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Evaluation of Bacterial Contamination and Safety of Bangladeshi Paper Currencies (Taka) Collected from Various Food Vendors

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Authors' contributions

This work was carried out in collaboration between all authors. Author SKM designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SH and MSR managed the analyses of the study. Author SH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Paper currency notes can act as transmission vehicle for microorganisms because of their widespread use and constant exchange from hand to hand. This study aimed at determining the level of bacterial contamination and the safety of the notes collected from some food vendors in Noakhali district, Bangladesh.
Methodology: A total of 20 currency notes (BDT 2, BDT 5, BDT 10, BDT 20) were collected at random from 5 different food vendors (Chotpoti, Chicken, Jhalmuri, Fish and Meat) at Noakhali, Bangladesh. Each sample was washed with Tryptic Soy Broth (TSB) and inoculated onto Nutrient Agar (NA) for total viable count. Standard microbiological and biochemical methods were used for the enumeration, isolation and characterization of pathogenic bacteria. Antibiotic susceptibility testing of isolated bacteria against commonly used antibiotic drugs was carried out through Clinical and Laboratory Standards Institute (CLSI) guidelines. Growth potential of the isolated bacteria observed in selected weaning foods (milk and mango juice).

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Results: About 90% of the currency notes were contaminated with bacteria. The highest amount of viable count (3.3×10^{10} CFU/ml) was found in BDT 10 of Chotpoti sample. A total of 30 bacterial isolates were identified from all the currencies. Of them, *Escherichia coli* (33.33%) was the most frequently isolated bacterial species followed by *Staphylococcus aureus* (26.66%), *Bacillus cereus* (13.33%), *Micrococcus* spp. (10%), *Klebsiella* spp. (3.33%), *Salmonella* spp. (3.33%), *Vibrio cholerae* (10%). Antibiotic sensitivity test reveals that, most of the isolates were highly resistant to vancomycin, ampicillin and penicillin, while no or little resistance was found against gentamicin, ciprofloxacin, tetracycline and chloramphenicol. In challenge study, *Salmonella* spp., *S. aureus* reached the level of infective dose within 6 or 12 hours of inoculation in respective foods. *Vibrio cholera* didn't reach this stage. The pH values of both food samples challenged with selected microorganism showed some variability because of fermentation.

Conclusion: This study revealed that paper currencies collected from food vendors in Noakhali, Bangladesh were contaminated with different pathogenic bacteria including multi drug resistant strains. Thus, it calls for awareness development on the potential risks associated with poor handling of paper currencies at all level of the food establishments.

Keywords: Paper currency; food vendors; bacterial contamination; antibiotic resistance; challenge test.

1. INTRODUCTION

Paper currencies are used as medium of exchange throughout the world. It is possible that paper currencies are being handled under unhygienic conditions and therefore possibly contaminated with different types of pathogenic microorganisms [1,2]. Paper currencies can act as vehicle for the transmission of pathogenic microorganisms in humans [3-6]. Previous data indicates that pathogens on paper currency could represent a potential cause of foodborne illness [7]. Food vendors rely heavily on paper currencies for exchange which ultimately results in high frequency of contact between the currencies and foods thus risking the safety of consumers [8,9]. According to several studies, many bacterial groups such as *Citrobacter* spp., *Mycobacterium leprae, Salmonella* spp., *Escherichia coli, Staphylococcus aureus, Klebsiella* spp., *Streptococcus* spp., *Vibrio cholerae* and *Pseudomonas aeroginosa* were found associated with paper currency notes [2, 10-12]. Moreover, the safety of public health could be under serious threat if the pathogenic bacteria isolated from paper currencies pose some other capabilities such as high growth potential in commonly used foods, antibiotic resistance properties etc. Recently, antimicrobial resistance capabilities of microorganisms have become a major public health concern in many regions of the world [13,14]. So investigation of the situation of antibiotic resistance capabilities in bacteria is essential to gauge the level of threat. There is still a dearth of comprehensive reports on microbial association in Bangladeshi paper currencies and related risk of this situation. Therefore, this study was designed to evaluate the microbial load and safety of Bangladeshi paper currencies collected from different food vendors in Noakhali district, Bangladesh.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 20 samples of Bangladeshi currency (Taka), comprising notes in four denominations (BDT 2, BDT 5, BDT 10 and BDT 20) were investigated in this experiment. The notes were collected from five different sources (Chotpoti (spicy peas and potato mix along with tamarind water) vendor, Chicken vendor, Jhalmuri (puffed rice which prepared with many spices) vendor, Fish vendor and Meat vendor) in Noakhali district, Bangladesh. Samples were collected with proper aseptic technique. To collect the paper currency, the individual food sellers were requested to drop currency notes into a sterile polythene bag. The bag was labeled accordingly, sealed and immediately transported to the laboratory of Microbiology Department at Noakhali Science and Technology University for microbiological analysis. Each paper currency notes was placed, aseptically, in a 25 ml test tube containing 10 ml Tryptic Soy Broth (TSB) and then shaken using vortex shaker for 1-2 minutes so that microbes adhered over the note surface came out and then the tubes were incubated at 30℃ for 0.5 hours. After this, the objects were taken out aseptically and then washed. The contents of test tubes were used for the detection of bacteria.

