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Original Research Article

Screening for microbial load and antibiotic resistance pattern in *Escherichia coli* isolated from paper currency circulating in Kushtia, Bangladesh

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ABSTRACT

Background: Paper currency is used for every type of commerce and plays an important role in the life of human beings. They are exchanged and come into contact with different environments and many different individuals during their circulation. Therefore, they can become contaminated with microorganisms and transfer bacteria across environments. The present study was aimed for quantitative assessment of microorganisms in circulated paper currency from Kushtia, Bangladesh and antibiotic resistant profiles of isolated *Escherichia coli*.
Methods: A total of 10 paper currency samples currently in circulation involving three denominations (5, 10 and 500) were randomly collected from individuals involved in various occupations including street beggar, local hotel, bus conductor, poultry seller, vegetable seller, fish seller, commercial bank, ATM booth, tea seller, grocery store in Kushtia city, Bangladesh. Selective culture and biochemical tests were performed for the isolation and identification of microbial pathogens. Antibiotic resistance profiles were evaluated for isolated *Escherichia coli* using Kirby-Bauer method according to CLSI guidelines.
Results: Aerobic mesophilic bacteria, *Enterobacteriaceae* and *Pseudomonas spp.* were the highest in paper currency from local hotel and ATM booth. *Enterobacteriaceae* (including coliforms) were predominantly present in paper currencies collected from local hotel, grocery, fish seller and beggar while *Pseudomonas spp.* were found in currency notes obtained from ATM booth, poultry farm, vegetable seller and local hotel. Antibiotic resistant profiles of *E. coli* isolated from local hotel currency showed that 50% of *E. coli* isolates were multidrug resistant. The highest resistant profile was observed against penicillin (95%) followed by polypeptide (75%), cephalosporin (50%), quinolone (30%) and sulfonamide (5%) groups of antibiotics.
Conclusions: Multiple antibiotic resistant pathogenic bacteria are prevalent in paper currency regardless of their sources. Paper currency could contribute in transmission of infectious disease as well as in antibiotic resistance, therefore, should be handled carefully.

Keywords: Antibiotic resistance, *Escherichia coli*, Microbial load, Paper currency

INTRODUCTION

People use paper currencies for swapping stuffs and services worldwide. During circulation, paper currencies could be handled by different individuals with different level of personal hygiene, making it a suitable medium for various microorganisms.[1] In addition, paper money are often counted with saliva, which contaminate the paper currency as well as the individual.[2] Several other reasons such as touching money after sneezing, coughing,

keeping money in filthy place, can also contribute to the contamination of paper currencies.[3] These contaminated currencies can act as transmission vehicle for pathogenic microbes and lead to microbial infections, i.e., tonsillitis, peptic ulcers, pneumonia, genital tract infections, gastroenteritis, tuberculosis, meningitis, etc.[4]

According to earlier studies, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella sp.*, *Klebsiella spp.*, *Streptococcus spp.*, *Mycobacterium spp.*, etc. were found associated with paper currency notes.[1,5] Moreover, paper currencies were also reported to be contaminated with fungi including yeast, *Aspergillus niger*, *Penicillium spp*., *Trichoderma spp.*, *Fusarium spp*., etc.[1,5] Importantly, contamination of paper currency is not confined to developing countries. The presence of pathogenic microorganisms such as *S. aureus*, *E. coli*, *K. enterobacter* on US currencies and coins was studied reported earlier.[4,6]

One of the major global challenges of 21st century is antibiotic resistance.[7,8] The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of current antibiotics.[9,10] The presence of multidrug resistant pathogenic bacteria on paper currency could therefore be a reason of public health risk. A recent study suggested the presence of antibiotic resistance *Escherichia coli* on Nigerian paper currency.[2] Pathogenic microorganisms were also found in paper currencies from different region of Bangladesh.[11]

Knowledge about the microbial diversity on currency notes in circulation can provide the basis for raise health consciousness in people during currency handling and effective control of infection transmission. To the best of the knowledge, paper money from Kushtia region, Bangladesh is not studied yet. Hence, the present study was undertaken to identify microbial contamination of Bangladeshi currency notes in circulation in southern region (Kushtia) of Bangladesh. In addition, antibiotic resistance pattern in isolated *E. coli* was also evaluated followed by the biochemical characterization.

METHODS

Collection of samples

Paper currency samples currently in circulation involving three denominations (5, 10 and 500) were randomly collected from individuals involved in various occupations including street beggar, local hotel, bus conductor, poultry farm, vegetable seller, fish seller, commercial bank, ATM booth, tea seller, grocery store in Kushtia city, Bangladesh. The samples were collected aseptically by letting the individuals to drop the paper notes into a sterile polybags. The polybags were promptly sealed, and the individuals were given a replacement equivalent paper currency. The polybags were immediately transported to Laboratory of Microbiology, Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh for microbial analysis.

