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Bacterial Population Associated with Fruits and Vegetables and its Treatment using Antimicrobial Rinsing

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A B S T R A C T

Fruits and vegetables are one of the essential diets of human being, which they are consumed widely. But, they are contaminated with different bacteria that may cause food borne illness. Bacteriological survey of 20 samples of fresh vegetables and fruits were done to study the density of microorganisms by standard plate count (SPC) method. The colony forming units per gram (cfu/g) of the vegetables were much less (7.2×10^5 to 30.4×10^5) in local market sample as compared to super market sample (12.0×10^5 to 20.6×10^5) and field sample (8.0×10^5 to 18.0×10^5). But in case of fruits higher cfu were obtained for the field sample (8×10^5) as compared to local market sample (4×10^5 to 5.5×10^5) and super market sample (2.5×10^5). Of the various pathogens identified from the surface of these samples *Escherichia coli, Staphylococcus aureus, Bacillus cerous* were found to be the pre-dominant species in most of the samples. It was observed that the *Bacillus, Enterobacter and Staphylococcus* were the dominant genera in vegetables. On the basis of antimicrobial rinsing of carrot, which was carrying the highest bacterial contamination, five different anti-microbial agents (Hydrogen peroxide, Sodium chloride, Calcium chloride, Sodium citrate and Ethanol) decreased the total number of microbial organisms' load of the carrot by 40-80 per cent. Hydrogen peroxide was, however, found to be the most effective, as rinsing treatment with it showed more reduction in total number of bacterial organisms in 80 per cent of samples. Hydrogen peroxide can thus be used as rinsing agent to reduce this type of superficial bacterial contamination so as to improve the quality of fresh vegetables and fruits for human consumption. The microflora of fruits and vegetables are one of the great concerns in our society as they can be of great risk for human health. Proper handling, hygiene, transportation and proper storage is necessary to avoid microbial food spoilage and related health risks.

Introduction

Fresh fruits and vegetables are an important source of nourishment, especially vitamins, and a vital ingredient in healthy and balanced diets. Fruits and vegetables are vital sources of Nutrients to human beings. They give the body the necessary vitamins, fats, minerals, and oil in the right proportion for human growth and development. Fruits and vegetables however, have serious challenges to their existence. These include changes in climatic condition, pests and microbial attack. Over the years, there has been an increase in the need to identify and isolate the microorganisms associated with the spoilage as a way of finding a means of controlling it (Akinyele and Akinkunmi, 2012). Fresh

produce is highly recommended in any diet, virtually without quantitative restriction (Silva *et al.,* 2007). Susceptibility of fruits and vegetables is largely due to differential chemical composition such as pH and moisture contents are associated with greater predisposition to microbial spoilage.

Cabbage (*Brassica oleracea capitata),* Brinjal (*Solanum melongena* L.), Tomato *Lycopersicon esculentum, (Solanaceae*), Cauliflower *(Brassica oleracea* var *Botrytis)* and Carrots *(Daucus carata L.)* are the important vegetables grown in India (Mansoor Hassan *et al.,* 2005; Wasim *et al.,* 2008; Felix and Mahendran, 2009). Ber (*Ziziphus mauritiana)*, Grapes (*Vitis*) and Chickoo (*Sapodilla*) has been recognised as a useful edible fruits since antiquity in India (Einsett and Pratt, 1975; Asam Ali *et al.,* 2006). Such and other fresh-cut vegetables are, therefore, offered as salads in more than 70 per cent of fast food establishments and family restaurants. These vegetables and fruits are, however, capable of causing human diseases while still on the plant in fields or orchids, or during harvesting, transport, processing, distribution and marketing, or in the home (FDA, 2000; Joshi, 2008). *Salmonellae* have been isolated from many types of raw fruits and vegetables (Beuchat, 1996; Wells and Butterfield, 1997). Bacteria such as *Clostridium botulinum, Bacillus cereus* and *Listeria monocytes,* all capable of causing illness, are normal in habitants of many soils, whereas *Salmonella, Shigella, Escherichia coli* and *Campylobacter* reside in the intestinal tracts of animal, including humans and more likely to contaminate raw fruits and vegetables through contact with their faeces, sewage, untreated irrigation water or surface water (Cliver, 1997; Speer, 1997). The incidence of food borne outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years (Mukherjee *et al.,* 2006). There is thus demand by the consumers to use only such fresh vegetables and fruits which are free from microbial contamination and safe for human consumption. In the developed world, the extensive use of antibiotics in agriculture, especially for prophylactic and growth promoting purposes, has generated much debate as to whether this practice contributes significantly to increased frequencies and dissemination of resistance genes into other ecosystem (Chikwendu *et el*., 2008). 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. Keeping the above facts in mind the present study on 'bacterial population associated with fresh fruits and vegetables and the treatment of carrot – a commonly used fresh vegetables as salad-using antimicrobial agents, so as to reduce the microbial contamination of the fresh fruits and vegetables before their consumption as raw, was designed with the following objectives.

