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Prevalence of multidrug resistant *Salmonella* spp. in raw vegetables in Jessore city, Bangladesh.

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Abstract

Food borne illness especially enteric fever is a common issue all over the world as well as in Bangladesh which results from consuming foods, vegetables or drinking beverages that are contaminated with infectious microorganisms. Antibiotic resistance of microorganisms is now a frequently asked public health concern and very tough to resolve the problem. The present study was conducted to evaluate microbiological quality of raw vegetables and their role as a source of antibiotic resistant bacteria specially *Salmonella* spp. A total of 9 types of vegetables were examined including Tomato, Carrot, Green chili, Cabbage, Cauliflower, Bean, Onion pulp, Red amaranth and Cucumber collected from open markets in Jessore town. *Salmonella* spp. were isolated using selective media such as XLD, BSA and BGA media and further biochemical tests were conducted to confirm their presence in selected vegetables. All the positive isolates were tested with commercially available antibiotic disc in Muller-Hinton agar media. Isolated *Salmonella* spp. were fully resistant to Ampicillin (10µg) and Streptomycin (10µg), moderately resistant to Gentamycin (10µg), partially resistant to Nalidixic acid (10µg) and Erythromycin (15µg) except Ciprofloxacin (30µg) and Tetracyclin (30µg) which were fully sensitive. Indiscriminate consumption of antibiotics and their disposal into the environment might be a better source of multiple antibiotic resistant bacteria in vegetables. These results suggest the necessity to follow the hygienic practices in handling vegetables in open markets as well as limitation of using compost prepared from human and animal excreta.

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Introduction

Fresh products like vegetables always play a great role in human diet. It is an essential source of vitamin, minerals, nutrients and antioxidants which are the health promoting factors for human being (Maffei *et al.,* 2013). Vegetables are the main vehicles of the food borne illness in many countries. Vegetables may be contaminated by bacteria, viruses or parasites (De Rover *et al.,* 1998). Surface of raw vegetables harbor many microorganisms sometimes it can makes micro colonies over the plants tissue (Szabo *et al.,* 2001).

Reports indicated the potentiality of vegetables containing Salmonellae on their surface which are mostly pathogenic (Salleh *et al*., 2003; Abadias *et al*., 2008). Most of the *Salmonella* spp. are pathogenic for human and vertebrates and causes many diseases. Infected humans and animals shed salmonellae into the environment via feces and reinfection may take place by ingestion of salmonellae contaminated food and water. About 16 million cases of typhoid fever occur annually while 1.3 billion gastroenteritis as well as 3 million cases of death occurs globally due to *Salmonella* contamination (Bhunia, 2008).

Some diverse factors may affect the variation of microbial profile of vegetables including normal micro flora of soil, animal manure derived flora, irrigation or sewage water, transportation and unconscious handling by retailers (Ray *et al.,* 2007; Oxfor *et al.,* 2009). Antibiotic abuse is increasing to date and that's why multidrug resistant bacteria are increasing day by day.

The frequent use of antimicrobial drug speeds up the drug-resistant strains in the environment. Among many developing countries, Bangladesh is now in most endangered condition due to frequently emergence of antibiotic resistant bacteria from hospital water and sewage samples (Ashfaq *et al.,* 2013). Jessore city is the most renowned place for the production and supplying vegetables all over the country. Due to lack of surveillance and inadequate screening of these raw vegetables, most outbreaks are becoming undetected and literature reports very insufficient information till now (Meher *et al.,* 2011).

Poor infection control practices, inadequate sanitary conditions and inappropriate food handling encourage the further spread of antimicrobial resistant (AMR) bacteria. In most of the cases, drug resistance can be occurred by the genetic change which may be influenced by the plasmid acquisition or transposon or chromosomal mutation (Warren, 2008; Winstaniey *et al.,* 2001).