Quantitative microbial analysis

Each paper note was soaked in 50 ml sterile distilled water for 15 m and thoroughly mixed using vortex. Then the samples were aseptically removed using forceps. After that, 100 µl aliquot of each sample was spread in duplicate on pre-solidified plates of nutrient agar (NA, HiMedia), eosin methylene blue (EMB, HiMedia), MacConkey agar (MCA, HiMedia), cetrimide agar (CEA, HiMedia), mannitol salt agar (MSA, Scharlau), salmonella-shigella agar (SSA; Oxoid) and potato dextrose agar (PDA, HiMedia) and incubated at 37ºC for 24 hours (except for PDA which was incubated up to 4 days at 30ºC) for counts of aerobic mesophilic bacteria, fecal Coliforms, *Enterobacteriaceae*, *Pseudomonas*, *Staphylococci*, *Salmonella and Shigella*, Fungus, respectively. The colonies those developed were counted and the total viable cells were calculated as CFU/currency notes.

Biochemical characterization

To confirm the identification of the isolates to the genus level, colonies of aerobic mesophilic bacteria with distinct morphological differences like color, size and shape were randomly picked from countable plates and characterized morphologically and biochemically. Morphological and biochemical tests were performed as described in Benson's microbiological applications lab manual.[12]

Antibiotic resistance pattern

Antibiotic resistance pattern in *E. coli* isolates were determined by Kirby–Bauer disc diffusion method based on Clinical and Laboratory Standards Institute (CLSI) guidelines.[13] Standard antibiotic discs (HiMedia, India) from five different classes used were Penicillin G (P, 10 µg), Amoxicillin (AMX, 30 µg), Co-trimoxazole (COT, 25 µg), Ciprofloxacin (CIP, 5 µg), Nalidixic Acid (NA, 30 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CTR, 30 µg), Colistin (CL, 10 µg), Polymixin B (PB, 300 µg). Briefly, bacterial inoculums were prepared by maintaining 0.5 McFarland standard and swabbed onto Muller-Hinton agar (Merck, Germany) plates (6 mm thick). Antibiotic discs were then placed on the inoculated plates and incubated at 37ºC for 22 hours. The zone of growth inhibition around each disc was measured by millimeter scale and used to categorize the selected bacterial isolates as sensitive, intermediate or resistant.

RESULTS

Microbial load in paper currency

A total of 10 paper notes were analyzed for determination of microbial load. Currency notes from all sources

showed uncountable number of viable colonies (Table 1). The counts of aerobic mesophilic bacteria, *Enterobacteriaceae and Pseudomonas spp.* were the highest in paper currency from local hotel and ATM booth, respectively. *Enterobacteriaceae* (including coliforms) were predominantly present in paper currencies collected from local hotel, grocery, fish seller and beggar while *Pseudomonas spp.* were found in currency notes obtained from ATM booth, poultry farm, vegetable seller and local hotel. However, hotel currencies were showed to have maximum coliform counts (Table 1).

Table 1: Microbial load (CFU/currency) in paper currencies from various sources.

Sources	Microbial count (CFU/currency)					
	TVC	*Enterobacteraceae*	Coliforms	*Pseudomonas*	*Staphylococcus*	Fungi
New taka	TNTC	Nil	Nil	4.20×10^4	ND	Nil
ATM booth	TNTC	Nil	Nil	TNTC	ND	Nil
Poultry farm	TNTC	Nil	Nil	113×10^4	ND	Nil
Vegetable seller	TNTC	Nil	Nil	2.60×10^4	ND	Nil
Local hotel	TNTC	TNTC	TNTC	120×10^4	ND	Nil
Grocery	TNTC	TNTC	0.80×10^4	Nil	Nil	ND
Fish seller	TNTC	TNTC	1.70×10^4	Nil	Nil	ND
Bus conductor	TNTC	Nil	Nil	Nil	Nil	ND
Street beggar	TNTC	2.00×10^4	0.90×10^4	Nil	Nil	ND
Tea seller	TNTC	TNTC	1.00×10^4	Nil	Nil	ND

*ND= Not done, TNTC= Too numerous to count, TVC=Total Viable Count.

Characterization of isolates

The morphological and biochemical characteristics of the tested isolates (Table 2) were typical properties of species *E. coli* (Isolate E1 and E2) and *P. aeruginosa* (Isolate P1 and P2), respectively.

Table 2: Morphological and biochemical features of selected *Escherichia coli* and *Pseudomonas spp.* isolates.

Morphological and biochemical tests	Presumptive *Escherichia coli*		Presumptive *Pseudomonas spp.*	
	Isolate E1	Isolate E2	Isolate P1	Isolate P2
Selective growth	EMB	EMB	CEA	CEA
Gram reaction	-(rod)	-(rod)	-(rod)	-(rod)
Motility test	+	+	+	+
Casease test	-	-	+	+
Oxidase test	-	-	+	+
Methyl red test	+	+	-	-
Voges-Proskauer test	+	+	+	+
Indole production	+	+	-	-
Citrate test	-	-	+	+
Urease test	-	-	-	-
Glucose	+(g)	+(g)	-	-
Sucrose	+(g)	+(g)	-	-
Lactose	+(g)	+(g)	-	-

