard and convenient method of assessing microbial population abundance [15], structure, and function in microbial communities [16, 17].

This study aimed to identify the bacterial isolates recovered from Sudanese banknotes using culture-based and 16S rRNA gene sequencing techniques.

2. Materials and Methods

2.1. Sources of banknotes

Prospective study was conducted in March 2016 in which a total of 45 Sudanese banknotes were randomly collected from different sources; Hospitals (f = 15), food sellers (f = 15), transporters (f = 15). Five 'mint' brand new notes were collected from the bank before being touched with bankers to be used as controls. These new banknotes were included in the study to ensure whether the banknotes are contaminated from their source or during handling in the circulation. The banknotes studied were two, five, ten, twenty and fifty Sudanese pounds. All banknotes were in good shape and not damaged and transported into sterile plastic petri dishes to the microbiological laboratory for bacterial isolation, identification and antibiotic sensitivity testing.

2.2. Phenotypic analysis of bacteria from Sudanese Banknotes

2.2.1. Bacterial extraction and biochemical tests

The banknotes were moistened with sterile distilled water, swabbed both sides by cotton-tipped swab and directly inoculated on 5% blood agar and MaCconkey agar plates. For bacterial growth observation, the inoculated plates were incubated aerobically at 37°C for 24 hrs. The cultural characteristic of the recovered contaminants of each banknote were examined and the suspected colonies were stained by gram-staining method. The gram-negative, rod-shaped bacilli and swarm colonies on media were sub-cultured in nutrient agar plates for further identification tests. Biochemical tests (catalase test, oxidase test, motility, indole test, urease, glucose fermentation test and lactose fermentation test) were carried out [17].

2.2.2. Susceptibility tests

Samples were tested for their susceptibility to these antibiotics: Amoxicillin (AMX) 25 µg, Amoxyclav (AMC) 30 µg, Cephalexin (CN) 30 µg, Cefuroxime (CXM) 30 µg, Ceftriaxone (CTR) 30 µg, Ceftazidime (CAZ) 30 µg, Gentamicin (GEN) 10 µg, Kanamycin (K) 30 µg, Co-trimoxazole (COT) 25 µg, Erythromycin (E) 5 µg, Azithromycin (AZM) 15 µg, Ciprofloxacin (CIP) 30 µg, Levofloxacin (LE) 5 µg, Nitrofurantoin (NIT) 200 µg, Chloramphenicol (C) 30 µg, and Meropenem (MEM) 10 µg using Kirby–Bauer disc diffusion method [18].

2.3. Genotypic analysis of bacteria from Sudanese Banknotes

2.3.1. DNA extraction

More resistant isolate recovered from examined Sudanese banknotes was subjected to genotypic analysis. Three colonies from pure sub-cultured isolate was suspended in 200µl 1x Phosphate Buffer Saline (PBS). Genomic DNA was extracted using chelex extraction protocol [19] and quantified using (GeneQuant, Amersham) according to manufacturer's protocol.

2.3.2. 16S rRNA gene amplification

Forward primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 1495R (5'-CTACGGCTACCTTGTTACGA-3') [20] were used to amplify 16S rRNA gene. The PCR reaction mixture (iTRON, Korea) were as follows: 2.5 unit Taq DNA polymerase, 2.5 mMmm dNTP, 1x reaction Buffer (10x), 1x Gel loading buffer, 5µl of template DNA, 1µL of primer (27F:10pmol/µ), 1µl of primer (1495R10 pmol/µl) and 15µl of distilled water mixed in a final volume of 25µl. The PCR conditions were; 94°C for 5 minute, 37 cycles of 94°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute, and final extension at 72°C for 10 minutes. Amplification was done using thermocycler system (BIO-RAD, USA).

2.3.3. Detection of amplified product by agarose gel electrophoresis

The PCR products were assessed by gel electrophoresis. One percent agarose gel with 0.5% ethidium bromide were mixed and 5µl of PCR product and ladder (iTRON, Korea) were transferred into separated wells in the gel. The electric current was allowed at 100 volts for 30 minute, while UV Trans-illuminator (UVP, USA) was used for the observation of DNA bands [21]. The obtained fragment was sent to be sequenced in Microgen company (Seoul, South Korea).