2.2 Total Viable Bacterial Count

The collected paper currency samples were then analyzed for total viable bacterial count. Accordingly, 1 ml of each paper currency vortexed sample were transferred into 9 ml of autoclaved distilled water and thoroughly mixed using vortex. The homogenates were diluted serially from 10^{-1} to 10^{-8} dilution and a volume of 0.1 ml aliquot of appropriate dilution was spread-plated in duplicate on Nutrient Agar media for bacterial enumeration. All plates are incubated at 37℃ for 24 hours.

2.3 Isolation and Identification of Bacteria

According to colony morphology in nutrient agar media followed by Gram staining it were plated on different selective media. Selective media used for isolation and identification of microorganisms include Mannitol salt agar (MSA), Xylose Lysine Deoxycholate (XLD) Agar, MacConkey Agar (MAC), Eosin Methylene Blue (EMB) Agar, Thiosulfate Citrate Bile Salts agar (TCBS), *Bacillus cereus* agar base media (BCAM). All media were prepared according to manufacturer's instructions (Himedia laboratories, India). Then isolates form these selective plates were subjected to further biochemical tests to characterize them up to the genus level. The biochemical tests include Triple Sugar Iron test, Simmons Citrate Agar test, catalase test, oxidase test, indole test, urease test and motility test.

2.4 Antibiotic Susceptibility Testing

Antibiotic sensitivity was tested by the standard agar disc diffusion technique on Mueller-Hinton agar using commercial antibiotic discs (Oxoid, UK) [15]. Using sterile swab stick, bacterial suspension was inoculated on Mueller Hinton Agar (MHA) medium. Plates were kept for drying about fifteen minutes and the following antibiotic discs (HiMedia Laboratories, India) were applied on the plate surface: Ampicillin (10 µg), Penicillin (10 µg), Tetracycline (30 µg), Vancomycin (10 µg), Chloramphenicol (10 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg). The plates were inversely incubated at 37℃ for 24 hours in aerobic condition. Susceptibility of bacterial isolates was recorded as 'sensitive' 'intermediate', or 'resistant' according to Clinical and Laboratory Standards Institute guideline [16].

2.5 *In vitro* Food Challenge Test of Bacteria Isolated from Paper Currency

In vitro food challenge test was performed to determine the growth potential of pathogenic bacteria isolated from paper currencies. The growth potential of *Staphylococcus aureus*, *Vibrio cholera*, and *Salmonella* sp. were assessed on selected weaning foods (milk and mango juice) by previously described method [13]. According to this method, 200 ml of each food items was vortexed separately and then placed into 80℃ for 10 minutes in a water bath to avoid any unwanted presence of microorganisms in tested food items. After that overnight grown 1 ml of each bacterial culture *(Staphylococcus aureus, Vibrio cholera* and *Salmonella* sp.) was added into 100 ml of each processed food sample then incubated for 30-32℃ for 24 hours. Initial inoculum level was determined by adding 10 ml of each inoculated food with 90 ml of Buffered Peptone Water (BPW) and 0.1 ml of appropriate dilution was spread plated on MSA for *S. aureus*, TCBS for *V. cholera* and XLD for *Salmonella* sp. A portion of food sample was further sampled aseptically from 0 to 24 hours at an interval of 6 hours [17]. The pH of each food sample was also measured using pH meter at 6 hours interval from 0 to 24 hours.

