Microbiological And Antibiotic Resistance Pattern Analysis Of Wash Water From Tea Vendors Of Urban Areas In Bangladesh

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Abstract: Tea is the most widely consumed beverage in the world next to water. For selling food and drinks in Bangladesh a large number of people are involved. This study was performed to determine the microbiological quality of tea vendors in different area of Dhaka city. A total of 50 street tea vendors were randomly selected for this study. Samples were collected from Tea cup, vendor hand and water of each tea vendors. All samples were assessed for the presence of total bacterial count, total Enterobacteriaceae, total coliform count and total Staphylococcus aureus. The presence of Salmonella and Vibrio species were determined following appropriate enrichment and culture method. Cup and hand samples collected showed significantly higher count of total heterotrophic bacteria. Prevalence of Vibrio spp. and Salmonella spp., Staphylo-coccus aureus has been on higher side as compare to the Enterobacters and Klebsiella. Antibiotic sensitivity pattern of the S. aureus isolates shows that the highest sensitivity was observed against Streptomycin whereas high resistance observed against Amoxicillin. Contamination of tea vendor samples with pathogenic and resistant bacteria indicate their poor quality and unacceptability as drinks. This could be a threat to the consumer's health and require immediate attention in order to control any outbreak of food and water borne diseases. Results of this concise study indicate that water testing is significant approach to ensure the supply and availability of contaminated-free water for tea preparation and processing.

Index Terms: Antibiotic Resistance, Bacteriological analysis, Hygienic Condition, pathogens

1 Introduction

Tea (Camellia sinensis) is the most consumed drink throughout the world [1]. According to Food and Agricul-ture Organization of United Nations (FAO), about 61.9 thousand tons of Tea is consumed per year in Bangladesh (Chang, 2014). In urban areas of Bangladesh, tea has be-come a fundamental element of daily life. Consumption tea is rapidly increasing which is vivid in Dhaka city, the capital of Bangladesh. Apart from its medicinal and re-freshing facet, tea finds the way in almost every family and individual through different means. This might be the rea-son of the presence of tea vendors in every nook and corner of Dhaka city. Both permanent and moving tea ven-dors are frequently seen in Dhaka city. Tea consumption in Bangladesh has increased several folds during winter sea-son (from October to February) for getting relieve from severe cold [2]. Simple preparation method, low price, availability and instant refreshment of Caffeine [3] have increased its popularity. From tea preparation to washing cups, water is the only essential element needed for tea vendors. Most often this water may not be safe for the con-sumers [4, 5]. The water is reused for washing tea cups thus serving cups can be contaminated. Consumption of tea prepared in such unhygienic environment can be the potential cause for out-break of severe waterborne diseases [6]. A number of stud-ies have revealed the presence of heavy bacterial contami-nation ranges from 30-57.5% which includes coliforms, feacal coliforms, Salmonella sp., Shigella sp., fecal strepto-cocci etc. [7, 8, 9, 10]. Popularity of tea in urban areas of Bangladesh prepared in unhygienic condition can be a po-tent source of such diseases. Presence of antibiotic resistant bacteria in contaminated water used by tea vendors is intensifying the complexity in this scenario [11]. Therefore, tea has become a prime concern as a conduit in food borne disease statistics [12, 13]. Unfortunately no significant re-search work has been carried out about consumers' safety and hygiene status of floating and permanent tea vendors in Bangladesh. Present study has been conducted to evaluate microbiological status of the wash water used by the tea vendors from various parts of Dhaka city, Bangla-desh.

2 Materials and Methods

2.1 Sampling area

In this study, 50 samples were randomly collected from different parts of Dhaka city. Sampling sites were Mouchak, Farmgate, Jatrabari, Nikunja, Khilkhet, Kallyanpur, Mo-hakhali, Airport, Sayedabad, and Shahajadpur. Multiple samples were randomly collected from each area. Samples were transported to the laboratory promptly after collection and processed immediately. All the research works have been carried out in Center of Excellence, Department of Microbiology, Primeasia University, Banani, Dhaka. This study was carried out from October 2015 to March 2016

2.2 Sample collection and preservation

All the samples were collected following standard methods of water sample collection for microbiological analysis. Clean and sterile screw capped tubes were used for sample collection and the samples were then immediately trans-ported to the laboratory in cooler box maintain temperature within 10°C. For long term preservation of isolated micro-organisms, 15% glycerol and Luria Broth containing young culture are mixed together thoroughly and kept at -30°C.

