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DETECTION OF FOOD BORNE BACTERIA FROM SALAD VEGETABLE ITEMS OF LOCAL MARKETS AND THEIR ANTIBIOGRAM PROFILE

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Abstract

The study was conducted to evaluate bacterial flora of salad vegetable items and for isolation, identification, characterization and antibiogram studies of the organisms obtained. For this, a total of 90 samples from mixed vegetables which are commonly used for salad such as tomato, lemon, green chili, coriander leaf, carrot and cucumber were collected from five different markets located in Mymensingh city. All the vegetables were highly contaminated with bacterial flora. Range of microbial count of tomato was log 6.276 CFU/ml to 6.543 CFU/ml, lemon was log 5.493 to 6.261 CFU/ml, green chili was log 5.205 to 5.64 CFU/ml, coriander leaf was log 7.055 to 7.759 CFU/ml, carrot was log 6.786 to 7.221 CFU/ml and cucumber was log 5.469 to 6.845 CFU/ml respectively. All these samples were also analyzed by culturing in different selective media such as Salmonella-Shigella (SS) agar, Eosin Methylene Blue (EMB) agar, Acetamide agar, M-Aeromonas agar, Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar and Nutrient agar. Biochemical tests were performed to identify the organisms. Out of 90 salads vegetable samples a total of 234 bacterial isolates of six genera were identified. *Pseudomonas* spp. (28.20%) was the most dominant followed by *Bacillus* spp. (20.94%), *Aeromonas* spp. (20.51%), *Vibrio* spp. (16.66%), *Salmonella* spp. (8.11%) and *E. coli* (5.55%) respectively. Results of antibiotic sensitivity test showed that, out of five antibiotics *Salmonella* sp., *Bacillus* sp., *Pseudomonas* sp., *Aeromonas* sp. and *E. coli* were equally sensitive to three antibiotics such as Chloramphenicol, Ciprofloxacin and Gentamicin and resistant to Ampicillin and Cefalexin respectively. Whereas *Vibrio* sp. was sensitive to Chloramphenicol and Gentamicin, intermediately sensitive to Ciprofloxacin and Ampicillin and resistant to Cefalexin. In this study the bacterial load was highest at Natunbazar and lowest bacterial load was recorded in KR market at BAU campus. These results suggest that the necessity to follow the hygienic practices of handling the salad vegetable items in local markets of Mymensingh city, Bangladesh

Keywords*:* Salad vegetables, Microbiological quality, Multi-drug resistant bacteria, Mymensingh city

INTRODUCTION

Vegetables are considered a good dietary source of nutrients, micronutrients, vitamins and fiber, needed vitally for health and well-being of humans. Well balanced diets, rich in vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies are reported to minimize the risk of several diseases (Kalia and Gupta, 2006). Tomato, cucumber, carrot, green chili, lemon and coriander leaf are recognized as some of the salad vegetables that are normally consumed raw. Their traditional use in preparing salads is familiar throughout the world. In recent days' increase in health awareness has led to consumption of minimally processed foods (Wells and Butterfield, 1997). Salad vegetables have thus become popular as it suits the present day necessity and does not need any elaborate preparations (Tournas, 2005).

Vegetables are widely exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. Microbial spoilage and contaminating pathogens pose a serious problem in food safety. Several outbreaks of gastroenteritis have been linked to the consumption of contaminated salad vegetables. The world's largest reported vegetables borne outbreak, occurred in Japan in 1996 in which 11,000 people affected and about 6,000 cultures were confirmed. The outbreak involved the death of the children and was caused by *E. coli* (Beuchat, 1996).

Usually four categories of microbiological quality have been determined based on standard plate counts, levels of indicator organisms and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory and potentially hazardous. Satisfactory results indicate good microbiological quality. No action required. Marginal results are border line in that they are within limits of acceptable microbiological quality but may indicate possible hygiene problems in the handling of the salad vegetables. Premises that regularly yield borderline results should have their vegetables handling controls investigated. Unsatisfactory results are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices. Potentially hazardous, the levels in this range may cause foodborne illness and immediate remedial action should be initiated. Limited works have been carried out to determine microbial contamination of vegetables in Bangladesh (Nipa *et al*, 2011; Rahman and Noor, 2012) but a comprehensive study needs to be performed to determine the wide range of bacteria associated with contamination of vegetables.

Materials and Methods

Collection of samples

A total of 90 salad vegetable samples such as tomatoes (n=15), lemon (n=15), green chili (n =15), coriander leaf (n=15), carrot (n=15) and cucumber (n=15) were collected from five different markets of Mymenshing Sadar Upazilla. The samples were transported to the bacteriology laboratory at 4°C temperature using an ice box.

