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Bacteriological Analysis of Fresh Vegetables from Main Market of Dehradun

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Abstract: Vegetables are one of the essential diets of human beings, which are consumed widely. But, they are contaminated with different bacteria that may cause food-borne illness. In order to identify them, bacteriological analysis was performed on five fresh vegetables (Potato, Tomato, Cauliflower, Cucumber and Spinach), collected from main vegetable Mandi at Dehradun. All vegetable samples were processed to identify total viable cells (cfu/ml). The highest total viable count was found in cucumber (5.8 X 10^8 cfu/ml) followed by Potato (5.0 X $10^{8)}$ cfu/ml, Cauliflower (4.0 X 10^8 cfu/ml), Tomato (4.2 X 10^8 cfu/ml) and spinach (3.8 X 10^8 cfu/ml). *Enterobacter aerogenes, Serratia entomophila, Bacillus cereus, Listeria monocytogenes, Proteus vulgaris and Micrococcu*s were identified on the basis of morphology (Gram's staining and negative-staining), Biochemical test (color formation on the Universal Food Pathogen detection kit and Enterobacteriaciae kit) and selective cum differential culture media (such as MacConkey Agar, Bile esculin agar, Bacillus cereus agar, EMB Agar, Mannitol salt agar media). It was observed that Bacillus, Listeria and Enterobacter were the dominating genera in vegetables. The antibiotic sensitivity test (MIC Value) of Azithromycin and Chloramphenicol was determined against identified bacteria. It was observed that MIC Value of Azithromycin against *Proteus vulgaris, Enterococcus faecalis*, and *Enterobacter aerogenes* was 4µg/ml, 1.2µg/ml and 0.12µg/ml respectively while no inhibition was observed against *Serratia entomophila.* Likewise, MIC Value of Chloramphenicol against *Enterobacter aerogens, Proteus vulgaris, Enterococcus faecalis and Serratia entomophila* was 8 µg/ml, 8 µg/ml, 1 µg/ml and 1 µg/ml respectively. The microbial flora of vegetables is of great concern in our society as they can be of great risk for human health. Proper vegetable handling, hygiene transportation and proper storage is necessary to avoid microbial food spoilage and related health risks.
Keywords: Food pathogens, vegetables, bacteriological analysis, microbial load, antibiotic sensitivity assay.

Introduction:

Vegetables are included among the basic and nutritious food for human beings. Vegetables are important protective food and highly beneficial for the maintenance of health and prevention of diseases. They contain valuable food ingredients which are essential for the proper function of the body. Vegetable contain various medicinal and therapeutic agent and are valued mainly for their high vitamin and mineral content. Studies have evaluated the association of fruit and vegetable consumption with the reduction of risk of specific diseases[1]. Being an edible part of plants, they are also full of vitamins and minerals. Fruits and vegetables normally carry a non-pathogenic epiphytic microflora[2]. The inner tissues of healthy plants and animals are free of microorganisms, however, the surfaces of raw vegetables and meats are contaminated with a variety of

microorganisms and this depends on the microbial population of the environment from which the food was taken, the condition of the raw product, the method of handling, the time and conditions of storage[3]. Pathogens from the human and animal reservoir as well as other environmental pathogens can be found at the time of consumption[4]. Enteric pathogens such as *Escherichia coli*, Salmonella and Shigella are among the greatest concerns during food-related outbreaks[5]. It was reported that salad vegetables such as carrots, radishes, tomatoes, lettuce, cabbage, Cucumber, coriander carries *Escherichia coli, Staphylococcus aureus, Enterobacter sp, Klebsiellia sp., Providencia sp, and Pseudomonas aeruginosa*[6].

Differences in microbial profiles of various fruits and vegetables result largely from unrelated factors such as resident micro flora in the soil, application of non-resident micro flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers[7].

The incidence of food borne outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years[8]. In India, food-borne diseases and infections are a serious health hazard causing large numbers of mortality and morbidity. Outbreaks of Hepatitis, Cholera and Botulism are reported every year leading to widespread loss of earnings, work output and physical sufferings.

In 2007, *Listeria monocytogenes* was isolated from 105 (5%) milk samples collected from 52 farms in Maharashtra. An outbreak of *Staphylococcus aureus* food poisoning due to contaminated "bhalla" (a snack made up of potato balls fried in vegetable oil) affected more than 100 children and adults in Madhya Pradesh in 2007. The food poisoning cases investigated during 2003–05 in Hyderabad are an infective and intoxicating type of food borne diseases caused by Salmonella sp. and *Staphylococcus aureus*. It is interesting that most of the food poisoning cases investigated is caused due to the organism *Staphylococcus aureus*[9]. Bacteria can cause severe food poisoning in humans with symptoms like stomach cramps, vomiting, loss of appetite, and fever. This food poisoning may occur as a result of eating food prepared with unclean cooking utensils, raw fish, raw fruits and vegetables and uncooked meats or eggs. Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to escalating healthcare costs worldwide.

