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Bacterial Contamination of Food Samples Sold in Local Market of Savar City, Bangladesh

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Abstract: To study the microbial quality and risk factors associated with various foods of local market samples were collected from open market of savar city. Nine food samples including fresh fruits, vegetable, chicken egg, ice cream and fruit juices were screened for the detection of potential pathogenic bacteria. A total of twenty three suspected colonies were selectively isolated from various food samples. Bacterial load of different food samples was determined by CFU. Different types of selective and differential media and different biochemical tests revealed five potential pathogenic bacteria such as *S. epidermidis, Staphylococcus aureus, Bacillus, E. coli, Pseudomonas sp.* API 20 E rapid enzymatic detection system was performed to identify *Enterobacteriaceae* family of bacteria on the basis of their enzyme substrate characteristics and changing the color of the substrate inserted in to the microtube. *Psuedomonas* and *E. coli* was pin point confirmed by this test. Ten different types of antibiotic discs were used to observe the antibiotic sensitivity pattern of the isolates. All the isolates showed resistance to all ten antibiotics except E1, C1, M1 (sensitive to Imipenem) and T1 (sensitive to Streptomycin).

Key words: Open market foods · Bacterial contamination · Antibiotic sensitivity · Bangladesh

INTRODUCTION

Foods occur in diverse forms comprising mainly of milk and milk products, cereal and cereal products, fruits and vegetables, meat and meat products, sea foods, sugar and sugar products. The food with its nutrients, apart from being consumed by human, also acts as an excellent media for the growth of spoilage and pathogenic microorganisms. Contaminated food is a common source of human infections. Therefore, microbial food safety is an increasing public health concern worldwide. It is estimated that each year in the United States there are approximately 76 million food borne illnesses caused by *Campylobacter spp*., non-typhoid *Salmonella*, pathogenic *E. coli* [1].

Food contamination with these pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing and handling or preparation. There are two types of retail markets, supermarkets and open markets. Street food vendors are a traditional and indigenous fast food approaching most countries of the South-East Asia Region including Bangladesh. They provide cheap and enjoyable food to millions of consumers [2]. Open markets are traditional open-air markets where foodstuffs are sold by individual vendors or farmers and are usually displayed unwrapped at ambient temperature; each vendor tends to sell similar types of food (such as vegetables, raw meats, fishes).The open markets are thought to have multiple sources of potential contamination (rodents, insects, sewage and water). The contamination and growth of bacteria may be controlled comparably well in open markets; however, the risk of contamination from humans or other routes remain [3].

Different fruits and vegetables are usually sold in open market in Bangladesh. Although many agricultural products are cooked prior to eating, many Southeast Asian cultures also consume uncooked produce either directly or as fresh condiments to other dishes, such as soups. Fresh fruits and vegetables have been known to

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be contaminated with various pathogenic microorganisms and serve as vehicles for human illness [4, 5]. Foods included in salad bars in retail shops [6] and vegetables in open markets [7] have been reported to be contaminated heavily with bacteria, with high aerobic plate count (APC) values and showing contamination with coliforms. Arecognized source for food-borne pathogens is fecal contamination of water used for irrigation, or for processing, of fresh produce [8].

Besides these, in many tropical countries, fruit juices are common beverages and are sold at all public places and roadsides shops. They are well recognized for their nutritive values, minerals and vitamin contents. However in view of their ready consumption, quick methods of cleaning , handling and extraction of the fruit juices are often prove to be an potential health hazard. As in open market different food samples including fruits, vegetables, fruit juices, ice creams, eggs are sold in open state so the possibility of microbial load is also very high in these samples. Surveys of agricultural produce, meats and shellfish have been conducted finding relatively high microbial loads in Southeast Asia [9, 10] indicating that contamination of water for agriculture and aquaculture, compounded by poor food handling during distribution, can have a negative impact on public health. Some have also investigated risk assessment models based on consumption of fresh produce [11].

In Bangladesh, most of food samples of daily necessaries are sold open in open market place. Moreover, no reports are available about the hygienic conditions of these food samples. So, investigation of food hygiene conditions based on scientific evidence is required for the food samples, especially in these markets, to improve the safety and suitability of retail foods sold in developing countries.

