The Bioscan 3 (4) : 485 - 488, 2008
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

EVALUATION OF MICROBIOLOGICAL SAFETY OF READY-TO-EAT SALAD VEGETABLES: A CASE STUDY OF AMRAVATI CITY (INDIA)

D. H. TAMBEKAR*, S. D. TAMBEKAR, D. V.DHANORKAR AND P. B. GULHANE
Post Graduate Department of Microbiology,
S.G.B. Amravati University, Amravati - 444 602 (INDIA)
E-mail: diliptambekar@rediffmail.com

KEY WORDS
Pathogens in Salad vegetables
Salmonella spp.
E.coli
Bacterial contamination

Received on :
23.03.2008
Accepted on :
27.10.2008

***Corresponding author**

ABSTRACT
Study was undertaken to evaluate bacteriological quality of ready-to-eat salad vegetables sold in local markets in Amravati city. A total of 42 different ready-to-eat salad vegetable samples were analysed and 75 different bacterial pathogens were isolated and identified by standard bacteriological techniques. The prominent pathogen was Salmonella spp. (33%) found in all vegetables followed by *Escherichia coli* (32%), *Proteus* spp. (15%), *P. aeruginosa* (13%), *S.aureus* (6%) and *Enterobacter* spp (1%). The highest (13%) bacterial contamination was recorded in coriander followed by spinach (11%), cauliflower, tomato, cucumber, radish and carrot (10%), cabbage and beet (8%), spring onion and least (7%) in fenugreek. The study concluded that, number of pathogenic bacteria due to various reasons and some times an important source of food borne illness to human especially when the salad vegetables are consumed raw.

INTRODUCTION

Consumption of ready to eat salad vegetables has increased in quantity and variety in recent years. Mixed salad is frequently served as an accompaniment to Indian meals, kebabs, pizzas, burger and other take away meals. Thus, ready-to-eat salad vegetables are generally considered safe to eat by consumers (European Commission, Health and Consumer Protection, Directorate-General, 2002, Mintell Market Intelligence Report, 2002; Dhiraputra *et al.*, 2005). But the numerous reports have indicated that raw vegetables may harbor potential food borne pathogens. The salad vegetables are consumed without any heat treatment, sometimes without washing and peeling and therefore the possibility of food borne diseases is more (Beuchat, 1996, Report of the Codex Committee on Food Hygiene, 2001). The vegetables can become contaminated with pathogenic microorganisms during growth, harvest and post harvest handling (McMahon and Wilson, 2001; Buck *et al.*, 2003; Iwona, *et al.*, 2007).

Since many vegetables and fruits are eaten raw, the use of untreated water to wash these foods can serve as a vehicle of transmission of pathogenic organisms (Banwart, 1998). Contact of fruits and vegetables by pickers and handlers at the time of harvest also offers a mechanism by which pathogens in feaces can contaminate raw produce. Some post harvest sources include feaces, human handling, harvesting equipment, transport containers, wild animal, insects, wash and rinse water, transport vehicles, improper storage, Improper packaging and improper handling after wholesale or retail purchase (Beuchat and Ryu, 1997; Johnston *et al.*, 2005). Food retailers endeavor to present fruit and vegetable products, within their stores in a manner that is appealing to consumers. This often requires growers to remove roots, exterior leaves and dirt and also includes an increasing trend of packaging the products, so they appear cleaner and more hygienic. This may or may not involve a washing step, which may or may not be carried out in a hygienic processing environment. Consumers can easily mistake these products as being ready to eat and therefore not wash them in home prior to consumption (Sivapalasingam *et al.*, 2004; Johnston *et al.*, 2006).

Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetables and fruits. Food borne outbreaks of infectious intestinal disease in England and Wales were associated with consumption of salad vegetables and fruit between 1992 and 2000 (Long *et al.*, 2002, Sagoo *et al.*, 2002). Lokmat daily news paper, (2002) reported that the local farmers in Amravati city are

using municipal waste water discharged in Ambanala for irrigation and washing purposes which is suspected source of contamination of microorganisms in vegetables. Therefore study was undertaken to evaluate bacteriological quality of ready-to-eat salad vegetables sold in local markets in Amravati city.