Although, newer antibiotics are available, but, emerging antimicrobial resistance is becoming an increasing problem in many pathogens throughout the world. For instance, *S. aureus* exhibits remarkable versatility in their behaviour towards antibiotics and its capacity to produce human diseases had not diminished even with the introduction of antibiotics[10]. Although, outbreaks of *S. aureus* resistant to Betalactam antibiotics have been frequently associated with devastating food borne infections. Vegetables are frequently consumed raw without being exposed to the processes that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce bacterial levels. Eating or drinking contaminated foods or drinks can cause food borne disease[11].

In Dehradun, it has been observed that the local residence consume the vegetables from a particular Mandi, which circulates the vegetables all over the city. And, it was suspected to be the primary source of contamination of microorganisms in fresh vegetables. Assessment of microbiological risk is an emerging tool for the evaluation of the safety of food and water supplies. Hence, the study was carried out to examine the floral microorganisms present over the vegetables.

Materials and Methods:

Media:

All the bacterial media used were procured from HiMedia i.e. Nutrient Agar, Nutrient Broth, MacConkey Agar, Bacillus cereus Agar, Bile Esculin Agar, Eosin- methylene blue agar, Triple sugar Iron agar, Mannitol salt agar etc.

Readymade Kits:

Hi25™ Enterobacteriaciae Identification Kit (HiMedia, KB003), HiDtect Universal Food pathogen Identification Disc (HiMedia, DT010), Grams Staining Kit (HiMedia, K001), Azithromycin AZM (Strip A: 128- 0.01 µg & Strip B: 2- 0.0001 µg) (HiMedia, MD004) and Chloramphenicol C (Stip A: 240-0.01 µg & Strip B: 8.0-0.001 µg) (HiMedia, MD004).

Methodology:

a) **Study Area:** The study was carried out of Main Sabzi Mandi, Dehradun City (Uttarakhand). Sample of fresh vegetables (Potato, Tomato, Cauliflower, Cucumber and Spinach) were analyzed five times at the regular interval of period.

b) Sample collection: In total, five commonly consumed fresh vegetables namely potato (*Solanum tuberosum*) (S1), tomato (*Solanum lycopersicum*) (S2), Cauliflower (Brassica *oleracea*) (S3), Cucumber (*Cucumis sativus*) (S4) and Spinach (*Spinacia oleracea*) (S5) were collected for the bacterial analysis (Fig. 1). Samples were collected in the sterile polythene zip bags to avoid any handling contamination and transported to laboratory for microbial analysis. The samples were collected twice monthly from the market. The samples were kept in the refrigerator at 4°C for later use.

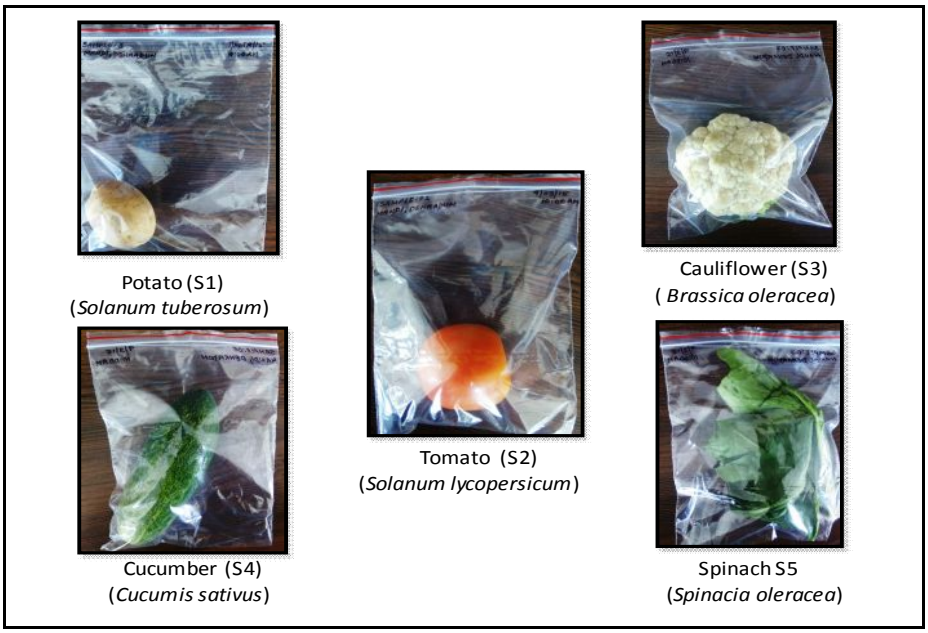


Figure 1: Vegetable collected for microbial analysis

c) Sample processing: The method followed in the present study is shown in Fig. 2. Twenty-five gram of each collected vegetable sample was weighed in sterile conditions and homogenized in sterile saline water using pestle and mortar for five minutes. All the sterile conditions were maintained throughout the process. The homogenates were collected in sterile tubes and stored at -20ºC for further use[13].