2.4. Bioinformatics analysis

The 16S rRNA gene sequence was analyzed using BLAST [22] with non-redundant (nr) NCBI GenBank database to find closely related bacterial 16S rRNA gene sequences. The 16S rRNA gene sequence of *Proteus* isolate was submitted to NCBI database with accession number (KY 039269). Nineteen sequences of high-quality 16S rRNA gene were selected from SILVA database [23] and subjected for multiple sequence alignment

using Clustal W program [24] within BioEdit software [25], with total alignment score 1661794. The phylogenetic tree was constructed according to maximum likelihood method using MEGA 7 software [26].

3. Results

3.1. Results of susceptibility tests

All *P. mirabilis* isolates were completely sensitive to Meropenem (MEM), Levofloxacin (LEV), Ciprofloxacin (CIP), Gentamicin (GEN), Ceftazidime (CAZ), Ceftriaxone (CTR), Cephalexin (CN), and Amoxyclav (AMC), as well as they were completely resistant to Azithromycin (AZM) and Erythromycin (E). However, resistant strains of *P. mirabilis* isolates to Kanamycin (K), Chloramphenicol (C), Nitrofurantoin (NIT), Co-trimoxazole (COT), Cefuroxime (CXM) and Amoxicillin (AMX) 25µg were also detected, as shown in Table 1.

Table 1: Susceptibility test of *P. mirabilis* isolates using disc diffusion assay.

antibiotics	MEM	C	LEV	CIP	K	GEN	NIT	COT	AZM	E	CAZ	CTR	CXM	CN	AMC	AMX
Sensitive isolates	100%	86%	100%	100%	57%	100%	0%	43%	0%	100%	0%	100%	57%	100%	100%	57%
Resistant isolates	0%	14%	0%	0%	14%	0%	57%	57%	100%	100%	0%	0%	43%	0%	0%	43%
Intermedia te isolates	0%	0%	0%	0%	29%	0%	43%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table 1 shows the results of susceptibility test of P. mirabilis isolates toward commonly used antibiotics using disc diffusion assay.

3.2. DNA quantification results

Table 2: DNA concentrations in µg/l using GeneQuant.

Sample No.	Protein µg/l	Ratio	ssDNA µg/l	Purity %
Sample 1	0.4	1.2	39.0	69
Sample 2	0.3	1.4	39.5	75
Sample 3	0.4	1.3	48.0	72
Sample 4	0.3	1.3	38.8	74
Sample 5	0.3	1.5	52.0	82

Table 2 illustrates the DNA concentrations of five samples using GeneQuant machine. Only Sample 5 was adopted for further processes.

3.3. Fragments separation using gel electrophoresis

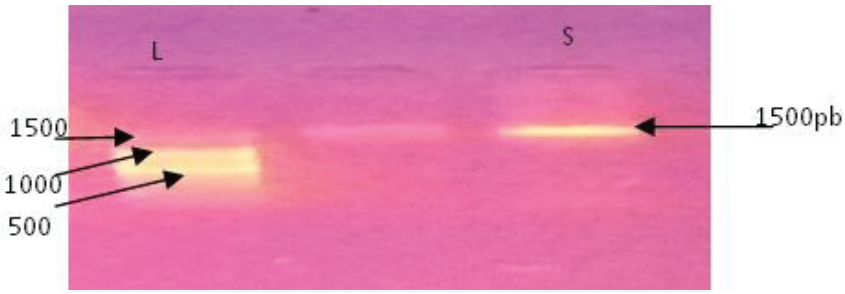

Figure 1: Amplified fragment detection, L: ladder (500 bp), S: Sample 5.

Figure 1 represents separated band of Sample 5 compared with bands of ladder using gel electrophoresis.