Isolation of bacteria

Samples were enriched in nutrient broth at 37°C for 24 hours. The overnight bacterial broths were streaked on SS agar (for *Salmonella* spp*)*, EMB (for *E. coli*), NA (for *Bacillus* spp.), MA agar (for *Aeromonas* spp.), AC agar (for *Pseudomonas* spp), TCBS agar (for *Vibrio* spp.) which were incubated at 37°C for 24 hours. Single colony was further subculture until pure culture was obtained.

Identification of bacteria

Colony characteristics of bacteria such as shape, size, surface texture, edge, elevation and color observed in pure culture, Gram's staining and biochemical tests (sugar fermentation, Methyl red, Voges-Proskauer and Indole production) were used for identification of bacteria (Cheesbrough, 1985).

Motility Profile

E. coli and *Bacillus* spp. were found non motile but *Salmonella* spp., *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. were found motile when examined their hanging drop slides under microscope.

Biochemical tests

Results of sugar fermentation tests using five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol are measured in Table 4. Acid and gas production was indicated by change of color of Phenol red from red to yellow and presence of gas bubbles in Durham's tube. Negative reaction was indicated by no change of color.

Antibiotic sensitivity test

Six isolates randomly selected from six genera were tested for antimicrobial drug susceptibility against 05 commonly used antibiotics such as Ampicillin (10 µg), Chloramphenicol (30 µg**)**, Ciprofloxacin (5 µg**)**, Gentamicin (10 µg) and Cefalexin (30 µg) by disc diffusion or Kirby-Bauer method (Bauer *et al*., 1966). Results of antibiotic sensitivity tests were recorded as sensitive, intermediate and resistant following the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2007).

RESULTS

Total viable count (TVC) of salad vegetables

The mean Log CFU of bacteria in tomato, lemon, green chili, coriander leaf, carrot and cucumber samples collected from different markets are presented in Table 1.

Table 1: Total viable count (TVC) per ml of total samples from different markets

Markets name	Tomato (mean log CFU±SD)	Lemon (mean log CFU±SD)	Green chili (mean log CFU±SD)	Coriander leaf (mean log CFU±SD)	Carrot (mean log CFU±SD)	Cucumber (mean log CFU±SD)
KR market	6.276 ± 0.177	5.493 ± 0.479	5.205 ± 0.074	7.055± 0.855	6.786 ± 0.543	5.469 ± 0.415
Sheshmore	6.415 ± 0.513	5.559 ± 0.506	5.556 ± 0.693	7.276± 0.100	6.989 ± 0.746	5.585 ± 0.546
Kewatkhali	6.485 ± 0.457	5.607 ± 0.470	5.62 ± 0.618	7.549± 0.394	7.134 ± 0.807	5.973± 0.505
Mesuabazar	6.302 ± 0.298	5.547 ± 0.369	5.545 ± 0.422	7.115± 0.834	6.927 ± 0.720	5.554 ± 0.501
Natunbazar	6.543 ± 0.531	6.261 ± 0.113	5.64 ± 0.571	7.759± 0.499	7.221 ± 0.966	6.845 ± 0.570

Isolation of bacteria

Six genera of bacteria such as: *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp. and *E. coli*, were isolated from different salad vegetables are presented in Table 2.

Table 2: Summary of isolation of bacteria from different salad vegetables

Name of samples	Bacterial genera (Number and percentages)					
	***Salmonella* spp. (n)**	***Bacillus* spp. (n)**	***Pseudomonas* spp. (n)**	***Aeromonas* spp. (n)**	***Vibrio* spp. (n)**	***E. coli* (n)**
Tomato (n=15)	5(33.33%)	6(40%)	9(60%)	7(46.66%)	6(40%)	ND
Lemon (n=15)	ND	8(53.33%)	7(46.66%)	5(33.33%)	5(33.33%)	ND
Green chili (n=15)	ND	9(60%)	13(86.66%)	10(66.66%)	8(53.33%)	4(26.66 %)
Coriander leaf (n=15)	6(40%)	11(73.33%)	12(80%)	9(60%)	9(60%)	3(20%)
Carrot (n=15)	5(33.33%)	9(60%)	14(93.33%)	11(73.33%)	7(46.66%)	6(40%)
Cucumber (n=15)	3(20%)	6(40%)	11(73.33%)	6(40%)	4(26.66)	ND

ND= Not Detected

Identification of bacteria

Identification of bacteria was performed by cultural characteristics, staining methods and biochemical tests. Summary of cultural characteristic, sugar fermentation and biochemical tests of *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp.and *E. coli* are presented in Table 3 and Table 4.