MATERIALS AND METHODS

Sample Collection: Eight fruit, fruit juice, egg and vegetable samples were collected from different local market of Savar area and transferred to laboratory. Raw foods were stored at 4°C without washing with water and alcohol and preserved for detailed study. Samples were analyzed within 24 hours.

Isolation of Bacteria: Bacterial enumeration and isolation was carried out by spread plate method in Nutrient Agar media (NA) [12] at adjusted pH 6. One g/ml of sample was suspended in 100 ml of sterile water in a conical flask and vortexes vigorously for five minutes. The plates were inoculated with 10-fold diluted solution and incubated (aerobically) at 37°C for 24 hours in an incubator (Memmert GmbH + Co Kg 8540 Sehwabach) at inverted condition. After incubation, plates having well discrete colonies were selected for counting. Discrete bacterial colonies were isolated immediately after counting. Based on distinct colony morphology, further selection was made and isolates were purified by repeated streaking as well as stored in NA slants at 4°C for further analysis.

Microbial Load Determination: Microbial load was determined from the total number of discrete colonies counted after incubation. Isolated colonies were counted in colony formation unit (CFU per gram) as follows- Number of CFU/ g = Number of CFU/ (Volume plated in ml × total dilution used) [42].

Identification of the Isolates: The selected bacterial colonies were observed to study various characters *viz.* color, form, elevation, margin, surface, optical characters etc. according to Eklund and Lankford (1967) [12]. Bacterial colonies were cultured on different selective and differential media such as: MSA, SSA, EMB, MacConkey, Bouillon agar, King's B, XLD, Simmon Citrate etc. Different biochemical tests (Casein test, Fermentation test, Indole test, Starch hydrolysis test, Catalase test) were also done. Results of the physiological and biochemical tests of selected isolates were analyzed following Bergey's Manual of Systematic Bacteriology [13], Bergey's Manual of Determinative Bacteriology [14], Manual of Microbiological Methods [15], Microbiological Methods [16] and Understanding Microbes [17]. Coagulase test and endospore staining were used as a confirmation test of bacterial genus or species of *Staphylococcus aureus* and *Bacillus* sp. API 20 E were used for confirmation of *Pseudomonas* sp. and *E. coli* [18].

Determination of Antibiotic Susceptibility: Isolates were tested for antibiotic susceptibility against Penicillin 10g, Amoxicillin 10 µg, Ciprofloxacin 5 µg, Imipenem 10µg, Gentamycin 120 µg, Streptomycin 10µg, Oxacillin 1µg, Sulphominazole 25µg, Oxytetracycline 30µg, Ceftriaxone 30 µg by the disc diffusion assay on Mueller-Hinton Agar (Difco, Detroit, MI) following the standard protocol [19]. Agar disc diffusion (Bauer-Kirby) susceptibility test was used to assess the sensitivity of antibiotics according to Bauer *et al.* [19].

RESULTS AND DISCUSSION

Street food is popular in urban settings in many countries. These foods, like others, can be exposed to harmful bacteria during the growing and harvesting process [2].There are many contributing factors associated with the safety of street food. In this study, the microbial quality of different street and local market foods were determined. A total of 19 bacterial isolates were obtained from local market samples. The bacterial loads of different samples are shown in Table 1. We found that mean heterotrophic bacterial load of the samples were ranged from $1.73x10^6$ to$75.9x10^6$CFU/g on NA media. Maximum heterotrophic bacterial counts were observed in the wild mango (Table 1).While minimum heterotrophic bacterial counts were observed in the Cucumber (Table 1) of the local market samples.

Most fruits contain bacterial counts up to 1.0×10^5 CFU/ cm^2 on their surfaces [20]. Our study showed the bacterial count of fruits and vegetables up to $75.9x10^6$ CFU/g which was higher than the previous study. Vegetables sold in open markets are produced by private farms that have poor management of distribution practices and are sold by different vendors. Therefore, the contamination of pathogenic bacteria may be due to unhygienic handling by workers, washing by contaminated water and improper irrigation by untreated water in the farms. Similar findings were observed by Beuchat, *et al*., Tambekar and Mundhana [21, 22, 23].Besides, unwrapped vegetables are often placed directly on benches at ambient temperature until consumption which facilitates bacterial contamination.