3.4. Multiple sequence alignment

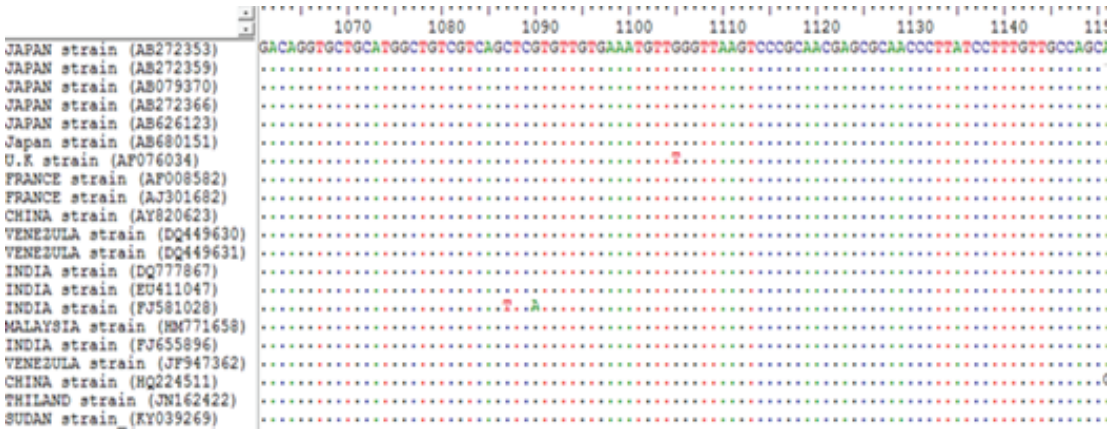

Figure 2: Multiple sequence alignment of 19 databases 16S rRNA genes with 16S rRNA gene of *P. mirabilis* isolate (KY 039269) from Sudan.

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3.5. Phylogenetic analysis

Evolutionary history was inferred using maximum likelihood method based on Tamura-Nei model. The tree was constructed by MEGA7 software [26]. 16S rRNA gene of *P. mirabilis* isolate from Sudanese notes (KY 039269) is closely related to16S rRNA gene of *P. mirabilis* isolate from India (EU411047) as shown in Figure 3.

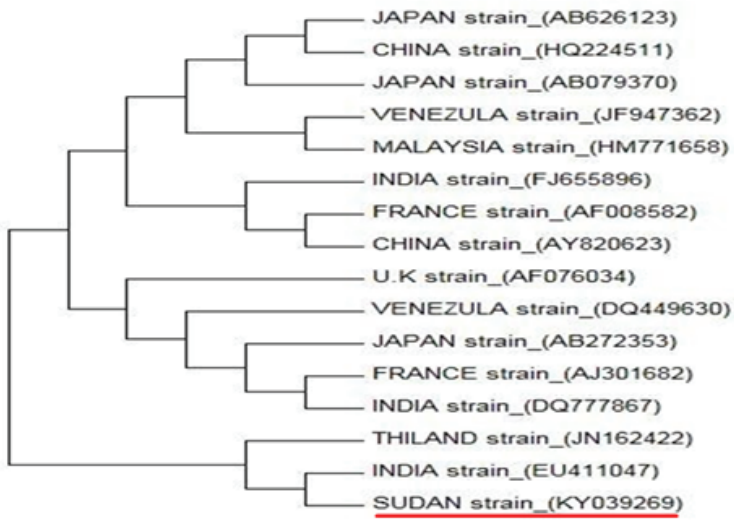

Figure 3: Phylogenetic tree of 16S rRNA gene of *P. mirabilis* isolate from Sudanese notes (KY 039269).

4. Discussion

The result of this study represents that 22.2% of tested Sudanese banknotes were contaminated by *P. mirabilis* that is agreed with results from India and Nigeria where it was found in banknotes at low frequency [27, 28]. Banknotes contamination rate is incredibly high at Khartoum transport circulation that may be due to more frequent exchange, poor hygiene and environmental conditions such as temperature and humidity [29]. The risk of transmission of pathogenic microorganisms and diseases by banknotes was reported worldwide, but most of these studies were performed in the tropical or sub-tropical regions of the world [30, 31]. Banknotes and coins were reported as carriers of potentially pathogenic microorganism since the beginning of the seventies [32, 33].