MATERIALS AND METHODS

Collection of Samples from Market Place: A total of 42 ready-to-eat salad vegetable samples of 11 different varieties were collected from local vegetable markets including Itwara market, Rajapeth market, Irwin square shop and Chaparashi pura market in Amravati city and analysed for its bacteriological quality (Table 1). These samples were collected in sterile polyethylene bags and were transported to laboratory on same day.

Sample analysis: A 25g of each vegetable was weighed and washed with sterile distilled water. Wash water (1mL) was diluted 10-fold in sterile distilled water; 0.5mL of this diluted water was inoculated in 4.5mL of Mac-Conkey broth and incubated at 37°C for 4-5h. After incubation, the loopful of growth from Mac-Conkey broth was subcultured on Cysteine Lactose Electrolyte Deficient (CLED) agar plates by streak- plate method. The plates were incubated at 37°C for 24h. Tentative identification of isolates were made by gram staining, motility, oxidase test and cultural characteristics on CLED such as yellow colored colonies of lactose fermenting *E.coli*, greenish colour colonies of *Proteus* spp. greenish blue or blue colonies of *Ps. aeruginosa*, mucoid yellow to whitish blue colonies of *Klebsiella* spp. and deep yellow opaque colonies of *S. aureus* (Hi-Media Manual, 2003). Confirmation of various bacterial pathogens were made by subculturing on Xylose Lysine Deoxycholate agar (XLD agar; M1108, Himedia, Mumbai), *Salmonella-Shigella* agar (S-S agar M108, Himedia, Mumbai) for *Salmonella*, Mannitol salt agar for *Staphylococcus aureus*, Cetrimide Agar for *Pseudomonas* spp. MacConkey agar for other enteric pathogens and various special biochemical tests. For confirmation of the pathogens, typical colonies were inoculated into Rapid Microbial Limit Test kits, which are a combination media in liquid and solid phase in a single bottle for simultaneous enrichment, isolation, and confirmation of pathogens. These MLT kits are recommended in diagnostic microbiology for accurate identification of pathogen and are supplied by Himedia Laboratories Limited, Mumbai, India. Biochemical tests were performed using conventional methods to confirm pathogen identification whenever necessary (Collee *et al.*, 1996).

Along with salad vegetable sample, information, or data on place, hygienic status of vendor, hygienic condition of vending site, cattle's wandering around shop, disposal of waste vegetables nearby were collected. All data were analysed with the Statistical Package for Social Sciences 15 for Windows (SPSS Inc.; Chicago, IL, USA) software.

RESULTS AND DISCUSSION

Many microorganisms can be present on raw fruits and vegetables. Since these products are frequently eaten raw, any pathogens present on these vegetables represent a potential risk to the consumer. The health of consumers can be adversely affected by consumption of microbiologically unsafe fruits and vegetables, as these salad vegetables are consumed without any heat treatment, sometimes without washing and peeling them. The microbiological contamination of fruits and vegetables can occur directly or indirectly from animals or insects, soil, manures, irrigated water and equipment used to grow these horticultural commodities as well as human handling along the food chain. The pathogenic microorganisms, which reside in intestinal tracts of animals or human, are more likely to contaminate the vegetables through feces, sewage, untreated irrigation water or surface water (Sivapalasingam, *et al.*, 2004).

A total of 42 different ready-to-eat salad vegetable samples which included cauliflower, cabbage, carrot, tomato, coriander, spinach, cucumber, beet, fenugreek radish and spring onion were analyzed (Table1). These vegetables were collected from Rajapeth market, Chaparashi pura, Irwin square and Itwara market in Amravati city. From these 42 samples, 75 different bacterial pathogens were isolated and identified. The vegetable samples collected from Chaparashi pura (29%) area showed maximum contamination followed by Itwara market (27%), Rajapeth market (25%) and Irwin square (19%). More contamination was found in vegetable samples collected from Chaparashi pura (29%) and Itwara market (27%), as they are more crowded than other areas (Table 1).