A survey of 100 street foods in 100 countries was conducted by WHO [24], revealed the major health threat facing comes from raw and undercooked food, infected food handlers and inadequate hygiene measures in processing and storing of such food. The water quality, hygienic conditions and the level of cleanliness may contribute to food poisoning [2]. The pathogenic bacteria present in different food samples of our present study include *S. aureus, S. epidermidis, Bacillus, E. coli* and *Pseudomonas* etc. The higher percentage of *Staphylococcus* spp. contamination in fish or seafood and vegetables in open market samples could be a result of human contamination of food samples, such as the direct touching of food by human hands [3].So, it is suggested that raw fruits and vegetables purchased from the open market can be associated with pathogenic microbial load, which should be cured before consuming them.

Table 1: Bacterial load of the local market samples

Sample	Bacterial load CFU/g or ml
Mango bar	$2x10^6$
Tamarind juice	$4 x10^6$
Egg	$5 x10^6$
Sugarcane juice	$71 x10^6$
Guava	$3 x10^6$
Ice cream	$3.38x10^6$
Cucumber	$1.73x10^6$
Wild mango	$75.9x10^6$

Physical hazards that contaminated fruits and vegetables during production or handlings are likely to be removed by proper washing and rinsing by suitable antimicrobial agents to reduce contamination before consuming [25].

Fruit juices sold by street venders have shown remarkable microbial contamination in our present study. There are many reports of food borne illness associated with the consumption of fruit juices of several places of India and elsewhere [26]. Contamination of fruit juices sold in restaurants, cafes and even road side's stalls are sometimes unacceptable for human consumption and creates significant health problems [27]. Use of unhygienic water preservation without refrigeration, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can also act as sources of contamination. The contamination of juices was also due to the use of unhygienic conditions of water storage and use of unclean utensils and unhygienic physical and biological contaminants [28]. Similar studies were done by many other workers. In India, the presence of Coliforms and Staphylococci in kinnow and mandarin juices in Patiala city were reported [29]. Similarly Coliforms were observed in fresh fruit and vegetable juices sold by the street vendors of Nagpur city [30]. These results are in agreement to our present study where sugarcane juice and tamarind juices showed the presence of *Bacillus, S. aureus, E. coli, Pseudomonas* sp. like pathogenic bacteria. Different fruit juices have shown to be potential sources of bacterial pathogens notable *E. coli* 0157:H7, species of *Salmonella, Shigella* and *Staphylococcus aureus* [20]. The presence of coagulase positive *Staphylococcus aureus* in juices indicates severe contamination through handling. The entry of *Staphylococcus aureus* in juices, may be attributed to contact with the outer surface of fruits during juicing, survival and growth of food borne pathogens on surfaces of fruits and vegetables have been demonstrated [31]. The ice and water added during preparation were likely to provide possible sources of additional bacterial contamination [32, 33, 34]. Furthermore, these fruit juices

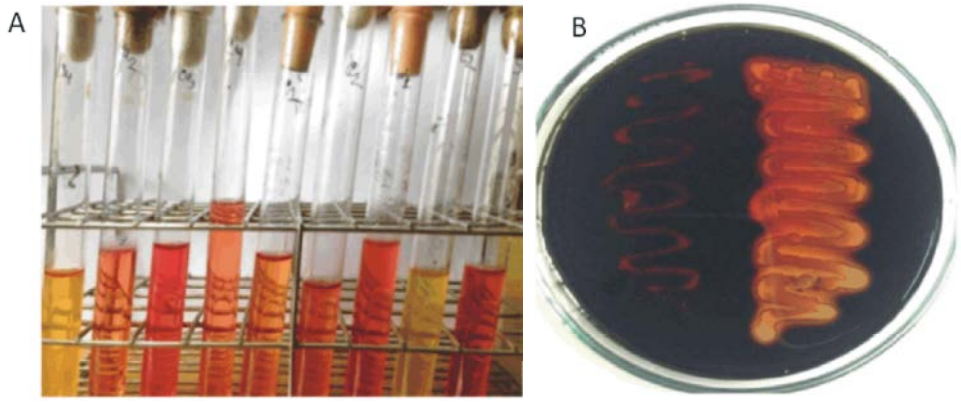

Plate 1: Fermentation test (positive *E. coli* yellow color tube) A, Starch hydrolysis test (B)