According to world health organization (WHO) the multidrug resistant microorganisms are more prevalent worldwide reported in the infectious disease report in 2000 (WHO 2000).

The current study focused on the quality of raw vegetables and salad agents from four different markets as they are sometimes eaten raw or uncooked and determination of *Salmonella* spp. which is very notorious to cause salmonellosis as well as enteric fever in mortals. Furthermore, the antibiotic resistance of salmonellae was also evaluated as the topic is very burning issue for third world countries like Bangladesh due to the indiscriminate use of antibiotics.

Methods and materials

Collection of Samples

A total of 45 samples of 9 different types of vegetables (Table 1.) were collected using sterile poly ethylene bags separately from 4 different markets in Jessore city (Borobazar, Palbari, Churamonkathi, Ambottola). The sampling time was October 2012 to February 2013. After collection all samples were insulated instantly in an ice box for keeping the temperature range from 4 to 6°C.

Within one hour of collection, the samples were taken into the laboratory and rinsed with 100 ml distilled water for each and then diluted 10 fold. After washing the surface of vegetables, 10 ml of washed aqueous suspension of each sample was mixed with 90 ml Luria Bertani (LB) broth and incubated at 37°C for 24 hours. Then one loop full culture was streaked to the selective media for isolation and identification of *Salmonella* spp.

Isolation and Identification

One loop full culture from LB broth was streaked over Nutrient Agar (NA) (Oxoid, England) surface and kept it for incubation overnight at 37°C. After proper incubation young colonies from the Nutrient agar surface were subsequently streaked over the selective media of *Salmonella* spp. named Xylose Lysine Deoxycholate (XLD) (Merck, Germany), Bismuth Sulfite Agar (BSA), Brilliant Green Agar (BGA) (Oxoid, England) and incubated at 37°C for 24 hour.

All salmonella positive cultures were further studied for biochemical analysis in conventional way for confirmation of *Salmonella* spp. Biochemical tests including Oxidase test, Catalase test, Motility test, TSI test, Urease test, MR-VP test, citrate utilization test, Casein hydrolysis test, Indole test, glucose fermentation test etc (Table 2). When the target strain was confirmed then all positive isolates were stocked in glycerol broth for further analysis.

Antimicrobial Susceptibility testing

For antimicrobial sensitivity testing the cotton bud containing viable culture was swabbed 3 times over the entire Muller Hinton agar (Oxoid, England) surface by disc diffusion technique (Bauer *et al.,* 1966) by rotating the plate approximately 60° each time to ensure an even distribution and confluent growth. Eight different commercial antibiotic discs were used. They included ciprofloxacin (Cip), erythromycin (Ery), ampicillin (Amp), gentamycin (Gen), Nalidixic acid (Nal), Streptomycin (Str) tetracycline (Tet), Chloramphenicol (Chl).

The discs were placed on Muller Hinton agar plate with not less than 2 cm apart and kept in refrigerator for 2 hours for proper absorption into the plate. The agar plates were then incubated at 37°C overnight. Diameter of clear zones around each antibiotic disc were measured and recorded.

Result

The result of this study highlighted the possibility of *Salmonella* spp. prevalence into the raw vegetables in a concerning amount. In this study, only *Salmonella* spp. was targeted because the pathogen is mostly responsible for acute gastroenteritis as well as enteric fever.

Within four months of time period all samples were collected, processed, experimented and analyzed. All 45 samples were periodically tested and grown in selective media for isolation of *Salmonella* spp. After recovery from selective media, 17 positive samples were selected (Table 1) for biochemical test and confirmed as positive isolates (Table 2).

Table 1. Number of vegetable samples positive and negative for *Salmonella* with sample no (s) of contaminated vegetables.