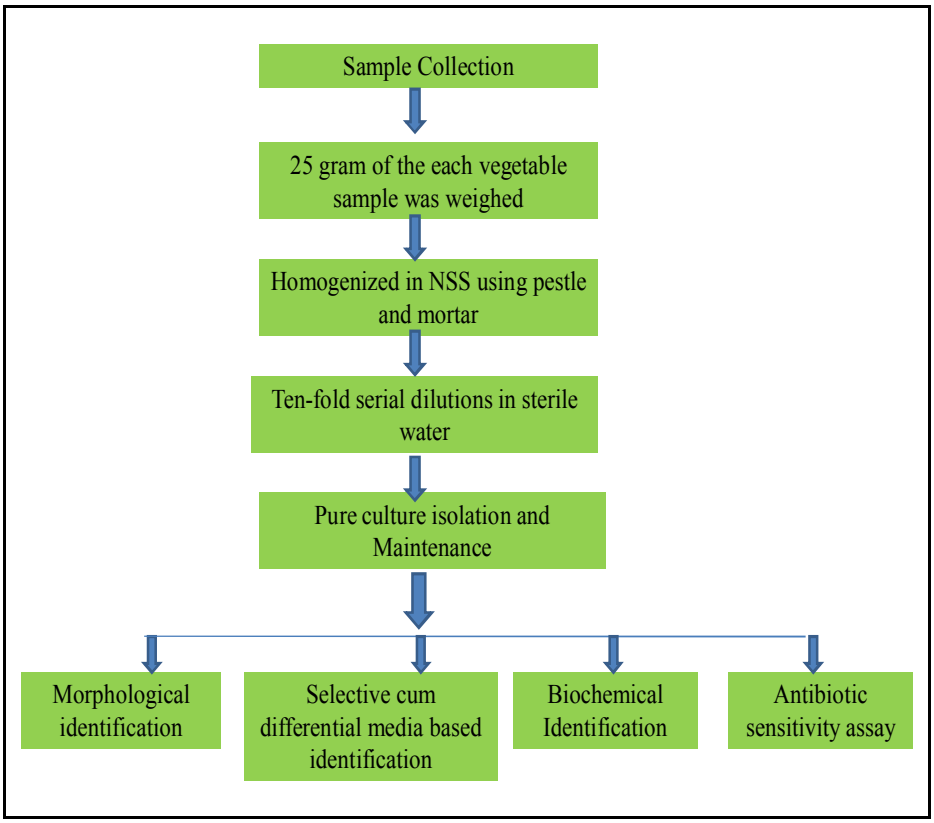


Figure 2: Flow diagram showing methodology used in the present study.

Isolation of Bacteria

One ml of each sample was serially tenfold diluted in sterile water up to 10^{-5} dilution. The amount of 0.1 ml at 10^{-5} dilution was spreaded over Nutrient agar media using sterile spreaders. The plates were incubated at 37 ºC for 12-24 hours for the appearance of colonies. Discrete colonies were sub-cultured in nutrient broth and streaked over different selective-cum-differential media agar plates i.e. MacConkey Agar, Bacillus Cereus Agar, EMB agar and Bile Esculin Agar, mannitol salt agar and were incubated at 37ºC for 12-24 hours. The pure bacterial colonies obtained were primary identified using morphological analysis. Each isolated pure culture was maintained at 4ºC for further analysis [2].

Total Plate Count of Bacteria (CFU/ml)

Microbial load in each vegetable sample was determined as CFU/ml and was calculated using formula[12].

Cfu/ml = {(No. of colonies X dilution factor) / volume of inoculums]

Identification of Microorganisms

a) **Morphological identification:** The isolated bacteria were identified on the basis of negative staining and Gram's-staining[12].

b) **Selective-cum-differential Agar media based identification**: The pure isolated colonies were grown on media like Bacillus cereus agar, Bile esculin agar, MacConkey agar, EMB agar, Mannitol Salt agar and were identified on the basis of characteristic growth appearance.

c) **Biochemical Identification:** The isolated bacterial colonies were confirmed by Biochemical kits (Universal Food pathogen Identification Disc, Hi2^{5TM} Enterobacteriaciae Identification Kit and TSI test) and the results were interpreted as per interpretation chart and identification index following kit protocol**.**

Antibiotic Sensitivity Test

The method was followed using kit protocol of HiMedia (MD004). MIC value of Azithromycin AZM (Strip A: 128- 0.01 µg & Strip B: 2- 0.0001 µg) (MD004) and Chloramphenicol C (Strip A: 240-0.01 µg & Strip B: 8.0-0.001 µg) (HiMedia, MD004). was determined by observing zone of inhibition (ZOI) against each bacterial isolate.