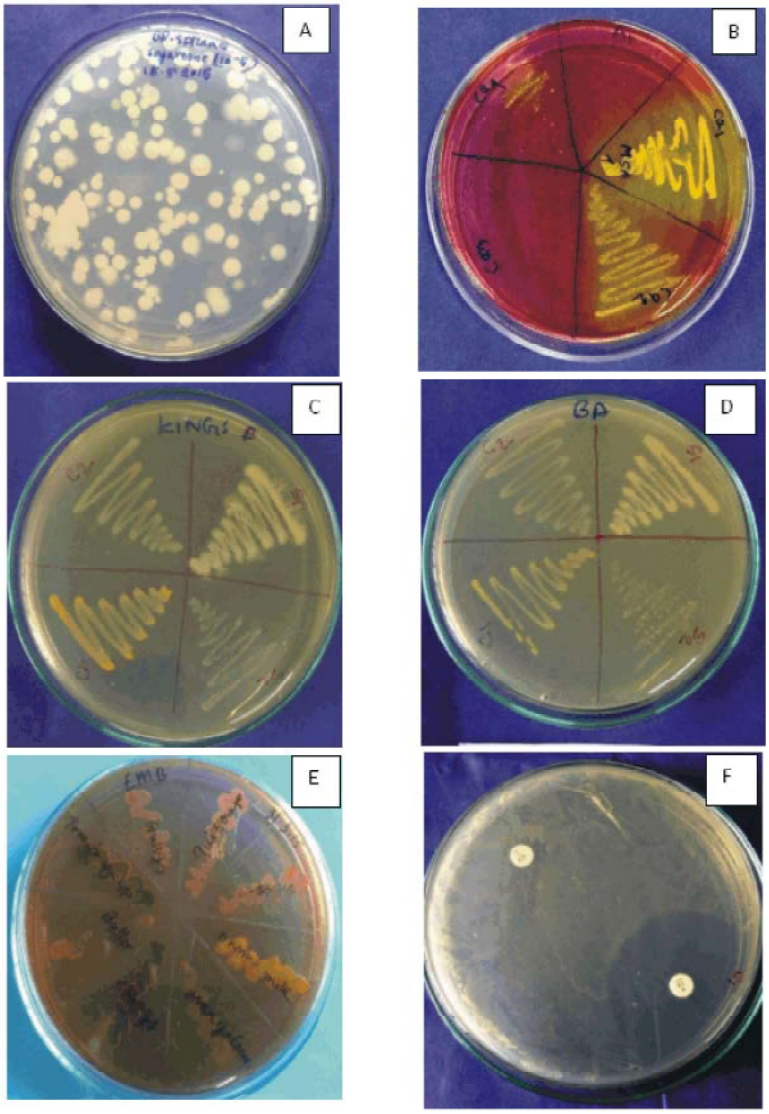

Plate 2: Bacterial isolates on nutrient agar (A), *Staphylococcus aureus* (yellow colonies) and *S. epidermis* (pink colonies) on MSA media (B), *Pseudomonas* sp. (greenish yellow) on Kings B media (C), *Bacillus* sp. (White colonies) on Bouillon agar media (D), *E. coli* (greenish metalic sheen) on EMB media (E) and antibiotic sensitivity test (F)