2.3 Bacterial count and Media

Membrane filtration method was followed for total viable count. 100 ml of samples were passed through sterile mem-brane of pore size 0.45 µl. Bacteria present in the sample will be retained on the surface of the membrane. The mem-brane was then transferred aseptically to Nutrient Agar, MacConkey Agar and Mannitol Salt Agar (MSA) for total viable count, total coliform count and total staphylococcal count. Media were incubated at 37° C for 16-24hr and co-lony count was recorded as CFU/ml. different types of me-dia as Nutrient Agar, Thiosulfate Citrate Bile salt Sucrose (TCBS) Agar, Mannitol Salt Agar (MSA), MacConkey Agar, Salmonella-Shigella (SS) Agar, Eosin Methylene Blue (EMB) Agar, Muller

Hinton Agar, Peptone Broth, and Lactose fermentation Broth were used.

2.4 Gram staining Biochemical characterization

Gram staining is the first step of detection and identification of bacteria. Upon smear preparation and heat fixation, Crystal violates (primary stain) was added to the sample and held for about 1 minute. Addition of mordant (Iodine) fol-lowing primary staining results in binding of Crystal violate and trapping it into the cell wall. After 45 seconds, mordant is washed away, decolorizing agent (Alcohol) is added for another 45 seconds and then counter stain (Safranin) is applied. The microscope gram positive cells appeared violet and gram negative cells looks red or pink. [14, 15]

2.5 Biochemical characterization

Biochemical test were carried out for final confirmation of bacteria recovered from different culture techniques. These biochemical tests include Catalase Test, Oxidase Test, Citrate Utilization Test, Urease Test, Indole Test, Methyl Red / Voges-Proskauer (MR/VP) Test, Triple Sugar Iron Agar Test and Hydrogen Sulfide test.

2.6 Antibiotic sensitivity assay

For determining susceptibility of Staphylococcus aureus against antimicrobial agent, in vitro agar disc-diffusion method was used which is known as the Kirby Bauer method [16]. Commercially available antibiotic discs, e.g. Ampicilin (AMP), Chloramphenicol (C), Tetracyclin (TE), Erythromycin (E), Azithromycin (AZM), Streptomycin (S), Gentamycin (GN), Nalidixic Acid (NA), Ciprofloxacin (CIP), Norfloxacin (NOR) were used in this study. A suspension of the test organism was prepared containing 103 to 105 cfu/ml. Turbidity of the culture broth was adjusted with normal saline to match the equivalent turbidity standard of McFarland (0.5 Standard). A sterile cotton swab was used to spread the bacterial suspension evenly over the entire surface of a Mueller-Hinton agar (pH 7.4) plate for obtain-ing uniform inoculum. Antibiotic discs were placed asepti-cally using sterile forceps on the surface of the inoculated plates and incubated at 37ºC for 18 to 24 hours

3 Results

3.1. Bacterial loads in water samples

Figure 1 demonstrated the bacterial loads in the water samples collected from both permanent tea vendors and mobile tea vendors. All the collected samples of both categories were found to be contaminated. However, bacterial loads are significantly higher in mobile vendors than permanent vendors.

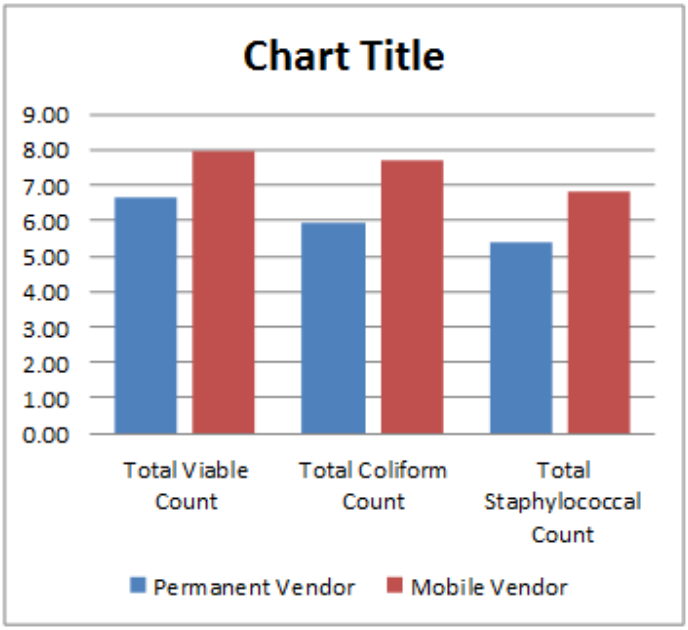

Figure 1. *Bacterial loads in water samples collected from permanent tea vendors and mobile tea vendors.*

3.2. Gram staining

From the gram staining result it was found that most of the isolates were gram positive and few numbers of isolates were gram negative. The results are given in Table 1.

3.4. Characterization of the Isolates

To characterize the water bacteria different types of selective media like MacConkey, TCBS, SS and MSA plates were used. In addition to this biochemical tests as citrate, indole, MR-VP, catalase, oxidase and urease had been performed. Based on this tests presumptive organisms like Escherichia coli, Vibrio spp., Salmonella typhi, Salmonella typhimurium and Staphylococcus aureus were identified. The result of characterization of isolated organisms shown in Table 2.