Results

Total plate count:

Numerous colonies were seen on NAM (Fig. 3). The total bacterial count was observed higher in the cucumber followed by Potato > Tomato > Cauliflower > Spinach (Table 1).

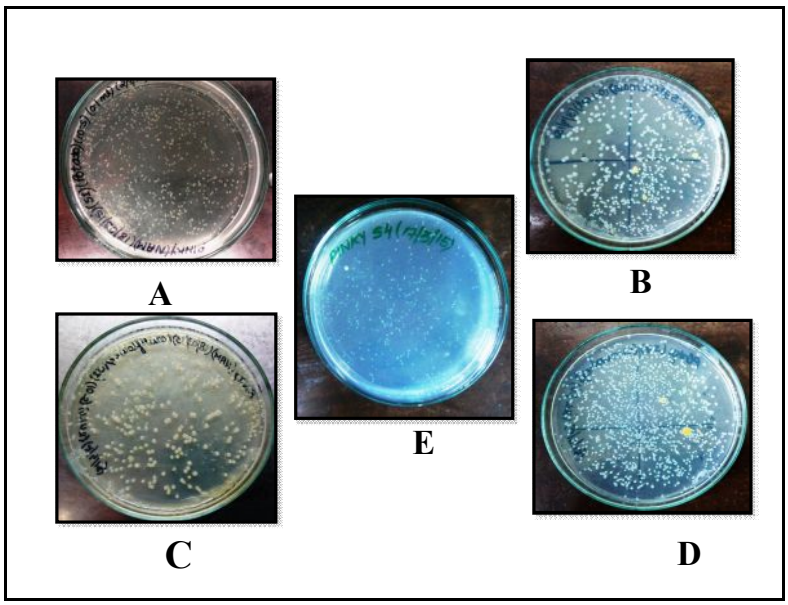


Figure 3: Microbial growth on Nutrient agar plates

A.Growth of Bacteria isolated from Potato, B. Growth of Bacteria isolated from Tomato, C. Growth of Bacteria isolated from Cauliflower, D. Growth of Bacteria isolated from Cucumber, E. Growth of Bacteria isolated from Spinach.

Table 1: Total viable count of vegetable count

S. No.	Vegetable Sample	Cfu/ml
1)	Potato (S1) (*Solanum tuberosum*)	5.0×10^8
2)	Tomato (S2)(*Solanum lycopersicum*)	4.20×10^8
3)	Cauliflower (S3) (*Brassica oleracea*)	4.0×10^8
4)	Cucumber (S4) (*Cucumis sativus*)	5.8×10^8
5)	Spinach (S5) (*Spinacia oleracea*)	3.8×10^8

Morphological Identification:

Microscopic identification of each bacterial isolate by negative staining and Gram's Staining revealed both bacilli and cocci form (Figure 4 and Table 2).