The 16S rRNA gene that is commonly used for identification and classification of microbes from environmental samples [34, 35] was used in this study for identification of banknotes isolates after biochemical tests. Banknotes-associated microorganisms detected previously using 16S rRNA gene [36, 37]. It can be used for identification for bacterial strains more accurately than it is possible with phenotypic analysis, allowing identification of strains that are poorly culturable or do not exhibit distinguishable phenotypic traits [12]. In this study, the most resistant isolate toward examined antibiotics was chosen for DNA extraction and sequencing for identification and also to detect changes in 16S rRNA sequence that may explain their resistance to these antibiotics [38]. After quantification of extracted DNA, gel electrophoresis and DNA sequencing,

the obtained 756 pb sequence with the aid of BLAST and multiple sequence alignment confirmed that the isolate was *P. mirabilis* with 100% identity to the database sequence. It is known that the full-length or near-full-length 16S rRNA gene sequences is crucial for making confident genus and species-level taxonomic placements [39]. The 16S rRNA gene could be used as phylogenetic marker because of its functional constancy and the presence of conserved and variable sequence regions evolving at very different rates. It is also critical for the concurrent universal amplification and measurement of both close and distant phylogenetic relationships. So, it can be used in the assignment of close relationships at the genus level [12], and in several cases at the species level [39, 40]. Phylogenetic analysis using maximum likelihood method based on Tamura-Nei model [26] showed that the closest strain to our isolate is *P. mirabilis* identified in India (EU411047). Sequencing independent techniques such as pulsed-field gel electrophoresis (PFGE) [41, 42], random amplified polymorphism deoxyribonucleic acid (RAPD) [43], and restriction fragment length polymorphism (RFLP) [44] have broad availability and cost lower than other typing methods; however, 16S rRNA sequencing has high discriminatory power, 100% typeability and good reproducibility [45].

P. mirabilis isolates were found to be resistant to some antibiotics. A lot of studies detected *P. mirabilis* as Extended-spectrum beta-lactamases ESBLs producer [46]. A number of studies had focused on antibiotic resistance among bacteria recovered from banknotes [47]. Isolates that are resistant to commonly used antibiotics represents risks and public-health hazards to the community and individuals handling banknotes [48].

5. Conclusion

Our study has shown that some currency notes circulated at Khartoum transportation are carriers of antimicrobial-resistant *P. mirabilis* that could be potential source for their transmission in public. Awareness about the health risk of contaminated currency and proper hand hygiene might be necessary. Notes sterilization and electronic credit cards use are recommended.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

The authors would like to thank the Africa City of technology for their support.

References

[1] Coker, C., Bakare, O. O., and Mobley, H. L. (2000). H-NS is a repressor of the *Proteus mirabilis* urease transcriptional activator gene ureR. *Journal of Bacteriology*, vol. 182, no. 9, pp. 2649–2653.

[2] Bahashwan, S. A. and H.M. El Shafey. (2013). Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. *European Scientific Journal*, vol. 9, no. 27.

[3] Woo, P. C. Y., Ng, K. H. L., Lau, S. K. P., et al. (2003). Usefulness of the MicroSeq 500 16S ribosomal DNA-based bacterial identification system for identification of clinically significant bacterial isolates with ambiguous biochemical profiles. *Journal of Clinical Microbiology*, vol. 4, no. 5, pp. 1996–2001.

[4] Tshikhudo, P., Nnzeru, R., Ntushelo, K., et al. (2013). Bacterial species identification getting easier. *African Journal of Biotechnology*, vol. 12, no. 41, pp. 5975–5982.

[5] Brisse, S., Stefani, S., Verhoef, J., et al. (2002). Comparative evaluation of the BD Phoenix and VITEK 2 automated instruments for identification of isolates of the *Burkholderia cepacia* complex. *Journal of Clinical Microbiology*, vol. 40, no. 5, pp. 1743–1748.

[6] Sneath, P. H. A. (1989). Analysis and interpretation of sequence data for bacterial systematics: The view of a numerical taxonomist. *Systematic and Applied Microbiology*, vol. 12, no. 1, pp. 15–31.

[7] Logan, N. A. (2009). *Bacterial Systematics.* John Wiley & Sons.

[8] Relman, D. A. (1999). The search for unrecognized pathogens. *Science,* 284, no. 5418, pp. 1308–1310.