3. RESULTS

3.1 Total Viable Bacterial Count

All the paper currencies were highly contaminated with different types of bacteria. Significantly higher bacterial concentration was detected in paper currency sampled from chotpoti vendor and fish vendor. Results of the viable bacterial count on paper notes revealed that the highest viable count was present in sample CP-10 (3.3×10^{10} CFU/ml) followed by sample JM-20 (4.8×10^{9}) and CP-5 (4.3×10^{9}). The lowest count was detected in sample CP-20, FS-10 which is too few to count that is less than 30 colonies in each 0.1ml of sample (Table 1).

3.2 Isolation and Identification of Bacteria

From a total of 20 different paper currency samples analyzed for the microbiological safety, a total of 30 bacterial colonies of 7 different genera were isolated from different food vendors (Table 2). Identification showed the presence of following bacteria by descending order of

percentage *Escherichia coli* 33.33%, *Staphylococcus aureus* 26.66%, *Bacillus cereus* 13.33%, *Micrococcus* sp. 10%, *Vibrio cholerae* 10%, *Klebsiella* sp. 3.33% and *Salmonella* sp. 3.33%.

Table 1. Total bacterial count in currency notes collected from various food vendors

Sample source	Sample name	Total bacterial count (CFU/ml)
Chicken vendor	CK-2	5.7×10^{7}
	CK-5	8.3×10^{6}
	CK-10	5.3×10^{7}
	CK-20	4.9×10^{7}
Chotpoti vendor	CP-2	6.7×10^{8}
	CP-5	4.3×10^{9}
	CP-10	3.3×10^{10}
	CP-20	6.3×10^{6}
Jhalmuri vendor	JM-2	7.8×10^{5}
	JM-5	6.6×10^{7}
	JM-10	3.9×10^{9}
	JM-20	4.8×10^{9}
Fish vendor	FS-2	4.2×10^{8}
	FS-5	7.9×10^{6}
	FS-10	1.2×10^{6}
	FS-20	3.1×10^{9}
Meat vendor	MT-2	3.8×10^{9}
	MT-5	3.3×10^{8}
	MT-10	7.5×10^{8}
	MT-20	3.2×10^{7}

3.3 Antibiotic Susceptibility Test

Table 3 shows the distribution of bacteria isolated from paper currencies according to their resistant pattern to antibiotics. In *Escherichia coli* group, the microorganisms were most resistant to penicillin (90%). In *Staphylococcus aureus* group, the microorganisms were most resistant to tetracycline and gentamycin group (37.5%) each. In *Bacillus cereus* group, the microorganisms were most resistant to ampicillin, vancomycin and chloramphenicol group (75%) each. In *Klebsiella* sp. group, the bacteria showed resistance to gentamycin only (100%). In *Micrococcus* sp. group, the microorganisms were most resistant to ampicillin, tetracycline and vancomycin group (66.7%) each. In *Salmonella* sp. group, the microorganisms were resistant to penicillin, tetracycline, vancomycin and chloramphenicol group (100%) each. In *Vibrio cholerae* group, the microorganisms were most resistant to tetracycline and chloramphenicol group (66.7%) each.

3.4 *In vitro* Food Challenge Test of Bacteria Isolated from Paper Currency

The count of *Salmonella* sp. in each food samples ranged from log 3.6 to log 3.84 at 0 hour (Fig. 1.a) *Salmonella* sp. were observed growing fast in milk (log 4.11CFU/ml) as compared to its growth in juice (log 3.87 CFU/ml) at 6 hours. The count increased in milk and juice, respectively, during the first 6 hours. The maximum growths attained were log 4.45 CFU/ml (milk) and log 4.27 CFU/ml (juice) at 24 hours (Fig. 1.a). Likewise, the growth potential of *S. aureus* isolated from paper currencies was assessed. Accordingly, higher growth rate was observed in juice (log 3.60 CFU/ml) than in milk (log 3.44 CFU/ml) at the 0 hours (Fig. 1.b). The counts increased by log 4.47 in milk and log 4.40 in juice at 24 hours. In case of *Vibrio cholera* the growth measurement was log 2.47 CFU/ml (in juice) and log 2.95 CFU/ml (in milk) at 0 hours. Growth pattern is promoted to log 3.53 (juice) and log 3.91 (milk) at the elevated time 24 hours (Fig. 1.c).