Sample type	Sample no(s)	Positive Sample No(s)	*Salmonella* spp.(a/N)*
Tomato	1-5	2,5	2/5
Carrot	6-10	7,8,10	3/5
Green chili	11-15	11	1/5
Cabbage	16-20	15,17	2/5
Cauliflower	21-25	21,22,23,25	4/5
Bean	26-30	27,29	2/5
Onion pulp	31-35	32	1/5
Red amaranth	36-40	37,38	2/5
Cucumber	41-50	_	0/5
			n=17/45

*(a/N): number of *Salmonella* contaminated vegetable(s)/ total number of vegetables experimented.

Table 2. Result showing the biochemical tests of all positive isolates.

Sample ID	Oxidase	Catalase	Motility	Citrate utilization	Urease	TSI	MR	VP	LDC	Casein hydrolysis	H2S production	Indole	Acid-Gas formation	Glucose fermentation
2	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
5	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
7	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
8	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
10	-	+	+	+	_	AA	+	_	+	_	+	_	+	+
11	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
15	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
17	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
21	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
22	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
23	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
25	-	+	+	+	_	AA	+	_	+	_	+	_	+	+
27	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
29	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
32	-	+	+	+	_	AA	+	_	+	+	+	_	+	+
37	-	+	+	+	_	AA	+	_	+	_	+	_	+	+
38	-	+	+	+	_	AA	+	_	+	+	+	_	+	+

AA= Alkaline slant and Acidic butt.

Following biochemical analysis all 17 positive isolates were picked up for arteriogram to observe their susceptibility to eight different antibiotics such as ciprofloxacin (5µg), erythromycin (15µg), ampicillin (10µg), gentamycin (10µg), nalidixic acid (30µg), Streptomycin (10µg) tetracycline (30µg), Chloramphenicol (30µg). The preparation of all tested organisms for antibiogram purpose was done according to McFarland 0.5 standard for maintaining the limit of cell concentration (Mcfarland J., 1907). Commercially prepared antibiotic discs were placed into the randomly selected colony swabbed previously in Muller Hinton agar plate and the zone of inhibition was measured following proper incubation (Table 3).

After measuring the zone of inhibition frequency of resistant, intermediate resistant and sensitiveness of tested antibiotics were obtained (Table 4). All positive isolates showed 100% resistant to ampicillin and streptomycin. Erythromycin and gentamycin were about to resistant and their percentage were 64.70 and 76.47. But it was also a matter of hope that Tetracycline and Chloramphenicol were still 100% effective against all *Salmonella* spp. whereas 76.47% isolates were sensitive to Ciprofloxacin which was almost in very prolific condition. Only Nalidixic acid showed intermediate resistance to handsome amount of the isolates and it was 47.05% (Fig 1).

Table 3. Antibiotic susceptibility pattern of *Salmonella* spp.isolated from vegetables.

Name ofantibiotics	Zone of Inhibition in diameter(mm)																			
	Randomly selected colonies																			
	11	2	38	27	15	7	25	17	32	21	23	15	5	10	29	22	8			
Amoxicilin (10µg)	0	0	0	0	9	0	11	0	0	0	10	0	0	0	8	0	0			
Ciprofloxacin (5µg)	24	20	27	28	19	25	27	26	31	18	28	29	21	32	23	19	29			
Erythromycin (15µg)	12	0	15	0	0	14	11	13	9	14	13	0	0	17	19	0	14			
Gentamycin (10 µg)	10	7	8	0	10	0	0	11	13	17	11	0	13	12	15	0	11			
Nalidixic acid (30µg)	16	18	19	14	15	21	12	16	12	22	14	11	14	20	13	19	17			
Streptomycin (10µg)	0	0	0	8	0	0	0	10	11	0	9	0	0	8	8	0	11			
Tetracyclin (30µg)	21	24	23	21	19	27	22	26	24	27	23	31	27	25	29	27	32			
Chloramohenicol (30µg)	22	27	29	24	28	28	21	27	27	25	30	26	29	22	29	31	28			

Table 4. Frequency of *Salmonella* isolates resistant, intermediate resistant and sensitive to some specific antibiotics.