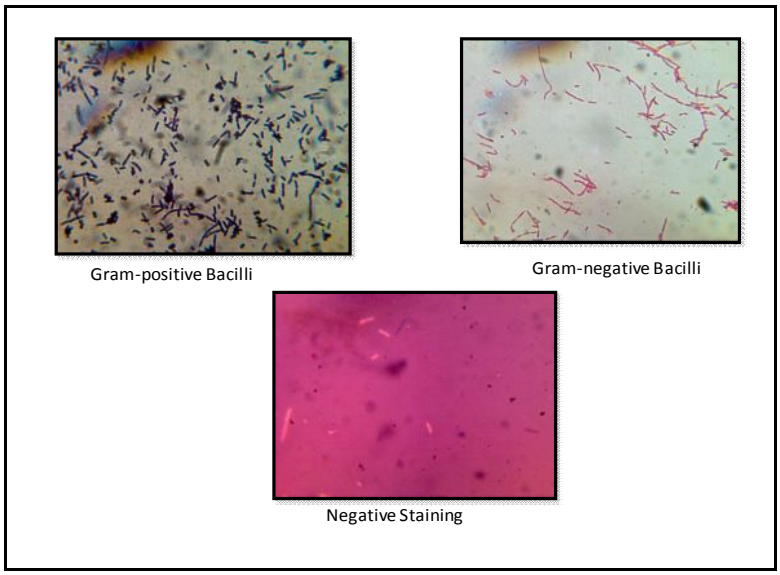


Figure 4. Bacilli as observed by Negative staining and Gram's staining

Table 2: Morphological Identification of obtained colonies

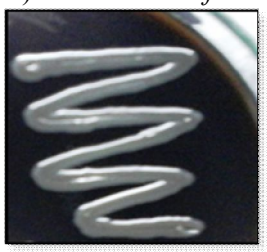
S. No.	Sample code name	Isolate code	Morphology characterization	Negative staining	Gram staining	Bacteria identified on the basis of selective cum differential media and Biochemical tests.
1	S-1	SP-1	Opaque, white waxy growth, abundant	Rods	Gram (-ve)	*Enterobacter*
		SP-2	White moist & glistening growth	Rods	Gram (+ve)	*Bacillus cereus*
		SP-3	Abundant, Opaque, Golden growth	cocci	Gram (+ve)	*Micro coccus*
		SP-4	Small, translucent, round, less growth	Rods	Gram (-ve)	*Serratia Entomophila*
		SP-5	Abundant, Round, opaque growth	Rods	Gram (+ve)	*Listeria monocytogenes*
		SP-6	Round, Smooth, opaque growth	Rods	Gram (-ve)	*Proteus vulgaris*
		SP-7	Opaque, round, translucent growth	Cocci	Gram (+ve)	*Enterococcus faecalis*

2	S-2	ST-1	Abundant, Opaque, Golden growth	cocci	Gram (+ve)	*Micro coccus*
		ST-2	Opaque, white waxy growth, abundant	Rods	Gram (-ve)	*Enterobacter*
		ST-3	White moist & glistening growth	Rods	Gram(+ve)	*Bacillus cereus*
		ST-4	Round, Smooth, opaque growth	Rods	Gram (-ve)	*Proteus vulgaris*
3	S-3	SCL-1	White moist & glistening growth	Rods	Gram (+ve)	*Bacillus cereus*
		SCL-2	Abundant, Opaque, Golden growth	cocci	Gram (+ve)	*Micro coccus*
		SCL-3	Opaque, white waxy growth, abundant	Rods	Gram (-ve)	*Enterobacter*
		SCL-4	Small, translucent, round, less growth	Rods	Gram (-ve)	*Serratia Entomophila*
		SCL-5	Round, Smooth, opaque growth	Rods	Gram (-ve)	*Proteus vulgaris*
4	S-4	SCU-1	Abundant, Opaque, Golden growth	cocci	Gram (+ve)	*Micro coccus*
		SCU-2	Opaque, white waxy growth, abundant	Rods	Gram (-ve)	*Enterobacter*
		SCU-3	White moist & glistening growth	Rods	Gram (+ve)	*Bacillus cereus*
		SCU-4	Abundant, Round, opaque growth	Rods	Gram (+ve)	*Listeria monocytogenes*
		SCU-5	Round, Smooth, opaque growth	Rods	Gram (-ve)	*Proteus vulgaris*
5	S-5	SSP-1	Opaque, white waxy growth, abundant	Rods	Gram (-Ve)	*Enterobacter*
		SSP-2	Abundant, Opaque, Golden growth	cocci	Gram (+ve)	*Micro coccus*
		SSP-3	White moist & glistening growth	Rods	Gram (+ve)	*Bacillus cereus*
		SSP-4	Opaque, round, translucent growth	Cocci	Gram (+ve)	*Enterococcus faecalis*
		SSP-5	Round, Smooth, opaque growth	Rods	Gram (-ve)	*Proteus vulgaris*

Selective-cum-differential Media based identification

Characteristics growth on selective cum differential media confirmed the presence of *Serratia entomophila, Bacillus cereus, Proteus vulgaris, Enterococcus faecalis, Enterobacter aerogenes* (Table 3).

Table 3. Characteristic growth on selective-cum-differential media

Bacteria Identified:	Selective Media Used:
1.) *Serratia entomophila*: 	Bile esculin agar (Himedia): Growth of bacteria other than streptococci D, which is identified as *Serratia entomophila* and confirmed by Enterobacteriaciae Identification kit.
2.) *Bacillus cereus* 	Bacillus cereus agar (Himedia): Appearance of blue colored colony on agar and confirmed by Catalase test and HiDtect Universal Food pathogen Identification Disc (DT010).
3.) *Proteus vulgaris* 	Growth of colorless colony as *Proteus vulgaris* on EMB Agar, which is confirmed by Himedia Enterobacteriaciae identification kit.
4.) *Enterococcus faecalis* 	Luxuriant growth and blackening of bile esculin agar medium around the colony indicated the positive reaction of *Enterococcus faecalis*.
5.) *Enterobacter aerogenes* 	Pink-colored colony without sheen over EMB Agar indicates the growth of *Enterobacter aerogenes*, which is further identified by Enterobacteriaciae Identification kit.
6.) *Micrococcus luteus* 	Yellow-pigmented coccus-shaped colonies over the nutrient agar medium indicate the presence of *Micrococcus luteus*.