Table 2. Percentage occurrence of different isolates from different food vendors (n=20)

Sample source	Name and no. of bacteria in percentage						
	***Escherichia coli* (n=10)**	***Staph. aureus* (n=8)**	***Bacillus cereus* (n=4)**	***Klebsiella* sp. (n=1)**	***Micrococcus* sp. (n=3)**	***Salmonella* sp. (n=1)**	***Vibrio cholerae* (n=3)**
Chotpoti vendor (n=4)	10	3.33	3.33	0	0	3.33	3.33
Chicken vendor (n=4)	3.33	10	3.33	0	0	0	0
Jhalmuri vendor (n=4)	6.67	6.67	3.33	0	3.33	0	6.67
Fish vendor (n=4)	10	3.33	0	0	3.33	0	0
Meat vendor (n=4)	3.33	3.33	3.33	3.33	3.33	0	0
Total	**33.33**	**26.66**	**13.33**	**3.33**	**10**	**3.33**	**10**

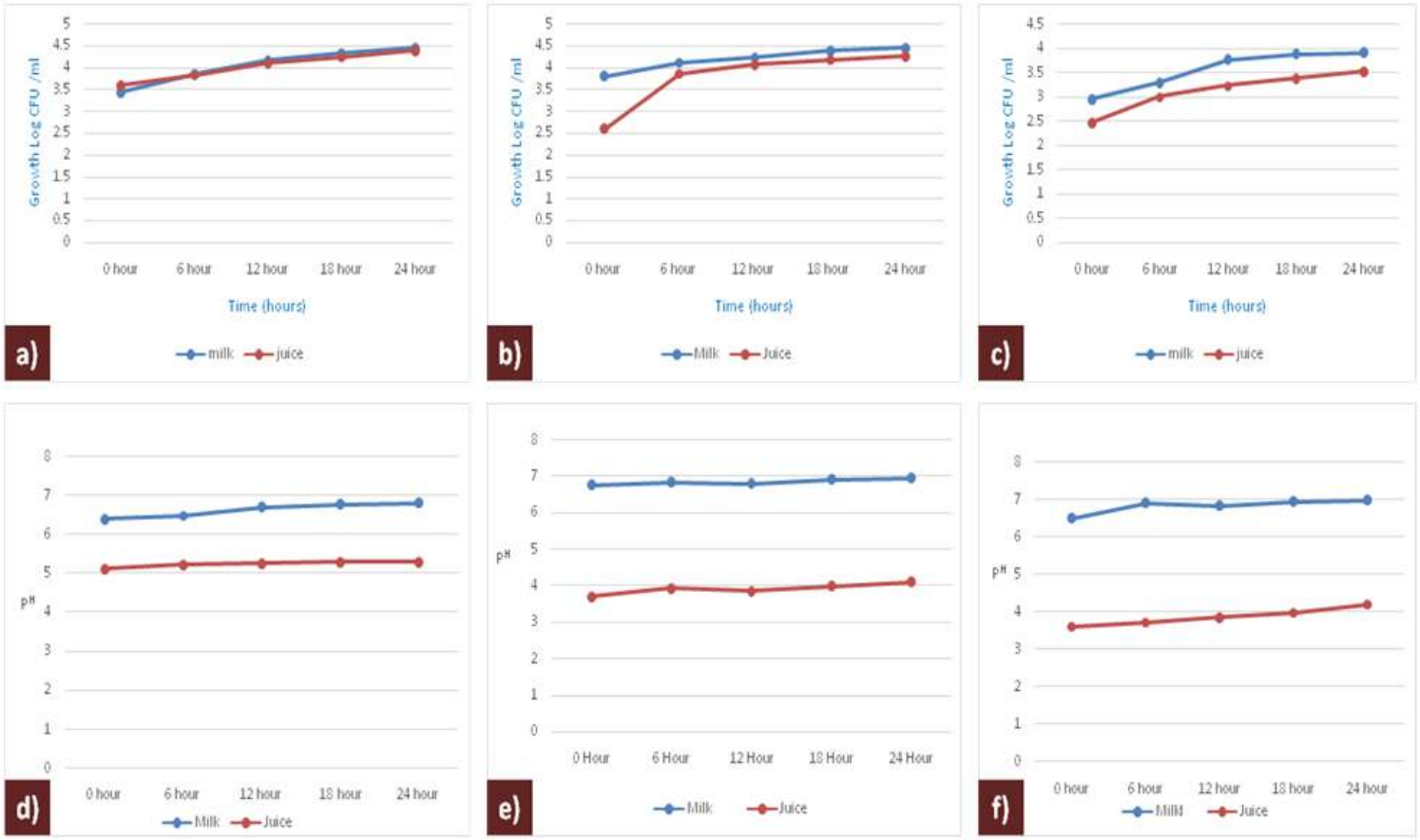

Fig. 1. The growth potential of a) *Salmonella* sp., b) *Stahphylococcus aureus,* c) *Vibrio cholerae* isolated from paper currencies in milk and juice and change in pH of milk and juice challenged with d) *Salmonella* sp., e) *Stahphylococcus aureus,* f) *Vibrio cholera*

Table 3. Antimicrobial susceptibility pattern of bacteria isolated from paper currency

Antibiotic	Percentage (%) of bacteria resistant to antibiotics						
	Escherichia coli (n=10)	*Staph. aureus* (n=8)	*Bacillus cereus* (n=4)	*Klebsiella* sp. (n=1)	*Micrococcus* sp. (n=3)	*Salmonella* sp. (n=1)	*Vibrio cholerae* (n=3)
Ampicillin	50	12.5	75	0	66.7	0	33.3
Penicillin	90	25	50	0	33.3	100	33.3
Tetracycline	60	37.5	25	0	66.7	100	66.7
Vancomycin	50	12.5	75	0	66.7	100	33.3
Chloramphenicol	60	25	75	0	33.3	100	66.7
Gentamycin	40	37.5	25	100	33.3	0	33.3
Ciprofloxacin	30	12.5	25	0	33.3	0	33.3

The pH values of both food samples challenged with *Salmonella* sp. were 6.3 (milk) and 3.6 (juice) at 0 hours (Fig. 1.d). There was initial rise in pH from 6.3 to 6.9 (in milk) and 3.6 to 3.9 (in juice) at 6 hours. However, the pH values dropped in both food samples from 6.9 to 6.83 in milk and 3.9 to 3.85 in juice at 12 hours. Nevertheless, it was raised afterwards up to 24 hours (Fig. 1.d). The pH values of both food samples challenged with *S. aureus* were 6.77 (milk) and 3.7 (juice) at 0 hours (Fig. 1.e). There was initial rise in pH from 6.7 to 6.99 (in milk) and 3.7 to 4.10 (in juice) at 24 hours (Fig. 1.e). *Vibrio cholera* was found with an initial pH 6.4 (in milk) and 5.12 (in juice) and afterwards up to 24 hours was 6.82 (in milk) and 5.3 (in juice) (Fig. 1.f). The pH values of both food samples challenged by the three pathogens showed some variability in due course of fermentation. However, the overall patterns were gradual increase in pH with drop in degree of acidification mainly due to depletion of carbohydrate sources and reversion towards utilization of proteins and/or release of other metabolites during the 24 hours period.