Table 3: Cultural characteristics of the bacterial isolates

Sl No.	Name of bacteria	Name of selective media	Colony characteristics
1	Salmonella spp.	SS agar	Opaque, smooth, round with black centered
2	Bacillus spp.	Nutrient agar	Thick, grayish-white or cream colored colonies were produced
3	Pseudomonas spp.	Acetamide agar	Purplish red colour colony
4	Aeromonas spp.	M-Aeromonas agar	Yellow brown colony
5	Vibrio spp.	TCBS agar	Yellow colony
6	*E.coli*	EMB agar	Metallic sheen(Greenish black) colony

SS=Salmonella Shigella agar; EMB=Eosin Methylene Blue; TCBS=Thiosulfate Citrate Bile Salts Sucrose

Table 4: Biochemical characteristics of the bacterial isolates

Carbohydrate fermentation test					MR test	VP test	Indole test	Interpretation
DX	ML	L	S	MN				
A	A	-	-	A	+	-	+	Salmonella spp.
AG	A	A	AG	AG	-	+	-	Bacillus spp.
A	A	-	-	-	-	-	+	Pseudomonas spp.
AG	-	-	-	-	+	-	+	Aeromonas spp.
A	A	-	A	A	+	-	+	Vibrio spp.
AG	AG	AG	AG	AG	+	-	+	*E.coli*

DX=Dextrose, ML=Maltose, L=Lactose, S=Sucrose, MN=Mannitol; A=Acid, AG= Acid & Gas; '+'=Positive; '- '=Negative; 'MR'=Methyl red; 'VP'=Voges Proskauer

Overall prevalence of *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp. *and E. coli*

Among 90 samples total number of isolates 234, Prevalence rate of *Salmonella* spp. was 8.11% (19 of 234). Whereas 20.9% (49of 234), 28.20% (66 of 234), 20.5% (48 of 234), 16.66% (39 of 234) and 5.5% (13 of 234) for *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp. and *E. coli* are shown in figure 1.

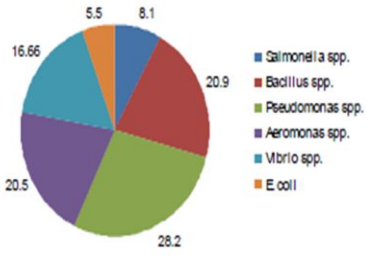

Fig. 1: The prevalence rate of *Salmonella spp., Bacillus spp., Pseudomonas spp., Aeromonas spp., Vibrio spp. and E. coli*

Antibiotics sensitivity tests

Summary of antibiogram profile of *Salmonella* sp., *Bacillus* sp., *Pseudomonas* sp., *Aeromonas* sp., *Vibrio* sp. and *E. coli.* against antibiotics are presented in Fig 2. *Salmonella* sp. was sensitive to 3 and resistant to 2 antibiotics, *Bacillus* sp. was sensitive to 3 and resistant to 2 antibiotics, *Pseudomonas* sp. was sensitive to 3 and resistant to 2 antibiotics, *Aeromonas* sp. was sensitive to 3 and resistant to 2 antibiotics, *Vibrio* sp. was sensitive to 2 and intermediately sensitive to 2 antibiotics but resistant to one antibiotic and *E. coli* was sensitive to 3 and resistant to 2 antibiotics.

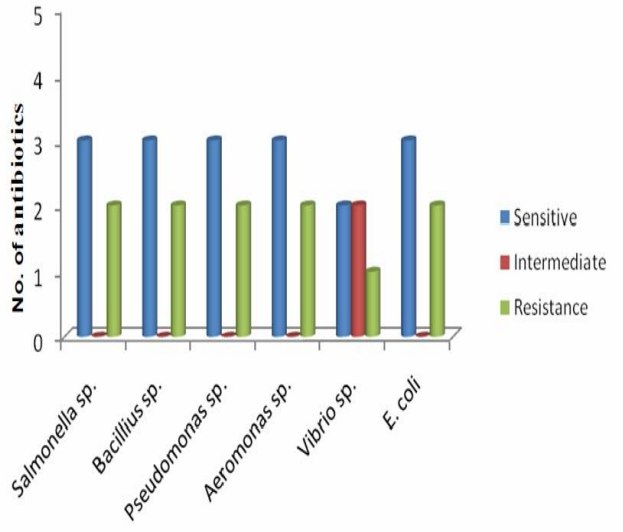

Fig. 2: Summary of antibiogram profile of *Salmonella sp., Bacillus sp., Pseudomonas,* sp., *Aeromonas sp., Vibrio sp. and E. coli against 5 antibiotics.*