Biochemical Identification

HiDtect Universal Food pathogen Identification Disc

Mixed bacterial population were identified as *Listeria monocytogenes, Bacillus cereus, Proteus vulgaris* on the basis of colour formation using Universal Food Pathogen identification disc.

Hi25™ Enterobacteriaceae Identification Kit

Bacterial genera were confirmed on the basis of 24 biochemical tests/ parameters using Himedia Biochemical test kits as shown in (Table 4 and 5).

Table 4: Identification of bacteria by biochemical tests

Sample code	24 Tests performed																							Organism identified
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
S-1	+	-	-	-	-	+	-	+	+	v	-	-	+	-	v	-	-	-	-	+	-	+	+	-	*Serratia entomophila*
S-2	-	-	-	+	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	+	-	+	+	-	*Proteus vulgaris*
S-3	+	+	+	-	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes*
S-4	-	-	-	+	+	+	+	+	-	+	+	-	+	-	+	-	-	-	-	+	-	+	+	-	*Proteus vulgaris*
S-5	+	+	+	-	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	*Enterobacter aerogenes*

Table 5: Identification index of various Enterobacteriaceae species

Test performed	Name	Test performed	Name
1	ONPG	**13**	Esculin hydrolysis
2	Lysine	**14**	Arabinsoe
3	Ornithine	**15**	Xylose
4	Urease	**16**	Adonitol
5	TDA	**17**	Rhamnose
6	Nitrate	**18**	Cellobiose
7	H2S	**19**	Melibiose
8	Citrate utilization	**20**	Saccharose
9	Voges proskauer's	**21**	Raffinose
10	Methyl red	**22**	Trehalose
11	Indole	**23**	Glucose
12	Malonate	**24**	Lactose

The most prevailed genera confirmed in different isolates were *Enterobacter aerogenes (30.6%)* > *Proteus vulgaris (25.6%)* > *Bacillus cereus (21.6%)* > *Listeria monocytogenes (11.8%)* > *Serratia entomophila (10.4%)*. It was observed that *Enterobacter aerogenes* was highly prevailed and *Serratia entomophila* was lowest (Fig. 5).

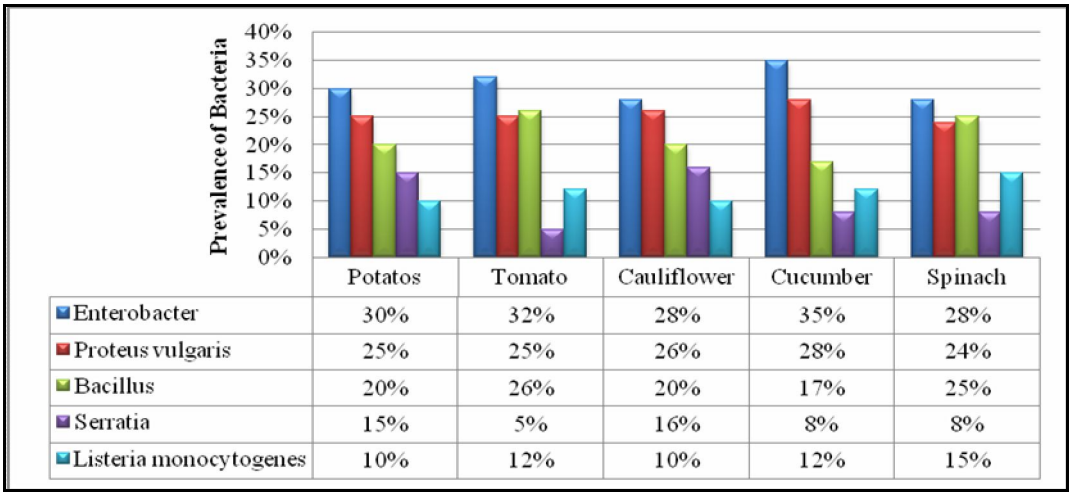
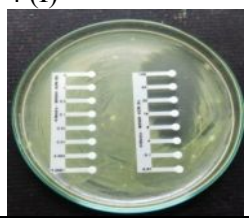
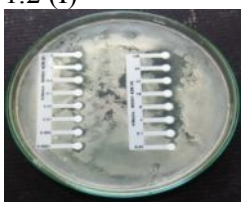
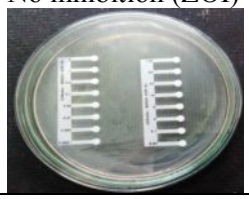


Figure 5: Prevalence of various bacteria in vegetables

Antibiotic Sensitivity Test

The antibiotic sensitivity test (MIC Value) was determined for Azithromycin and Chloramphenicol against identified bacteria. It was observed that MIC Value of Azithromycin against *Proteus vulgaris, Enterococcus faecalis* and *Enterobacter aerogenes* was 4µg/ml, 1.2µg/ml and 0.12µg/ml respectively while no inhibition was observed against *Serratia Entomophila*. Likewise, MIC Value of Chloramphenicol against *Enterobacter aerogens, Proteus vulgaris, Enterococcus faecalis and Serratia entomophila* was 8 µg/ml, 8 µg/ml, 1 µg/ml and 1 µg/ml respectively (Table 6 and Fig. 6).