Isolation and enumeration of bacteria in different samples of fresh fruits and vegetables.

Morphological, biochemical and physiological characterization of bacterial strains.

Antimicrobial profile of surface micro flora of fruits and vegetables.

Effect of different antimicrobial agents on fruits and vegetables.

Materials and Methods

Three fruits namely Ber, Grapes and Chickoo and five vegetables namely Cabbage, Carrot,

Brinjal, Tomato and Cauliflower were selected in the present study. The samples were collected from Local market (LM), Super market(SM) and Field (F), thorough survey of market was carried out in the month of February and March, for the collection of the samples of fresh fruits and vegetables available in the market so as to check the variation in the superficial bacterial contamination and reduce the same through antimicrobial rinsing treatment.

Following methodologies were used to check the variation in bacterial population load and its antimicrobial treatment.

Isolation and estimation of bacterial population

For the isolation of bacterial strain from different samples, serial dilutions of the samples were prepared.

The respected sample was suspended in 90ml distilled water and serial dilution was prepared. 0.1 ml sample was taken, spread it on different selective media, and incubated the plates for 24 hours at 37°.

Calculation of colony forming unit

After the appearance of colonies on the plates the cfu of each soil sample were calculated using the formula:

$$CFU = \frac{\text{Number of colonies appearing on the plate}}{\text{Volume of sample taken}} \times \text{Dilution factor}$$

Morphological characterization

The isolated strains were characterized on the basis of different morphological and physiological characterization as mentioned in Bergey's Manual of Systematic Bacteriology.

Antibiotic resistance profile (Bauer *et al.,* 1966)

Antibiotic resistance of selected pathogenic isolates was determined by disc diffusion assay on Mueller Hinton agar as per Kirby Bauer method.

Effect of antimicrobial agents on bacterial population (Patel and Joshi, 2008)

The effect of different antimicrobial agents on the bacterial load of the local market sample of carrot was determined. Sample was treated with five different antimicrobial agents with varying concentration ranging from 0.5-3%.

Results and Discussion

The present study entitled Bacterial population associated with fruits and vegetables and its treatment using antimicrobial rinsing was carried out to study the micro flora associated with fruits and vegetables and reducing their superficial contamination with antimicrobial rinsing. A comparison among the total microbial load of fruits and vegetables from various markets and field sample was also carried out. Twenty samples were collected from local market, super market and field, and bacterial population was isolated from them. The bacterial strains were characterized on the basis of various morphological and biochemical tests. The strains were screened for antibiotic sensitivity against various antibiotics. Further, treatment of fruits and vegetables was also carried out to study the reduction in total bacterial load. The results of present study are summarized as under:

Data presented in Table 1showed that among vegetable samples the highest cfu (colony forming units) count was obtained for local market samples of carrot i.e. 30.4×10^5 on NAM as compared to super market (20.6×10^5)

and field sample (18.0×10^5). Among fruits the cfu highest count was obtained for field sample i.e. 8×10^5 (Table 2) as compared to local market sample (5.5×10^5) and super market sample (2.5×10^5). Similar finding were observed by Ibrahim *et al.,* (2009), Patel and Joshi (2008) who observed high contamination and high number of pathogens on the surface of fresh vegetables and fruits in case of local market samples (log 9-10 cfu/ml) as compared to super market samples (log 5-6 cfu/ml).

A total of twenty one bacterial strains were isolated in the present study. On the basis of morphological and biochemical tests ten strains namely VTL4, VCBL7, VTM8, VCL9, VCAM10, VCBM11, FBL18, FGRL17, FGRL15 and FBL21 strains isolated were identified as *E. coli*. Patel and Joshi (2008) also detected the prevalence of the members of family *Enterobacteriaceae* among the bacterial population isolated from fruits and vegetables. While, VBL1, VTL5,

VCM6, VTF13, FCHL14, FGRL19, VCF12 and FCHL16 were identified as *Staphylococcus aureus* and VCM3, VCAL2, FCHL20 were characterized as *Bacillus cereus* (Table 4). Viswanathan and Kaur (2001) also reported the presence of *E. coli* and *Staphylococcus aureus* in vegetables and fruits. Several other workers have also detected *Staphylococcus aureus* from carrot and ready-to-eat vegetable salads (Abdelnoor *et al.,* 1983; Houang *et al.,* 1991) (Fig. 1–3).