Summary of antibiogram profile of Ampicillin (AMP), Chloramphenicol (C), Ciprofloxacin (CIP), Gentamicin (GEN) and Cefalexin (CN) against *Salmonella* sp., *Bacillus* sp., *Pseudomonas* sp., *Aeromonas* sp., *Vibrio* sp. and *E. coli* are presented in Fig. 3. All bacterial isolates (n=6) were sensitive to 2 antibiotics such as Chloramphenicol and Gentamicin. Five isolates were sensitive to Ciprofloxacin. All isolates were resistant to Cefalexin. Five isolates were resistant to Ampicillin. One isolates were intermediately sensitive to Ciprofloxacin and Ampicillin.

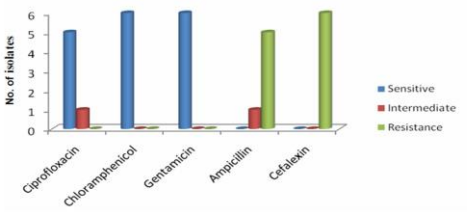

Fig. 3: Antibiogram profiles of bacteria isolated from salad vegetables.

Discussion

The present research work has been chosen to measure microbial loads in salad vegetables items sold in five markets in Mymensingh city and isolate and identify bacteria from salad vegetables items. High total viable count (TVC) indicates unsafe condition and therefore the occurrence of possible contamination.

In case of tomatoes washing sample, the highest TVC mean value was found at Natunbazar where it was log 6.543 CFU/ml and the lowest value was at KR market which was log 6.276 CFU/ml. The TVC value was lower to moderate in the vegetables sample Mesuabazar, Sheshmor and Kewatkhali.

The result of microbial load of lemon washing samples has also shown the similar condition. Lemon collected from Natunbazar with lower hygienic condition was evidenced consistently high mean value (log 6.21 CFU/ml). On the other hand, the lowest bacterial load of lemon washing sample was observed at KR market (log 5.493 CFU/ml). The mean values of TVC of green chili was found highest at Natunbazar where it was log 5.64 CFU/ml and the lowest at KR market which was log 5.205 CFU/ml. The TVC value was lower to moderate in the vegetables sample Mesuabazar (log 5.545), Sheshmor (log 5.556) and Kewatkhali (log 5.62). In case of coriander leaf washing sample, the highest mean values of TVC was found at Natunbazar (log 7.759 CFU/ml) and the lowest value was at KR market where it was log 7.005 CFU/ml.

The obtained TVC mean value of lemon, green chili and coriander leaf washing samples of the present study are in close agreement with the findings of Nipa *et al.* (2011). She also reported that, total bacterial population was log 5.62 CFU/ml, log 4.80 CFU/ml and log 5.5 CFU/ml for lemon, green chili and coriander leaf sample respectively.

Unfortunately, or it might be the real situation that the mean values of TVC of carrot and cucumber washing samples has also depicted the same hygienic level as found in tomatoes, lemon, green chili or coriander leaf washing samples. Carrot collected from Natunbazar with lower hygienic condition was evidently high mean value (log 7.221 CFU/ml) and the lowest at KR market (log 6.786 CFU/ml. In case of cucumber washing sample, the highest TVC mean value was found at Natunbazar where it was log 6.854 CFU/ml and the lowest was at KR market where it was log 5.469 CFU/ml. The TVC value was lower to moderate in the vegetables sample Mesuabazar, Sheshmor and Kewatkhali.

The obtained TVC mean value of carrot and cucumber washing samples of the present study are in close agreement with the findings of Uzeh *et al.* (2009). He also reported that, total bacterial population was log 6.77 CFU/ml and log 3.11 CFU/ml for carrot and cucumber samples respectively. This variation may be due to variation in maintained sanitary, handling and preservation condition of vegetables. Doores (1983) suggested to pay careful attention to maintain a microbiologically stable environment to achieve high quality in raw vegetables and processed products.

In this study, the colonies of *Salmonella* spp. on SS agar plate were opaque, translucent with black centers which were similar to the findings of other authors (Cheesbrough, 1985). In Gram's staining bacteria exhibited short rods, Gram negative, single or paired in arrangement. Similar findings were also reported by Buxton and Fraser (1977). Sugar fermentation tests profile of *Salmonella* spp. in the present study showed similarities with the findings of other researchers (Cowan, 1985). Morphology and staining characteristics of *Bacillus* spp. recorded in this study are in agreement with the finding of the other researchers (Merchant and Packer, 1967). Biochemical test of *Bacillus* spp. was able to ferment the five basic sugars by producing acid (Granum, 2001**)**. All the isolates fermented dextrose, sucrose, lactose, maltose and mannitol with the production of acid within 24h-48h of incubation.