Table 6: Antibiotic Sensitivity assay of selective dominating bacteria

S.No.	Name of the organism	MIC value (µg/ml) of Azithromycin	MIC Value (µg/ml) of Chloramphenicol
1	*Enterobacter aerogenes*	0.1 (S) 	8 (S) 
2	*Proteus vulgaris*	4 (I) 	8 (S) 
3	*Enterococcus faecalis*	1.2 (I) 	1.0 (S) 
4	*Serratia entomophila*	No inhibition (ZOI) 	1 (S) 

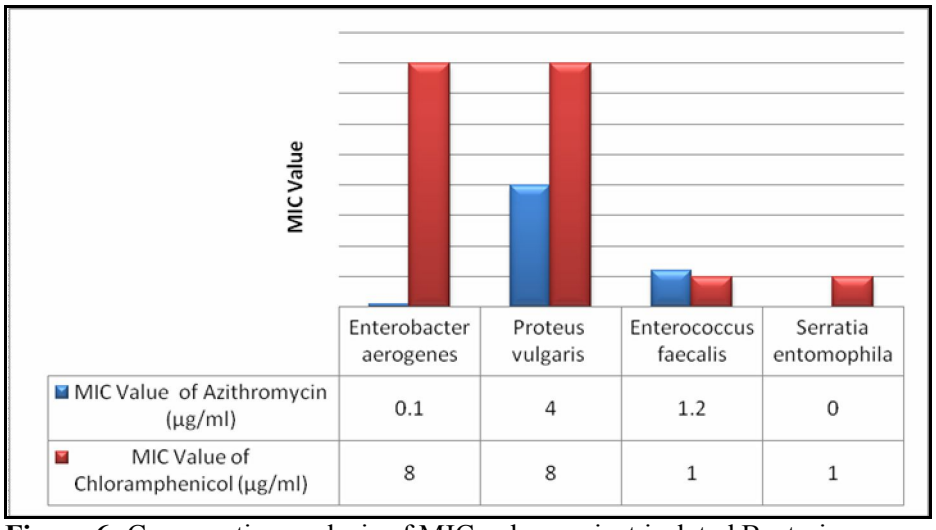


Figure 6: Comparative analysis of MIC value against isolated Bacteria

Discussion:

Vegetables are consumed widely as an energy source by human beings. They are rich in vitamins and minerals. Raw vegetables are consumed as salad too. But, there are many pathogenic microorganisms reside over them too, which can cause many food-borne infections such as listeriosis, salmonellosis etc.

In the present study, five fresh vegetables were analyzed for presence of microorganisms. It was observed that the CFU/ml count ranges from $3X10^8$cfu/ml to 5.8 X 10^8cfu/ml, which clearly indicates that all the samples contained acceptable number of microbial load of 10^8cfu/ml. It is observed that the results of this study correlates with the works of Uzeh, *et al*[13] and Bukar *et al*[14], where bacterial count was found between 10^8 – 10^9cfu/ml in commonly consumed fresh fruits and vegetables. This study also shows the presence of more than four predominating pathogenic bacteria, with Enterobacter genera being more dominating. Bacillus species are being part of the natural flora and are among the most privileged spoilage vegetables bacteria, though some Bacillus species (*B. cereus*) are capable of causing food borne illness[15]. This species is commonly found in soil and may contaminate fruits and vegetables during harvesting and also the presence of this pathogen in fresh fruits and vegetables may cause diarrhoea (due to enterotoxins) or vomiting (Emetic toxin). In order to observe the prevalence of bacteria in fresh vegetables, study of different vegetables was carried out. Isolates were recovered from the five samples of vegetables. The most common bacteria found were identified as *Serratia entomophila, Bacillus cereus, Proteus vulgaris, Enterococcus faecalis, Enterobacter aerogenes* and the prevalence of *Enterobacter* was highest.