Table 1. *Gram staining of recovered microorganisms Characterization of isolated organism by biochemical tests.*

Samples name	*Salmonella* spp. (on SS media)	*Vibrio* spp. (on TCBS media)	*Escherichia coli* (on MacConkey media)
Kollyanpur	Rod, Pink, (-)	Rod, Pink, (-)	Cocci, Purple, (+)
Nikunjo	--------	---------	Cocci, Purple, (+)
Banani	--------	Cocci, Pink, (+)	Rod, Pink, (-)
Jatrabari	Rod, Purple, (-)	Rod, Cocci, Pink, (-)	Cocci, Purple, (+)
Shaydabad	Rod, Cocci, (-)	Cocci, Pink, (-)	Spiral, Violet, (+)
Farmgate	Cocci, Purple, (+)	Cocci, Rod, Pink, (-)	Cocci, Purple, (+)
Mouchak	Rod, Pink, (-)		Cocci, Pink, (+)

Table 2. *Characterization of isolated organism by biochemical tests*

Colonies on	TSI		Gas	H_2S	Citrate Utilization	Indole Production	MR test	VP test	Catalase test	Oxidase test	Urease activity	Suspected Organism
	Slant	Butt										
MacConkey (Pink red)	acid	acid	+	-	+	+	+	-	+	+	-	*Escherichia coli*
TCBS (Green)	acid	acid	-	+	-	-	-	+	-	+	-	*Vibrio* spp.
SS Agar (Black center)	acid	acid	-	-	-	+	+	-	-	+	-	*Salmonella typhi*
	acid	acid	-	+	+	-	+	-	+	-	-	*Salmonella typhimurium*
MSA (Yellow)	acid	acid	-	-	-	-	+	-	+	-	+	*Staphylococcus aureus*

From Table 3 it has been observed that percentages of the presence of Vibrio spp. and Salmonella spp. are significantly higher in mobile vendors than permanent vendors.

Table 3*: Presence of pathogenic bacteria in the collected samples*

Sample ID	Permanent Vendor		Sample ID	Mobile Vendor	
	Vibrio spp.	*Salmonella* spp.		*Vibrio* spp.	*Salmonella* spp.
S_1P	Absent	Absent	S_1M	Absent	**Present**
S_2P	Absent	Absent	S_2M	Absent	Absent
S_3P	**Present**	Absent	S_3M	**Present**	Absent
S_4P	Absent	Absent	S_4M	**Present**	Absent
S_5P	Absent	Absent	S_5M	Absent	Absent
S_6P	**Present**	**Present**	S_6M	Absent	Absent
S_7P	Absent	Absent	S_7M	Absent	Absent
S_8P	Absent	Absent	S_8M	**Present**	**Present**
S_9P	**Present**	Absent	S_9M	Absent	Absent
$S_{10}P$	Absent	Absent	$S_{10}M$	**Present**	**Present**
$S_{11}P$	**Present**	**Present**	$S_{11}M$	Absent	Absent
$S_{12}P$	Absent	Absent	$S_{12}M$	**Present**	Absent
$S_{13}P$	Absent	Absent	$S_{13}M$	Absent	Absent
$S_{14}P$	Absent	Absent	$S_{14}M$	**Present**	Absent
Percentage	**29%**	**14%**	**Percentage**	**43%**	**21%**

3.5. Antibiotic sensitivity pattern

As the antibiotic resistance mostly found in case of S. aureus therefore the study was designed to observe the resistance pattern of this particular bacterial isolates only. From the From the demographic data it was found that the mobile tea vendors are mostly uneducated, they used rough methods and work under crude unsanitary conditions which might be the cause of heavy contaminations of pathogenic bacteria in mobile tea vendors compared to the permanent ones. In table 4, the concentration of used antibiotics and mean diameter of zone inhibition showed.