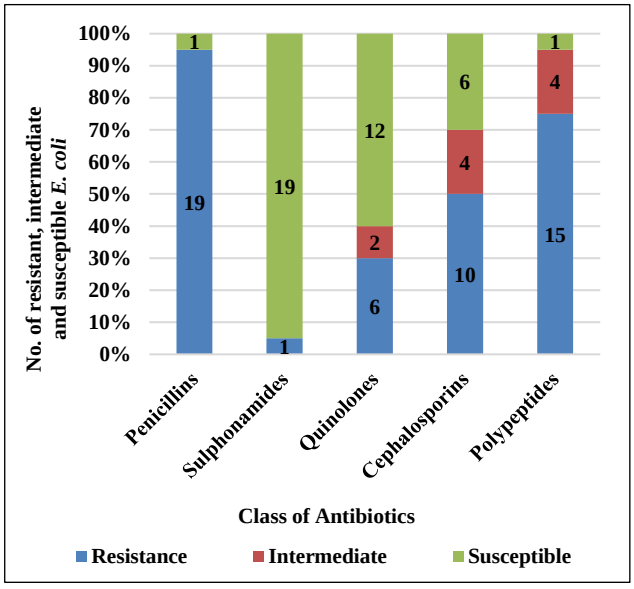

Figure 1: Antibiotic resistance pattern in isolated *Escherichia coli*.

Antibiotic resistance

E. coli isolates from local hotel currencies were evaluated for their resistance patterns against commonly used antibiotics. The highest resistant profile was observed against penicillin (95%) followed by polypeptide (75%), cephalosporin (50%), quinolone (30%) and sulfonamide (5%) groups of antibiotics (Figure 1). They all showed

100% resistant profile against ceftazidime, penicillin G, and colistin.

DISCUSSION

Due to longer exposure to different individuals and conditions, paper currency harbors different types of pathogenic microorganisms which demands public health concern.[1] In addition, drug resistance properties can be accumulated in microorganisms on paper currencies. In this study, pathogenic microorganisms in paper currencies from different sources were isolated and evaluated for their antibiotic resistance profile.

Enterobacteraceae and coliforms were found in notes from local hotel, grocery, fish seller, street beggar and tea seller. On the other hand, *Pseudomonas* was found in notes from ATM booth, poultry farm, vegetable seller, local hotel, even the Bank. Coliform bacteria and *Pseudomonas spp.* were present in 50% sample. The presence of coliform in paper currency is of particular importance because coliform and fecal coliform bacteria are generally present in feces of animal.[14] Therefore, presence of fecal coliforms in paper currency indicates fecal contamination which could possibly arise from handling of money with dirty hands. Microbial contaminations of paper currencies were also previously investigated. In a previous study, *Escherichia coli* (85.71%), *Klebsiella spp.* (92.85%), *Staphylococcus aureus* (53.84%), *Salmonella spp.* (42.85%), *Vibrio cholera* (28.57%), *Bacillus spp*. (25%) and *Pseudomonas spp.* (28.57%) were present on Bangladeshi paper currency.[11] The predominant presence of *Staphylococcus spp.* (34.06%) followed by *Bacillus spp.* (31.88%), *Enterobacteraceae* (13.39%), *Micrococcus spp.* (9.55%) and *Streptococcus spp.* (9.03%) isolated from Ethiopian currencies was also reported.[1] However, no sample in this study was found to contain *Staphylococcus spp.* and fungus.

Antibiotic resistant profiles of *E. coli* isolated from local hotel currency showed that 50% of *E. coli* isolates were multidrug resistant. All *E. coli* isolates were complete (100%) resistant to ceftazidime, penicillin G, and colistin. However, antibiotics from sulfonamide class were the most potent against the tested *E. coli* isolates. Antibiotic resistance patterns in microorganisms obtained from paper currency were also evaluated before.[2,15,16] Moses et al, found 85.7% of *E. coli* were resistant to ceftazidime but 100% susceptible to ceftazidime.[2] Their findings coincide with the present investigation.

Therefore, quantitative microbiological analysis suggests that paper currency circulating in Kushtia region contaminated with various pathogenic bacteria and multidrug resistant fecal coliform, *E. coli*. The observed high antibiotic resistance of *E. coli* isolates obtained from local hotel in Kushtia could be indicated to the abuse of antibiotics in this area which is a great concern for public health now. This study also revealed that colistin, penicillin G and ceftazidime are no longer effective for treating diseases caused by *E. coli* in this area.

CONCLUSION

The present study clearly exhibited that paper currencies collected from local hotel, beggar, fish seller, tea seller, grocery in Kushtia city, Bangladesh were highly contaminated with pathogenic bacteria. In particular, local hotel paper currency contains pathogenic *Escherichia coli* and *Pseudomonas spp.* Poor handling practices and personal hygiene could contribute to the observed microbial counts. Furthermore, 50% of the *Escherichia coli* isolates were multi-drug resistant including latest generation of antibiotics. Therefore, development of awareness on the potential risks associated with poor handling of paper currencies at all level are necessary.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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