In this study, the colonies of *Pseudomonas* spp. on Acetamide agar plate were purplish red colour which was similar to the findings of Izumi *et al.* (2004**)**. The result of Gram's staining was also in agreement with the report of Izumi *et al.* (2004). Sugar fermentation and catalase test are positive of *Pseudomonas* spp. in the present study. In this study, the colonies of *Aeromonas* spp. on M-Aeromonas agar plate were yellow-brown colour. In Gram's staining bacteria exhibited short rods, Gram negative, single, paired arrangement. Sugar fermentation and catalase test are positive of *Aeromonas* spp. in the present study.

In this study, the colonies of *Vibrio* sp 5 3S agar plate which were similar to the findings of other authors Khan *et al.*, (2007). In Gram's staining bacteria exhibited curved rod shaped appearance which was supported by other researchers (Faruque *et al.*, 2008; Kaper *et al*., 1995). In this study *Vibrio* spp. produced acid in dextrose, maltose, mannitol and sucrose and negative to VP test (Kaper *et al*., 1995).

In this study, different selective and differential agars are used for isolation of *E. coli* from samples. Colony characteristics of *E. coli* observed in EMB agar were similar to the findings of other authors Sharada *et al.* (1999)**.** Morphologically *E. coli* were Gram negative short rod arranged in single or paired and motile. Several researchers also described similar cultural, staining and motility characteristics of *E. coli* Thomas *et al.* (2005). The identified bacteria were re-confirmed through the use of different sugar fermentation and other biochemical tests which were found similar with the findings of other researchers Thomas *et al.* (2005).

In the present study, antibiotic sensitivity or resistance pattern (antibiogram profile) of isolated bacteria was investigated against five commonly used antibiotics (Chloramphenicol, Gentamicin, Ciprofloxacin, Cefalexin and

Ampicillin) using disc diffusion method (Bauer *et al.*, 1966). The results of antibiogram showed that *Salmonella*, *Bacillus*, *Pseudomonas*, *Aeromonas* and *E. coli* were almost sensitive to Chloramphenicol, Gentamicin and Ciprofloxacin, only *Vibrio* spp. were intermediately sensitive to Ciprofloxacin. Whereas, almost all the bacterial isolates showed resistance pattern to Cefalexin and Ampicillin, except *Vibrio*, which showed completely resistance to only Cefalexin, but intermediately sensitive to Ampicillin.

Nawas *et al.* (2012) found multiple drug resistance (MDR) in 39 and 51 isolates of *Salmonella* and *Vibrio* isolates, respectively among a total of 102 isolates, using disc diffusion method. The author reported that *Salmonella* spp. from salad and water showed resistance against Amoxicillin (75%), Cephradine and Cefalexin (68.75%). 85.71% *Vibrio* spp. isolated from salad and water were resistant to Amoxicillin respectively. The antibiogram profile of *Bacillus* spp. was almost similar to the findings of Whong and Kwaga (2007), where the author found that all *B. cereus* isolates were found to be susceptible to Ciprofloxacin, Chloramphenicol and Ofloxacin and overall resistance to Penicillin G (82%), Cefotaxime (56.7%), Ceftriaxone (53.3%) and Ampicillin (44%) were most frequent.

Łaniewska-Trokenheim *et al*. (2006) examined the antibiotic resistance pattern of *E. coli* and found that the highest number of isolated strains were resistant to Ampicillin (81.9%), whereas a lower number of the strains exhibited resistance to the Chloramphenicol (19.8%). *A*ntibiotic sensitivity test of the *Pseudomonas fluorescens* isolates were conducted by Foysal *et al.* (2011) using disc diffusion method for seven antibiotics, where all of the isolates were found to be sensitive only against Streptomycin and Gentamicin but, most of the isolates (80%) were found resistant to Chloramphenicol less similar with the findings of antibiogram profile of *Pseudomonas* spp. conducted in the present study.

CONCLUSIONS

A significantly high bacterial load was recorded in all vegetable samples in the study. Six genera of bacteria were isolated such as *Salmonella* spp., *Bacillus* spp., *Pseudomonas* spp., *Aeromonas* spp., *Vibrio* spp. and *E. coli.* However, *E. coli* was not found in tomatoes, lemon & cucumber and *Salmonella* spp. was not found in lemon & green chili. Antibiogram profiles of this study indicated that all six bacterial genera of vegetables are multidrug resistant which may cause human food-born infection and intoxication.

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