[9] Olsen, G. J. and Woese, C. R. (1993). Ribosomal RNA: A key to phylogeny. *FASEB Journal*, vol. 7, no. 1, pp. 113–123.

[10] Van De Peer, Y., Chapelle, S., De Wachter, R., et al. (1996). A quantitative map of nucleotide substitution rates in bacterial rRNA. *Nucleic Acids Research*, vol. 24, no. 17, pp. 3381–3391.

[11] Liao, D. (2000). Gene conversion drives within genic sequences: Concerted evolution of ribosomal RNA genes in Bacteria and Archaea. *Journal of Molecular Evolution*, vol. 51, pp. 305–317.

[12] Clarridge, J. E. (2004). Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clinical Microbiology Reviews*, vol. 17, no. 4, pp. 840–862.

[13] Relman, D. A. and Falkow, S. (1992). Identification of uncultured microorganisms: Expanding the spectrum of characterized microbial pathogens. *Infectious Agents and Disease*, vol. 1, no. 5, pp. 245–253.

[14] Patel, J. B. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *Molecular Diagnosis*, vol. 6, no. 4, pp. 313–321.

[15] Sogin, M. L., Morrison, H. G., Huber, J. A., et al. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere," in *Proceedings of the National Academy of Sciences*, vol. 103, no. 32, pp. 12115–12120.

[16] Harker, A. R. and Crandall, K. A. (2014). Life in extreme environments: Microbial diversity in Great Salt. *Extremophiles*, vol. 1904, pp. 525–535.

[17] Cheesbrough, M. (2000). *District Laboratory Practices in Tropical Countries* (second edition). Cambridge University Press.

[18] Forbes, B. A., Sahm, D. F., and Weissfeld, A. S. (2007). *Bailey & Scott's Diagnostic* Microbiology (twelfth edition). Elsevier Inc.

[19] Giraffa, G., Rossetti, L., and Neviani, E. (2000). An evaluation of chelex-based DNA purification protocols for the typing of lactic acid bacteria. *Journal of Microbiological Methods*, vol. 42, pp. 175–184.

[20] Lal, S., Mistry, K. N., and Patel, J. G. (2013). Evaluation of amplified rDNA restriction analysis (ARDRA) for identification and characterization of arsenite resistant. *International Science Press*, vol. 6, no. 1, pp. 12–20.

[21] Lee, P. Y., Costumbrado, J., Hsu, C., et al. (2012). Agarose gel electrophoresis for the separation of DNA fragments. *Journal of Visualized Experiments*, vol. 62, pp. 1–5.

[22] Johnson, M., Zaretskaya, I., Raytselis, Y., et al. (2008). NCBI BLAST: A better web interface. *Nucleic Acids Research*, vol. 36, pp. 5–9.

[23] Quast, C., Pruesse, E., Yilmaz, P., et al. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, vol. 41, pp. 590–596.

[24] Larkin, M. A., Blackshields, G., Brown, N. P., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics Applications Note*, vol. 23, no. 21, pp. 2947–2948.

[25] Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*, vol. 41, pp. 95–98.

[26] Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, vol. 33, no. 7, pp. 1870–1874.

[27] Thiruvengadam, S., Shreenidhi, K. S., Vidhyalakshmi, H., et al. (2014). A study of bacterial profiling on coins and currencies under circulation and identifying the virulence gene in Chennai (TN). *International Journal of ChemTech Research*, vol. 6, no. 9, pp. 4108–4114.

[28] State, O. and State, E. (2014). Antibiotic resistance and public health perspective of bacterial contamination of Nigerian currency. *Advances in Life Science and Technology*, vol. 24, pp. 4–15.

[29] Daeschel, M. A. and Fleming, H. P. (1984). Selection of lactic acid bacteria for use in vegetable fermentations. *Food Microbiology*, vol. 1, no. 4, pp. 303–313.

[30] Ahmed, S. U., Parveen, S., Nasreen, T., et al. (2010). Evaluation of the microbial contamination of Bangladesh Paper currency notes (Taka) in circulation. *Advances in Biological Research*, vol. 4, no. 5, pp. 266–271.