Antibiotics	Antibiotic sensitivity pattern (%) of *Salmonella* spp.		
	Resistant (%)	Intermediate resistant (%)	Sensitive (%)
Amoxicillin (10µg)	100	0	0
Ciprofloxacin (5µg)	0	23.53	76.47
Erythromycin (15µg)	64.70	35.30	0
Gentamycin (10 µg)	76.47	11.76	11.76
Nalidixic acid(30µg)	23.53	47.05	29.41
Streptomycin (10µg)	100	0	0
Tetracyclin (30µg)	0	0	100
Chloramphenicol (30µg)	0	0	100

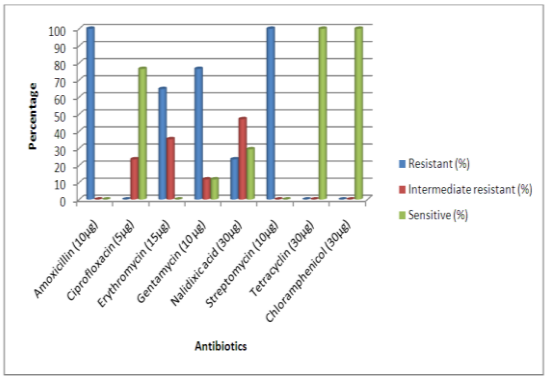

Fig. 1. Diagram of antibiotic resistant percentage of *Salmonella* spp. from different vegetable samples.

Discussion

Microbial resistance of a wide range of drugs is an unavoidable issue all over the world (Garau, 1994). Apart from vegetables multidrug resistant *Salmonella* spp. also found in another sources like hospital waste water, pond water, poultry meat and feces and ready to eat food products reported by David *et al.*, (2001) and Iruka *et al.,* (2005). The current study revealed that about 38.78% vegetable samples from different markets in Jessore city were highly contaminated with *Salmonella* spp within 45 samples. As nine types of vegetable samples were tested, all but cucumber was not totally contaminated with *Salmonella* spp. which was a significant result of this study comparing to Meher *et al.*, (2011). Additionally, in green chili and onion pulp samples we found very little amount of *Salmonella* spp. present on them. On the other hand, cauliflower and carrot samples were mostly contaminated with the suspected strain. It would be a reason that, these vegetables were very close proximity to soil or fertilizer used for their cultivation was originated from human or animal excreta as most *Salmonella* spp. is fecal origin (Saddik *et al.*, 1985).

Moreover, most of these isolated microorganisms showed resistance to a lot of antibiotics which was a matter of concern. In our study there were two leafy vegetables cabbage and red amaranth, which may harbor or shed *Salmonella* spp. due to the morphology, size and rough surface of leaves reported by some authors (Seow *et al.,* 2012; Aycicek *et al.*, 2006; Abadias *et al.,* 2008).

Multidrug resistant phenotype of *Salmonella* server has also been reported in many reports worldwide (Zhao *et al.,* 2003; Ponce *et al.,* 2008). In this study it was also showed the activity of Ampicillin and Streptomycin has totally run down and the effect of Gentamycin as well as Erythromycin to the tested strain was about to extinct. Ampicillin belongs to amino penicillin group whose function is to inhibit cell wall synthesis of bacteria but Streptomycin and Gentamycin belongs to aminoglycoside group whose function is to subside the protein synthesis of *Enterobacteriaceae* (CLSI, 2007). On the other hand Nalidixic acid is the first generation antibiotic belongs to quinolone group was less effective than ciprofloxacin which is the second generation antibiotic of fluoroquinolone group showed higher sensitivity against *Salmonella* spp (CLSI, 2007). Abundant use of antimicrobial agents in both veterinary and human medication is widely believed to be account for multidrug resistance which is the alarming public health concern (Kusumaningrum *et al.,* 2012).