Staphylococcus spp. and *E. coli* have been reported to be the major microbes associated with the contamination of the vegetables and fruits (Ibeyessie, 2007; Ibrahim *et al.,* 2009). VCM3, VCAL2, FCHL20 were identified as *Bacillus cereus*. Portnoy *et al.,* (1976) observed the presence of *Bacillus* spp. while working with raw vegetables and fruits. Presence of spore formers can be attributed to the fact that spore-forming organisms can attach to vegetables grown near the soil for e.g. *Bacillus cereus* (Linton, 2003).

Table.1 Colony forming unit of vegetables samples collected from different markets

SR. NO	VEGETABLES	Cfu ($x10^5$)			
		NAM	MCA	EMB	MSA
LOCAL MARKET					
1	Cabbage	7.2	-	3.2	3.5
2	Cauliflower	13.6	1.1	1.3	2.3
3	Carrot	30.4	6.2	-	4.5
4	Tomato	13.2	6	1.5	2.5
5	Brinjal	23.2	3	-	3.0
SUPER MARKET					
1	Cabbage	20.6	-	3.0	1.1
2	Cauliflower	12.0	1	-	1.2
3	Carrot	17.6	2.4	-	4.4
4	Tomato	16.8	1.4	2.1	1
5	Brinjl	12.0	-	2	1.0
FIELD					
1	Cabbage	16.0	2.5	-	1.4
2	Cauliflower	8.0	3.5	-	2.5
3	Carrot	14.0	3.4	-	3.0
4	Tomato	18.0	6.0	-	2.2
5	Brinjal	-	-	1.0	1.9

NAM= Nutrient agar methylene. MCA= MacConkey agar. EMB = Eosine methylene blue Agar medium. MSA = Mannitol Salt Agar

Table.2 Colony forming unit (cfu) of fruit samples collected from different markets

SR.NO	FRUIT SAMPLE	Cfu (x10$^{5)}$)			
		NAM	MCA	EMB	MSA
LOCAL MARKET					
1	Ber	4	-	3.4	-
2	Grapes	5.5	3.8	1.5	1.4
3	Chickho	1.9	4.0	1	2
SUPER MARKET					
1	Grapes	2.5	-	2.2	1.0
FIELD SAMPLE					
1	Ber	8	-	-	9

Table.3 Colony morphology of bacterial strains

Sr.no	Strain	Colony morphology	Medium
1	VBL1	Yellow colour, entire, circular	MS
2	VCAL2	Abundant, circular with greenish margin	EMB
3	VCM3	Yellow colour, entire, circular	MS
4	VTL4	Abundant, circular with greenish margin	EMB
5	VTL5	Yellow colour, entire, circular	MS
6	VCM6	Yellow colour, entire, circular	MS
7	VCBL7	Yellow colour, entire, circular	MS
8	VTM8	Yellow colour, entire, circular	MS
9	VCL9	Yellow colour, entire, circular	MS
10	VCAM10	Yellow colour, entire, circular	MS
11	VCBM11	Yellow colour, entire, circular	MS
12	VCF12	Yellow colour, entire, circular	MS
13	VTF13	Yellow colour, entire, circular	MS
14	FCHL14	Yellow colour, entire, circular	MS
15	FGRL15	Abundant, circular, with greenish margin	EMB
16	FCHL16	Yellow colour, entire, circular	MS
17	FGRL17	Pinkish pigment, round entire,	MCA
18	FBL18	Pinkish pigment, round entire	MCA
19	FGRL19	Yellow colour, entire, circular	MS
20	FCHL20	White, oval, moist, round in shape	NA
21	FBL21	Abundant, circular, with greenish margin	EMB

Table.4 Actual per cent cfu (cell density) decrease

Compounds (Concentration 3%)	Range of % cell density decrease
Hydrogen peroxide	50
Sodium chloride	48
Calcium chloride	13
Sodium citrate	13
Ethanol	30