Out of 75 different pathogens; *Salmonella* spp. (33%) was predominant and found in all vegetables followed by *Escherichia coli* (32%), *Proteus* spp. (15%), *P. aeruginosa*

Table 1: Vegetable samples collected from different vegetable markets

Area	A	B	C	D	E	F	G	H	I	J	K	L
Rajapeth Market	1	1	1	1	1	1	1	1	1	1	1	19(25%)
Irwin Square	1	1	1	1	1	1	1	0	1	0	1	14(19%)
Chaparashii Pura	1	1	1	1	1	1	1	1	1	1	1	22(29%)
Itwara Market	1	1	1	1	1	1	1	1	1	1	1	20(27%)
Total	4	4	4	4	4	4	4	3	4	3	4	75

A = Cauliflower; B = Cabbage; C = Coriander; D = Tomato; E = Cucumber; F = Carrot; G = Spinach; H = Spring onion; I = Beet; J = Beet; K = Radish; L = Pathogen Isolated

Table 2: Bacterial pathogens isolated from various vegetables

Vegetable	*Salmonella* spp.	*E. coli*	*Proteus* spp.	*Pseudomonas aeruginosa*	*S. aureus*	*Enterobacter* spp.
Cabbage	4(16%)	2(8%)	0	0	0	0
Cucumber	3(12%)	3(13%)	0	1(10%)	0	0
Spring onion	3(12%)	2(8%)	0	0	0	0
Beet	2(8%)	3(13%)	0	1(10%)	0	0
Coriander	3(12%)	1(4%)	1(9%)	3(30%)	2(50%)	0
Tomato	2(8%)	2(8%)	1(9%)	1(10%)	1(25%)	0
Carrot	2(8%)	2(8%)	1(9%)	0	1(25%)	1(100%)
Flower	2(8%)	3(13%)	2(18%)	0	0	0
Spinach	2(8%)	3(13%)	2(18%)	1(10%)	0	0
Fenugreek	1(4%)	1(4%)	2(18%)	1(10%)	0	0
Radish	1(4%)	2(8%)	2(18%)	2(20%)	0	0
Total	25 (33%)	24 (32%)	11 (17%)	10(13%)	4 (5%)	1(1%)

(13%), *S.aureus* (6%) and *Enterobacter* spp. (1%). Tambekar and Mundhada (2006) in their studies reported 6% *Salmonella* spp whereas present study revealed the occurrence of 33%. The vegetables irrigated and washed with Municipal wastewater discharged in Ambanala in Amravati city is suspected to be primary source of *Salmonella* spp. on vegetables (Lokmat daily news paper, 2002). Sagoo *et al.*, (2001) in their studies reported 2% *Escherichia coli* in salad vegetable but present study showed 32% *E. coli*. *E. coli* are inhabitant of intestinal tract of human and animals and indicated the used of contaminated water for either irrigation or washing the vegetables.

The highest (13%) bacterial contamination was recorded in coriander followed by spinach (11%), cauliflower, tomato, cucumber, radish and carrot (10%), cabbage and beet (8%), spring onion and least (7%) in fenugreek. *P.aeruginosa* and *Salmonella* spp (30%) were predominantly found on coriander. The spinach was mostly contaminated with *E. coli* (Table 2). Both these vegetables are dwarf and grown in close contact of soil, which may be contaminated with fecal matter and untreated water (Sagoo *et al.*, 2002).