It was observed that in Bangladesh the basic design of all open markets where raw vegetables, fish, meats etc. are sold are as in same organization and style as these products are sold with close vicinity and swarming flies might be the source of bacterial contamination (Meher *et al.,* 2011).

In another report it was found that the prevalence of *Salmonella* spp. was lower in amount in fresh and minimally processed vegetables (Maryam *et al.,* 2014). In this study most of the selected vegetables were salad agents which are normally eaten at uncooked conditions or sometimes cooked very slightly which is not enough for removal of all microorganisms. Some vegetables like cabbage, onion pulp are used as decorating agents on salad and salmonella contamination might come from it. Moreover, the experimented area was the hotspot for vegetable cultivation, processing and production. So any pathogenic bacterial contamination was not expected at all. *Salmonella* spp. was present in all types of vegetables except cucumber and from that point of view we can suggest that the contact of soil is directly related to salmonella contamination. Multi drug resistance in *Salmonella* spp. might come from contaminated water during irrigation, processing and washing for selling to the market (Singh *et al.,* 2006). However, the source of that contamination in vegetables were not identified but assumed that source of *Salmonella* spp. were fecal origin and transferred by irrigation water and compost manure (Ray *et al.,* 2007). Furthermore, the contaminated samples were also 100% resistant to a wide range of antibiotic groups which was never expected. Farmers, vegetable handlers as well as open market retailers should be more conscious about the issue and it would be come true by giving them proper training about how to handle vegetables, what is the minimum limit of using compost and what is the minimum time of preserving vegetables.

Conclusion

The present study demonstrated the occurrence of multiple antibiotic resistances among different isolates of *Salmonella* spp. from raw vegetables and salad agents in Jessore city, Bangladesh. Thus, intensive surveillance is needed to detect the emerging phenomena of antimicrobial resistance in developing countries. Vegetables can be a good source of multidrug resistant Salmonellae to the human which causes a lot of complexities into the body. As some vegetable are eaten raw or lightly cooked the anchoring microorganisms can get a good chance for entrance into the human intestine. Except *Salmonella* spp. there could be a lot of microorganisms present which might also cause a lot of diseases. So, adequate washing of vegetable with fresh water or with food grade chemicals and maintenance of appropriate temperature is highly needed before consumption to eliminate the microbial load from these food items.

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References

Abadias M, Usall J, Anguera M, Solsona C, Viñas I. 2008. Microbiological quality of fresh, minimally processed fruit and vegetables, and sprouts from retail establishments. International Journal of Food Microbiology **123,** 121-129.

Ashfaq KMA, Pijush S, Majharul IM, Kant OR, Chandra BG. 2013. Screening of antibiotic resistant Gram negative bacteria and plasmid profiling of multi-drug resistant isolates present in sewage associated with health care centers. International Journal of Medical Research & Health Sciences **2(4),** 923-930.

Aycicek HÃ, Oguz U, Karci K. 2006. Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. Int J Hyg Environ Health **209**, 197-201.

Bauer AW, Kirby MDK, Sherries JC and Truck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Patho l**45,** 493-496.

Bhunia AK. 2008. Foodborne microbial pathogens: Mechanisms and pathogenesis. United States of America: Springer Science + Business Media LLC.

Clinical and laboratory standards Institute. 2007. Performance standards for antimicrobial susceptibility testing; seventh informational supplement. CLSI document M100-S17 [ISBN 1-56238-625-5].

David GW, Shaohua Z, Robert S, Sherry A, Sharon F, Sheng C, Patrick FM, Shawn M, David DW, and Jianghong M. 2001. The Isolation of Antibiotic-Resistant Salmonella from Retail Ground Meats. N Engl J Med. **345**,1147-1154.

De Rover C. 1998. Microbial safety evaluations and recommendations on fresh produce. Food Control **9(6),** 321-347.

Garao J. 1994. B lactamases: Current situation and clinical importance. Intensive Care Med **20(3),** 5-9.