4. DISCUSSION

Paper currency is commonly contaminated with bacteria and contaminated currency is identified as a potential public health hazard due to easy transmission of pathogens by circulating banknotes [18,19]. Because of its rough surface microorganisms can easily get in and survive at this surface, remain stable up to 72 hours and still cultivable after a week [20]. Human occupational activities, without proper hygienic practices, especially those involving simultaneous money handling, could lead to the risk of infections. The results of this study revealed that currency notes are commonly contaminated with various bacteria. We found that 100% of tested currency notes were contaminated with bacteria, which is agreed with the results obtained by other studies [21,22].

This study described the presence of the bacteria including *E. coli, Klebsiella* spp., *Staphylococcus aureus, Salmonella* sp., *Bacillus cereus*, *Vibrio cholerae, Micrococcus* sp. in bank notes of Noakhali, Bangladesh which is supported by several previous studies [12,23]. The colon-inhabitant normal microbiota, *E. coli* (33.33%), was the most common isolated bacterium. This situation may be attributed to the possibility that some people disregard hand wash after using toilets. Such finding is in line with what has been reported that currency notes are contaminated with entering pathogens [24]. The results explain that paper currency can act as a potential source of enteric diseases. *E. coli* was found almost in all sources and mostly in 2 Taka notes which may be due to the frequent circulation [11,25]. *Staphylococcus aureus* (26.66%) was recorded as the second highest percentage in this study which produces many toxins responsible for toxic shock syndrome. *Staphylococcus aureus* is commonly present on the skin and in the nasal passage of human and its presence in paper currency is also abundant [23,26]. Average percentage of *Bacillus cereus* in this study is 13.33%. This genus comprise a vast group hardy spore forming species that live in soil and are found in the environment could also be transferred on money due to its placement on dirty surfaces or handling with dirty hands. *Bacillus* produces an emetic exotoxin capable of

inducing disease in man [27]. In this study, *Salmonella* spp. and *Klebsiella* spp. were less prevalent in all of the paper currencies.