The contamination in salad vegetables was high in crowded area (81%) whereas 19% in less crowded area. Hygienic condition of vending site plays an important role in occurrence of pathogens in vegetables. A maximum contamination was recorded at places where hygienic condition of vending site was poor (34%) as compared to fair hygienic condition (31%) and good hygienic condition (10%) of vending site. The contamination was more where the personal hygienic condition of vendor was poor (59%) than the places where the personal hygienic condition of vendor was fair (29%) and good (12%). The vegetable markets, where cattle were wandering showed (81%) more contamination whereas least contamination was recorded at the places where no cattle around. These animals usually harbor many pathogenic microorganisms on their skin, dung, nasal passages and tails, through which contamination may take place (Khetarpual, 2006). The markets where the disposal of waste vegetables was nearby shop showed 61% contamination while 39% when the disposal away from shop (Table 3).

Conclusions and Recommendations

The present study concluded that all salad vegetables were contaminated with 75 bacterial pathogens. The most predominant pathogen was *Salmonella* spp. (33%). The vegetables were contaminated with these pathogens as most of the farmers use the water discharged in Ambanala in Amravati city for irrigation of vegetable crops. The water in Ambanala is most polluted and contains sewage and

Table 3: Bacterial pathogens isolated from different areas in various conditions

Parameter	Type	*Salmonella* spp.	*E. coli*	*Proteus* spp.	*Ps. aeruginosa*	*S. aureus*	*Enterobacter* spp.	Total
Place of shop	Crowded	21(84%)	19(79%)	8(73%)	8(80%)	4(100%)	1(100%)	61 (81%)
	Less crowded	4(16%)	5(21%)	3(27%)	2(20%)	0	0	14 (19%)
Hygienic	Fair	8(32%)	11(46%)	5(45%)	5(50%)	1(25%)	1(100%)	31 (41%)
condition of	Poor	13(52%)	11(46%)	2(18%)	5(50%)	3(75%)		34 (45%)
vending site	Good	4(16%)	2(8%)	4(36%)		0	0	10 (14%)
Personal	Fair	9(36%)	4(17%)	3(27%)	3(30%)	3(75%)		22 (29%)
hygiene of	Poor	14(56%)	16(67%)	7(64%)	5(50%)	1(25%)	1(100%)	44 (59%)
vendor	Good	2(8%)	4(17%)	1(9%)	2(20%)	0	0	9 (12%)
Cattle present	Present	21(84%)	19(79%)	8(73%)	8(80%)	4(100%)	1(100%)	61 (81%)
around shop	Absent	4(16%)	5(21%)	3(27%)	2(20%)	0	0	14 (19%)
Disposal of waste	Nearby shop	14(56%)	14(58%)	6(55%)	8(80%)	3(75%)	1 (100%)	46 (61%)
vegetables	Away from shop	11(44%)	10(42%)	5(45%)	2(20%)	1(25%)	0	29 (39%)

drainage of Amravati city. Also, vendors use the water for sprinkling on vegetable to keep them fresh, which may be contaminated. Improper storage of vegetables in markets, personal hygiene of vegetable vendor and disposal of waste vegetables near by the shop, which attract cattle and flies, was added cause of contamination in the vegetables.

Thus, it was found that, the vegetables were contaminated by various microorganisms due to various reasons and therefore may be an important source of food borne illness to human especially when the salad vegetables are consumed raw. Therefore, to improve safety of vegetables, the most efficient way is to rely on a system, which reduces the risk factors during production and handling of vegetables. Provision should be made for disposal of waste vegetable in market. The entry of cattle in vegetable market should be prohibited. All the vegetables should be washed vigorously with running safe water before consumption to reduce microbial flora. Government intervention is also required to protect consumer and to ensure that standard of safety of such foods in attainable in the context prevailing local situation.

REFERENCES

Banwart, G. J., 1998. Sources of Microorganisms, 165-194, Chapman and Hall, Basic food microbiology. 1st Indian edition, Delhi, CBS Publishers and Distributors.

Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. Food prods.* **59:** 204-216.

Beuchat, L. R. and Ryu, J. H. 1997. Produce handling and processing practices. Emerging Infectious Diseases, 3, 4.

Buck, J. W., Walcott, R. R. and Beuchat, L. R. 2003. Recent trends in microbiological safety of fruits and vegetables. Online. Plant Health Progress doi: 10.1094/PHP-2003-0121-01-RV.