Iruka NO, Ramanan L, Zulfiqar AB, Adriano GD, Philip J, Thomas FOB, Ariel PM, Keith PK. 2005. Antimicrobial resistance in developing countries. Part I: recent trends and current status, The Lancet Infectious Diseases **5(8)**, 481-493.

Kusumaningrum HD, Suliantari and Dewanti-Hariyadi R. 2012.Multidrug resistance among different serotypes of *Salmonella* isolates from fresh products in Indonesia. International Food Research Journal **19(1)**, 57-63.

Maryam ZJ, Masud Y, Mohamad EG, Negin N, Mohammad RP, Gholam RJK. 2014. Microbial Evaluation of Fresh, Minimally-processed Vegetables and Bagged Sprouts from Chain Supermarkets. J Health Popul Nutr **32(3)**, 391-399.

Maffei DF, Silveira NFA, Catanozi MPLM. 2013. Microbiological quality of organic and conventional vegetables sold in Brazil. Food Control **29**, 226-30.

McFarland J.1907. Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc **14**,1176-8.

Meher NN, Reaz MM, Mahmudul HM, Fakruddin M, Saiful I, Habibur R. Bhuiyan and Asif I. 2011. Prevalence of Multi Drug Resistant Bacteria on Raw Salad Vegetables Sold in Major Markets of Chittagong City, Bangladesh. Middle-East Journal of Scientific Research **10(1),** 70-77.

Oxfor MO, Okorie VC, Ibeawuchi II, Ihejirika GO, Obilo OP and Dialoke SA. 2009. Microbial contaminants in fresh Tomato Wash Water and Food Safety Considerations in south-Eastern Nigeria. Life Sci. J **1**, 80-82.

Ponce E, Khan AA, Cheng C, Summage-West C Cernigli CE. 2008. Prevalence and characterization of *Salmonella enterica* serovar Weltevreden from imported seafood. Food Microbiology **25,** 29-35.

Ray B, Bhunia AK. 2007. Fundamental Food Microbiology. 4th Edn., CRC Press USA pp. 492.

Saddik MF, EI-Sherbeeny MR, Bryan FL. 1985. Microbiological profiles of Egeyptian vegetables and salads. J. Food Prot **48,** 883-886.

Salleh NA, Rusul G, Hassan Z, Reezal A, Isa SH, Nishibuchi M and Radu S. 2003. Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. Food Control **14,** 475-479.

Seow J, Ágoston R, Phua L, Yuk H-G. 2012. Microbiological quality of fresh vegetables and fruits sold in Singapore. Food Control **25**, 39-44.

Singh BR, Singh P, Verma A, Agrawal S, Babu N, Chandra, Agarwal RK. 2006. A study on prevalence of multi-drug-resistant (MDR) Salmonella in water sprinkled on fresh vegetables in Bareilly, Moradabad, and Kanpur (northern Indian cities). J Public Health **14**, 125-131.

Szabo EA, Coventry MJ. 2001. Vegetables and vegetable products. In C.J. Moir, *et al*., (eds) Spoilage of Processed Foods; Causes and Diagnosis. AIFST Inc. (NSW Branch) Food Microbiology Group. Waterloo.

Warren Levinson. 2008. Review of Medical Microbiology and Immunology 10th Edn, The McGraw-Hill Companies Inc 85-93.

Winstaniey C. Hart CA. 2001. Secretin systems and pathogenicity islands. J Med Microbiol **413,** 848-852.

World health Organization. 2000. Overcoming Antimicrobial Resistance World Health Report on Infectious Disease.
www.who.int/infectious -disease-report/2000/indxrpt2000_text.html).

Zhao S, Datta AR, Ayers S, Friedman S, Walker RD. and White DG. 2003. *Salmonella* serovars isolated from imported foods. International Journal of Food Microbiology **84**, 87-92.
DOI: 10.5958/j.2319-5886.2.4.148