Different studies indicated that the denomination of paper currencies have a direct correlation with degree of contamination as lower denomination notes had the most contaminants. Ahmed et al. 2010 showed that only 2 taka, 5 taka and 10 taka contained high load of bacteria. Present study reveals that not only these notes but also 20 taka notes were contaminated with high amount of microorganisms. The climatic and environmental conditions of the tropical countries favor the growth of many pathogenic bacteria and inadequate water and sanitation, high population density, scarcity of health care facilities and poor education make the population more prone to infectious diseases and make the situation more complex. For instance, isolates of various bacterial species recorded high rates of resistance collectively as against ampicillin, penicillin, tetracycline, vancomycin and chloramphenicol respectively which is not an uncommon phenomenon worldwide [28-31]. Antibiotics like ciprofloxacin and gentamicin collectively expressed little resistance rates in this study. Multidrug-resistant bacteria pose a big challenge to human survival and continued existence in relation to bacterial infection and diseases. The observed high antibiotic resistances in this study could be attributed to the abuse of antibiotics in this country which is a great concern for public health now [31]. The results from this study shows that Bangladeshi currency notes in circulation are contaminated with various bacteria most of which are resistant to commonly used antibiotics and therefore represent risks and public health hazards to the community and individuals handling currency notes.

The challenge studies showed that the inoculated *Salmonella* spp. reached the level of infective dose ($>10^3$) in cow milk within 6 hours and in mango juice within 12 hours. The maximum count obtained was log 4.27 CFU/ml (juice) and log 4.45 CFU/ml (milk) within 24 hours. The observation of the present study was similar with previous findings [5,32]. From these observations, it could be considered that within the indicated incubation hours, ingestion of following foods contaminated with sufficient number of *Salmonella* could induce disease symptoms such as diarrhea, vomiting and fever [33]. The growth potential of *S. aureus* increased considerably in milk and in juice from 0 to 6 hours and reached the infective dose within 12 hours in both food samples. The minimum infective dose for *S. aureus* is 10^3-10^7 [34]. The growth potential of *Vibrio cholera* increased considerably in milk and in juice but does not reach the infective dose in both food samples. The minimum infective dose for *Vibrio cholera* is $>10^5$. In this study, the challenged pathogens showed an initial upward growth pattern with the increase in pH and afterwards a steady growth pattern. This pH change could be due to the change of carbon and nitrogen source in the medium. However, optimum pH is not the sole requirement for the growth of bacteria as there could be an interaction of other intrinsic and extrinsic factors that influences the survival and growth of bacteria in a medium [35].

One of the limitations of this study could be that small sample size may not demonstrate the clear picture, so the results cannot be generalized. The discrepancy in the bacterial pattern may be attributed to the regional variation of bacterial profile and habits of the local people. Inability to quantify the cell numbers of the bacterial agents and failure to take into account the possible presence of other categories of potential pathogens, such as viruses and fungi that might contaminate currency notes could be the other limitation.

5. CONCLUSION

All paper currencies examined in this study were found to be contaminated with different bacteria including potentially pathogenic *Salmonella* spp., *S. aureus* and *Vibrio cholerae*. The presence of pathogenic bacteria could cause different types of foodborne diseases such as typhoid fever, food poisoning and cholera. Moreover, multiple antibiotic resistant bacteria from paper currency indicate that these currencies might act as vector in the transmission of pathogenic microorganisms, as well as in the spread of antibiotic resistant strains in the community and environment. Pathogenic test strains isolated from paper currencies were found growing to infective dose within 12-18 hours indicating that paper currencies are among the risk factors to human health. Therefore handling of paper currency deserves special attention. Periodic microbiological evaluation and frequent awareness development efforts are recommended to ameliorate the existing poor hygienic practices during handling paper currencies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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