Antibiotic Susceptibility test was performed to check the sensitivity of the isolates. It was observed that MIC Value of Azithromycin was comparatively higher (4µg/ml) against *Proteus vulgaris* while no inhibition was observed against *Serratia entomophila*. Likewise, MIC Value of Chloramphenicol was comparatively higher (8 µg/ml) against *Enterobacter aerogens, Proteus vulgaris*. Although, newer antibiotics are available, but emerging antimicrobial resistance is becoming an increasing problem in many pathogens throughout the world. For instance, *S. aureus* exhibits remarkable versatility in their behavior towards antibiotics and its capacity to produce human diseases had not diminished even with the introduction of antibiotics[10]. The variation in the susceptibility of these organisms towards antibiotics may be connected to their previous exposure to the antibiotics and thereby varying the degree of resistance in addition to this the Gram reaction of the organisms also influences their susceptibility to the antibiotics used[16].

Conclusion:

On the basis of the present study it was concluded that six bacterial isolates i.e. *Bacillus, Enterobacter, Micrococcus, Listeria monocytogenes, Proteus vulgaris and Serratia* was identified from the fresh vegetables. Among the above *Proteus vulgaris* and Enterobacter were the dominant species. Bacterial contamination may be present due to improper handling, unhygienic transportation condition and improper storage. Other source could be the result of post harvesting processing and unhygienic distribution. Therefore, all control measures must be taken to avoid food spoilage or food borne infections.

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Conflict of Interest:

Authors declare no conflict of interest.

References:

1. Hung HC, Joshipura KJ, Jiang RH, Hunter D, Smith SA. Fruits and Vegetable intake and the risk of major chronic disease. Journal of National Cancer Institute, (2004); 95:157- 164.
2. Ray Bibek. Fundamental Food Microbiology. Third Edition (2004). Londan: CRC Press
3. Ofor MO, Okorie VC, Ibeawuchi II, Ihejirika GO, Obilo OP, Dialoke SA. Microbial Contaminants in Fresh Tomato Wash Water and Food Safety Considerations in South-Eastern Nigeria (2009). Life Sci. J.1:80-82.

4. Aboh Mercy Itohan, Oladosu Peters and Ibrahim Kolo Bacterial contaminants of salad vegetables in Abuja Municipal Area Council, Nigeria. Malaysian Journal of Microbiology (2010).
5. Margaret Barth, Thomas R. Hankinson, Hong Zhuang, and Frederick Breidt.Microbiological Spoilage of Fruits and Vegetables. Food Microbiology and Food Safety (2009), Springer Science+Business Media, LLC.
6. R. V. Sudershan, R. Naveen Kumar, L. Kashinath, V. Bhaskar, and K. Polasa. Foodborne Infections and Intoxications in Hyderabad ,India. Hindawi Publishing Corporation Epidemiology Research International Volume (2014), Article ID 942961, 5 pages.
7. Pelczar, M.,J, Chan, E.C.S.,and Krieg, N.,R. Microbiology 5th edition (2006) Tata McGraw-Hill Publishing Company Limited, New Delhi.
8. Mukherjee, A., Speh, D., Jones, A.T., Buesing, K.M., Diez-Gonzalez, F. Longitudinal microbiological survey of fresh produce grown by farmers in the upper Journal of Food Protection 69 (2006), 1928–1936.
9. Vishwanathan, P and Kaur R. Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. Int. J. Hyg. Environ. Health. (2001); 203: 205-213.
10. Obiazi H. A. K., Nmorsi, O. P. G, Ekundayo A.O. and Ukwandu N.C.D. Prevalence and antibiotic susceptibility pattern of Staphylococcus aureus from clinical isolates grown at 37 and 44 ºC from Irrua, Nigeria African Journal of Microbiology Research (2007) pp. 057-060.
11. Adebayo-Tayo BC, Odu NN, Esen CU, Okonko IO. Microorganisms Associated With Spoilage of Stored Vegetables In Uyo Metropolis, Akwa Ibom State, Nigeria .Nature and Science, 2012;10(3).
12. Prescott LM, Harley JP, Klein DA.,Microbiology, 5th Edition (2002)., McGraw-Hill, New York.
13. Uzeh, R.E., F.A. Alade and M. Bankole. The microbial quality of pre-packed mixed vegetables salad in some retail outlets in Lagos, Nigeria (2009). Afr. J. Food Sci., 3(9): 270-272.
14. Bukar A, Uba A, Oyeyi TI,Occurrence of some Enteropathogenic bacteria in some minimally and fully processed ready - to - eat foods in Kano metropolis, Nigeria (2010). Afr. J. Food Sci. 4(2): 032-036.
15. Vanderzant C, Splittstoesser DF. Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, (1992).Washington D. C
16. Buhlmann, M., Bogli-Stuber, K., Droz, S., Muhlemann, K. Rapid screening for carriage of methicillin-resistant *Staphylococcus aureus* by PCR and associated costs (2008). J. Clini. Microbiology. 46(7): 2151-2154.

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