Table 4: *Antibiotic sensitivity pattern of isolated S. aureus.*

Name of Antibiotics	Mean Diameter (mm) of Zone of Inhibition								
	Randomly selected colonies								
Oxacillin (1µg)	13 (S)	10 (I)	14 (S)	14 (S)	21 (S)	13 (S)	16 (S)	16 (S)	19 (S)
Tetracyclin (30µg)	21 (S)	18 (I)	30 (S)	16 (I)	16 (I)	28 (S)	14 (I)	34 (S)	11 (I)
Ciprofloxacin (5µg)	19 (I)	21 (S)	6 (R)	30 (S)	24 (S)	7 (R)	21 (S)	17 (I)	27 (S)
Chloramphenicol (30µg)	20 (S)	31 (S)	15 (I)	12 (R)	25 (S)	16 (I)	10 (R)	24 (S)	29 (S)
Amoxicillin (10µg)	18(R)	11(R)	20 (S)	29 (S)	23 (S)	15 (R)	22 (S)	10(R)	16(R)
Erythromycin(15µg)	28 (S)	18 (S)	9 (R)	11 (R)	14 (I)	23 (S)	21 (I)	12(R)	10(R)
Streptomycin (10µg)	34 (S)	21 (S)	21 (S)	29 (S)	23 (S)	30 (S)	22(S)	21 (S)	21 (S)
Gentamycin (10µg)	23 (S)	10(R)	21 (S)	19 (S)	21 (S)	21 (S)	27 (S)	24 (S)	22 (S)
Ceftazidime (30µg)	16 (I)	26 (S)	12(R)	17 (I)	17 (I)	27 (S)	20 (I)	15 (I)	18 (I)

Table 5: *Frequency of the S. aureus isolates resistant, intermediately sensitive and sensitive to some specific antibiotics*

Name of Antibiotics	Antibiotic sensitivity pattern (%) of isolated *S. aureus*		
	Sensitive	Intermediately sensitive	Resistant
Oxacillin (1µg)	89%	11%	0%
Tetracyclin (30µg)	45%	55%	0%
Ciprofloxacin (5µg)	56%	22%	22%
Chloramphenicol (30µg)	56%	22%	22%
Amoxicillin (10µg)	45%	0%	55%
Erythromycin(15µg)	34%	44%	45%
Streptomycin (10µg)	100%	0%	0%
Gentamycin (10µg)	89%	0%	11%
Ceftazidime (30µg)	22%	67%	11%

From Table 5, it was observed that antibiotic sensitivity pattern of the S. aureus isolates were sensitive to most of the antibiotics and the highest sensitivity was observed against Streptomycin whereas high resistance observed against Amoxicillin. From the demographic data it was found that the mobile tea vendors are mostly uneducated, they used rough methods and work under crude unsanitary conditions which might be the cause of heavy contaminations of pathogenic bacteria in mobile tea vendors compared to the permanent one.

4 Discussion

After performing gram staining and biochemical tests, it has been observed that Vibrio spp. and Salmonella spp. are most prominently present in the collected samples. Moreover, Staphylococcus aureus has also been isolated from a large number of samples which indicates the poor quality of the wash water used by the tea vendors. However, the scenario in case of permanent tea vendors is comparatively better than the mobile tea vendors as lower number of microbial con-taminants have been isolated [17]. Table 1, Table 2 and Table 3 show the type of microorganisms isolated, their presumptive identification and the frequency of the presence of bacteria. This study shows that the quality of the wash water used by both permanent and mobile vendors of the urban areas of Bangladesh is not of good quality. Presence of the pathogenic and indicator bacteria reveals the poor hygi-enic condition of the vendors which is more prominent in mobile vendors in comparison with permanent vendors. The sources of contamination of the water could be the bucket, hand of the user, utensils or unhygienic environment. In case of mobile vendor the number of Vibrio found 43% where in permanent vendor the number was 29% only. Antibiotic sensitivity pattern of the S. aureus isolates shows that the isolates are sensitive to most of the antibiotics and the highest sensitivity was observed against Streptomycin of about 100%, whereas high resistance observed against Amoxicillin of about 55%. Chance of spreading water borne diseases in the urban people can be higher due to these mo-bile vendors as they are more popular and easily available to the consumers. However, risk of spreading water borne diseases by the permanent vendor couldn't be ignored de-spite of having lower microbial load. Hygiene status of the permanent vendors and water quality used there should be improved [18]. Findings of the present study demonstrate that wash water used by the tea vendors of Dhaka city con-tains various pathogenic organisms and these can be a po-tential hazard to the health of the inhabitants. This study also reveals the lack of hygiene practice and knowledge about food safety among tea vendors. Consumption of this popular but poorly hygienic drink could pose to a high risk to health. Tea is one of the most popular drinks among urban people of Bangladesh and tea vending is one of the prime sources of employment in low-income people living in Dhaka city. So, tea vending cannot be stopped but quality of the vended tea and hygiene condition of the tea vendors can be improved [19, 20, 21]. Health education, educating food safety, use of quality water for tea preparation, improving personal hygiene and proper waste disposal could ameliorate the situation and reduce health hazard.

5 Conclusion

The present study reveals that Tea sold on Dhaka are likely to be a potential hazard to the health of the Community people in Dhaka as it contains some pathogenic organisms with antibiotic resistance properties. Therefore, health edu-cation of the vendors on personal hygiene, safer food han-dling practice and the proper disposal of waste would im-prove food quality and thereby reduce the risk of contami-nation of street Tea vendor.

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