[31] Enemuor, S. C., Victor, P. I., and Oguntibeju, O. O. (2012). Microbial contamination of currency counting machines and counting room environment in selected commercial banks. *Scientific Research and Essays*, vol. 7, no. 14, pp. 1508–1511.

[32] New, O. K., Win, P. P., Han, A. M., et al. (1989). Contamination of currency notes with enteric bacterial pathogens. *Journal of Diarrheal Diseases Research*, vol. 7, pp. 92–94.

[33] Jiang, X. and Doyle, M. P. (1999). Fate of Escherichia coli O157:H7 and Salmonella Enteritidis on currency. *Journal of Food Protection*, vol. 62, No. 7, pp. 805–807.

[34] Macrae, A. (2000). The use of 16S rDNA methods in soil microbial ecology. *Brazilian Journal of Microbiology*, vol. 31, pp. 77–82.

[35] Schmidt, T. M., Delong, E. F., and Pace, N. R. (1991). Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. *Journal of Bacteriology*, vol. 173, no. 14, pp. 4371–4378.

[36] Surfaces, C., Santo, C. E., Morais, P. V., et al. (2010). Isolation and characterization of bacteria resistant to metallic copper surfaces. *Applied and Environmental Microbiology*, vol. 76, no. 5, pp. 1341–1348.

[37] Kalita, M., Szysz, M. P., and Szewczuk, A. T. (2013). Isolation of cultivable microorganisms from Polish notes and coins. *Polish Journal of Microbiology*, vol. 62, no. 3, pp. 281–286.

[38] Doi, Y. and Arakawa, Y. (2007). 16S Ribosomal RNA methylation: Emerging resistance mechanism against aminoglycosides. *Antimicrobial Resistance*, vol. 45, no. 1, pp. 88–94.

[39] Fournier, P. E. and Raoult, D. (2011). Prospects for the future using genomics and proteomics in clinical microbiology. *Annual Review of Microbiology*, vol. 65, pp. 169–188.

[40] Conlan, S., Kong, H. H., and Segre, J. A. (2012). Species-level analysis of DNA sequence data from the NIH Human Microbiome Project. *PloS One*, vol. 7, no. 10, p. 47075.

[41] Ellistrem, M. C. and Stout, J. E. (2000). Simplified protocol for pulsed-field gel electrophoresis analysis of *Streptococcus pneumoniae*. *Journal of Clinical Microbiology*, vol. 38, no. 1, pp. 351–353.

[42] Trindade, P. A., McCulloch, J. A., Oliveira, G. A., et al. (2003). Molecular techniques for MRSA typing: Current issues and perspectives. *The Brazilian Journal of Infectious Diseases*, vol. 7, no. 1, pp. 32–43.

[43] Babalola, O. O. (2003). Molecular techniques: An overview of methods for the detection of bacteria. *African Journal of Biotechnology*, vol. 2, pp. 710–713.

[44] Young, N. D. and Tanksley, S. D. (1989). Restriction fragment length polymorphism maps and the concept of graphical genotypes. *TAG Theoretical and Applied Genetics*, vol. 77, no. 1, pp. 95–101.

[45] Wassenaar, T. M. (2000). Genotyping of Campylobacter spp. *Applied and Environmental Microbiology*, vol. 66, no. 1, pp. 1–9.

[46] Ahmed, O. B. and Mashat, B. H. (2015). Occurrence of ESBL, MRSA and VRE pathogens in contaminated banknotes in Makkah, Saudi Arabia. *Global Advanced Research Journal of Microbiology*, vol. 4, no. 9, pp. 27–30.

[47] Gabriel, E. M., Coffey, A., and O'Mahony, J. M. (2013). Investigation into the prevalence, persistence and antibiotic resistance profiles of *Staphylococci* isolated from euro currency. *Journal of Applied Microbiology*, vol. 115, no. 2, pp. 565–571.

[48] Alemu, A. (2014). Microbial Contamination of Currency Notes and Coins in Circulation: A Potential Public Health Hazard. *Biomedicine and Biotechnology*, vol. 2, no. 3, pp. 46–53.