Table 2: Biochemical characteristics of the bacterial isolates

Sample	Isolates	Gram staining	Catalase test	Indole test	Fermentation test	Starch hydrolysis	Coagulase test	Endospore staining
Mango bar	M1	+	+	-	-	+	-	+
	M2	-	+	-	-	-	NA	NA
	M3	+	+	-	-	-	-	-
Tamarind juice	T1	+	+	-	-	+	-	+
	T2	+	+	-	-	+	-	+
	T3	-	+	+	+	+	NA	NA
	T4	+	+	-	-	-	+	-
Egg	E1	+	+	-	-	+	-	+
	E2	-	+	+	+	-	NA	NA
Sugarcane juice	S1	+	+	-	-	+	-	+
	S2	-	+	-	-	-	NA	NA
Guava	G1	+	+	-	-	-	+	-
	G2	-	+	-	-	-	NA	NA
Ice cream	Ic1	+	+	-	-	+	-	+
	Ic2	-	+	-	-	-	NA	NA
Guava	C1	+	+	-	-	-	-	-
	C2	+	+	-	-	-	+	-
Wild mango	Wm1	+	+	-	-	-	+	-
	Wm2	+	+	-	-	-	+	-

*NA= Not Available

Table 3: Antibiotic sensitivity of bacterial isolates. R, resistance and S, susceptibility to indicated antibiotics

Isolates	P-10	OT-30	0X-1	IPM-10	CRO-30	S-10	RL-25	CIP-5	AML-10	CN-120
E1	R	R	R	S	R	R	R	R	R	R
E2	R	R	R	R	R	R	R	R	R	R
C1	R	R	R	S	R	R	R	R	I	R
T1	R	R	R	R	R	S	R	R	R	R
M1	R	R	R	S	R	R	R	R	R	R
W1	R	R	R	R	R	R	R	R	R	R

were left in ambient temperature which may have led to the proliferation of contaminating bacteria resulting in increased bacterial counts [33, 35].

Moreover, egg sold by street venders also showed the presence of pathogenic microorganisms. A study on eggs reported that enteric bacteria like *Salmonella, E. coli, Listeria,* etc., could contaminate eggs and may cause egg-borne diseases [36, 37]. Some global epidemics have also been linked with egg consumption and known to cause egg-borne pathogens present in poultry eggs and their contents [38, 39]. In our present study, *E. coli* and *Bacillus* was identified from egg samples. Food poisoning associated with egg-borne pathogens may cause severe morbidity or mortality with diarrhea, vomiting, nausea and abdominal cramps [40].

These microbes present in different local market samples are generally associated with some acute chronic diseases. These organisms are highly pathogenic and may cause serious diseases in human beings [27]. For example *Pseudomonas* is an opportunistic human pathogen and a common food spoiler. *Stapholococcus* is generally related to gastrointestinal infections because of its ability to produce very strong toxins. The group members of *Enterobacteriaceae* are widely known for intestinal infection and diarrhea [20].

The antibiotic susceptibility pattern of different bacterial isolates is shown in Table 3. Bacterial isolates E1, C1 and M1 showed susceptibility to the antibiotics Imipenem and T1 isolate showed susceptibility to the antibiotic Streptomycin and resistance to all other antibiotics. Except them all other isolates showed resistance to all the ten antibiotics. These findings showed high resistance of the identified bacteria against different antibiotics. It also suggests that an antibiotic resistant bacteria is increasing which is alarming to us.

Therefore, necessary control measures should be taken to minimize the pathogenic bacterial load in the open market samples. The presence of pathogenic micro flora on the surface of fresh fruits and vegetables indicates the necessity for observing hygienic conditions during production as such type of contamination can occur from soil, water, waste and humans. Further processing of these products is compulsory for raw consumption to ensure their quality and safety to the

user. This could be done by proper washing with water and pretreatment by different antimicrobial agents to decrease the density of microbial contaminants from the surface of the fresh produce.

The Government intervention is also required to protect the consumer and to ensure the quality of the products. Ensuring food safety starts with production, at the farm level. The microbial and chemical risks could be introduced at the farm-level (e.g. using water contaminated by industrial waste or poultry farm waste for irrigation of crops). Good agricultural practices should be applied to reduce microbial and chemical hazards.

CONCLUSION

Microbial spoilage can be minimized or delayed by good hygiene practices, careful handling of food and proper transportation and storage conditions (temperature and humidity).Since many types of contamination and spoilage begin at the farm, the use of non-contaminated water for irrigation and antibiotic-free fertilizer mixes is essential to minimize cross contamination. In addition, quick marketing and high wash water quality are also recommended to avoid further loss of such commodities.

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