Collee, J. G., Duguid, J. P. Fraser, A. G. and Marmion, B. P. 1996. Enterobacteriaceae – *Escherichia, Klebsiella, Proteus* and Other Genera, 380-381, In: Collee, J. G. J. P. Duguid, A. G. Fraser and B. P. Marmion (Edn), Practical medical microbiology. 14th Edn. Honkleongi Mackie and Mac-Cortney, Lonyman Group UK Ltd.

Dhiraputra, C. C. Tiensasitorn, Techachaiwiwat, W., Jirapanakorn, N., Kachintorn, K. and Danchaivijitra, S. 2005. Bacterial contamination of vegetables served in hospitals. *J. Med. Assoc. Thai.* **88(10):** 42-8.

European Commission, Health and Consumer Protection, Directorate-General, 2002. Risk Profile on the Microbiological Contamination of Fruits and Vegetables Eaten Raw. Report of the Scientific Committee on Food, SCF/CS/FMH/SURF/Final, 29 April 2002

Hi-Media manual for Microbiology and Cell Culture Laboratory Practices, 2003. Hi-media Laboratories, Pvt. Ltd, Mumbai. 69.

Iwona Warminska-Radyko, Lucja Laniewska-Trokenheim, Marta Mikes, 2007. Microbiological contamination of vegetable salads, Pol. *J. Natur. Sc.* **22(4):** 733-741.

Johnston, L. M., Jaykus, L. A., Moll, D., Anciso, J., Mora, B. and Moe, C. L. 2006. A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *Int. J. Food Microbiol.* **112(2):** 83-95.

Johnston, L. M., Jaykus, L. A., Moll, D., Martinez, M.C., Anciso, J. Mora, B. and Moe, C. L. 2005. A field study of the microbiological quality of fresh produce. *J. Food Prot.* **68(9):** 1840-7.

Khetarpual, N., 2006. The Role of Microorganisms in Foods, 20-73. N. Khetarpual, Food Microbiology. Daya Publishing House, New Delhi.

Lokmat Daily News Paper, 2002. Typhoid increased in Amravati District 14th Dec., 1.

Long, S. M., Adak, G. K., Brien, S. J. O'. and Gillespie, I. A. 2002. General outbreaks of infectious intestinal disease linked with salad vegetables and fruit, England and Wales. (1992-2000). *Comm. Dis. Pub. Health.* **5:**101-105.

McMahon, M. A. S. and Wilson, I. G. 2001. The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *Intl. J. Food Microbiol.* **53:** 155-162.

Mintell Market Intelligence Report, 2002. Fresh Fruits and vegetables, May 2000.

Report of the Codex committee on Food Hygiene, 2001. Risk Profile on Microbiological Contamination of Fruits and Vegetables Eaten Raw. Europe, 151- 156

Sagoo, S. K., Little, C. L. and Mitchell, R. T. 2001. The microbiological examination of ready to eat organic vegetables from retail establishments. LACOTS/PHIS Co-ordinate Food Liaison Group Studies, PHLS Environmental Surveillance Unit, 61 Colin dales Avenue, London NW9 5EQ.

Sagoo, S. K., Little, C. L. and Mitchell, R. T. 2002. The Microbiological Examination of open Ready to at Prepared Salad Vegetables from Catering and Retail Premises. LACOTS/PHIS Co-ordinate Food Liaison Group Studies, PHLS Environmental Surveillance Unit, 61 Colin dales Avenue, London NW9 5EQ.

Sivapalasingam, S., Friedman, C. R., Cohen, L. and Tauxe, R. V. 2004. Fresh produce: A growing cause of outbreaks of food borne illness in the United States, 1973 through 1997. *J. Food. Prot.* **67(10):** 2342-2353.

Tambekar, D. H. and Mundhada R. H. 2006. Bacteriological quality of salad vegetables sold in Amravati city. *J. Biological sciences.* **6 (1):** 28-30.