Fig.1 Growth of VBL1 on MSA   **Fig.2** Growth of FGRL15 on EMB
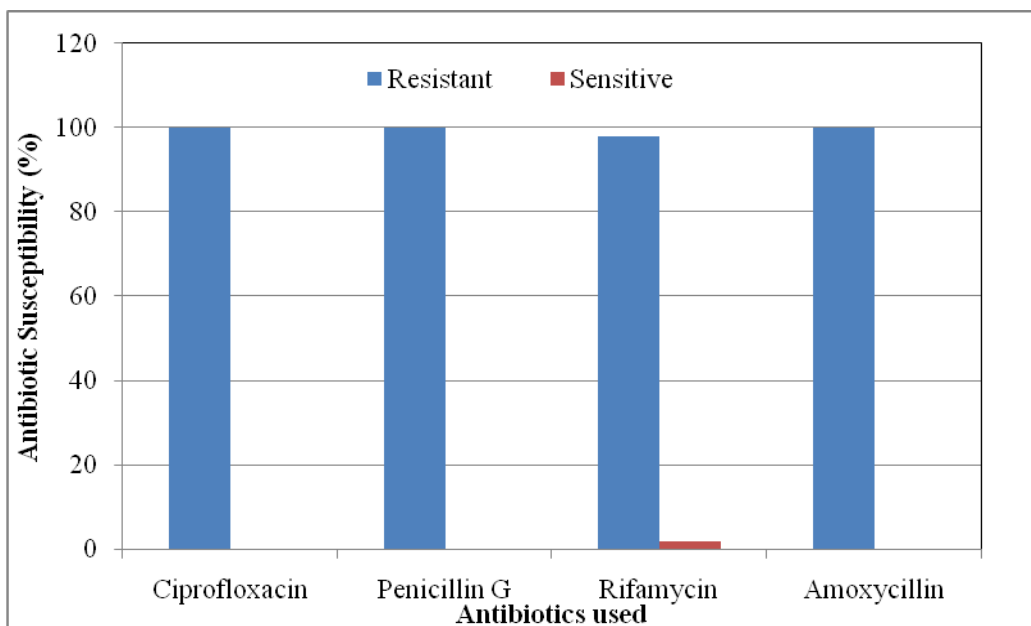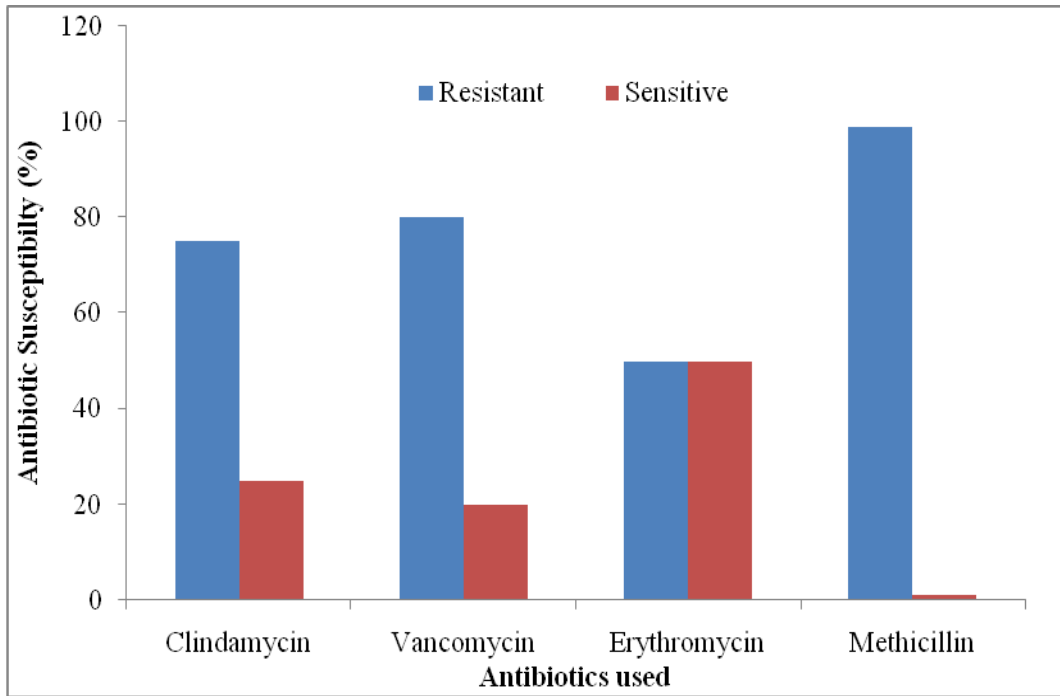

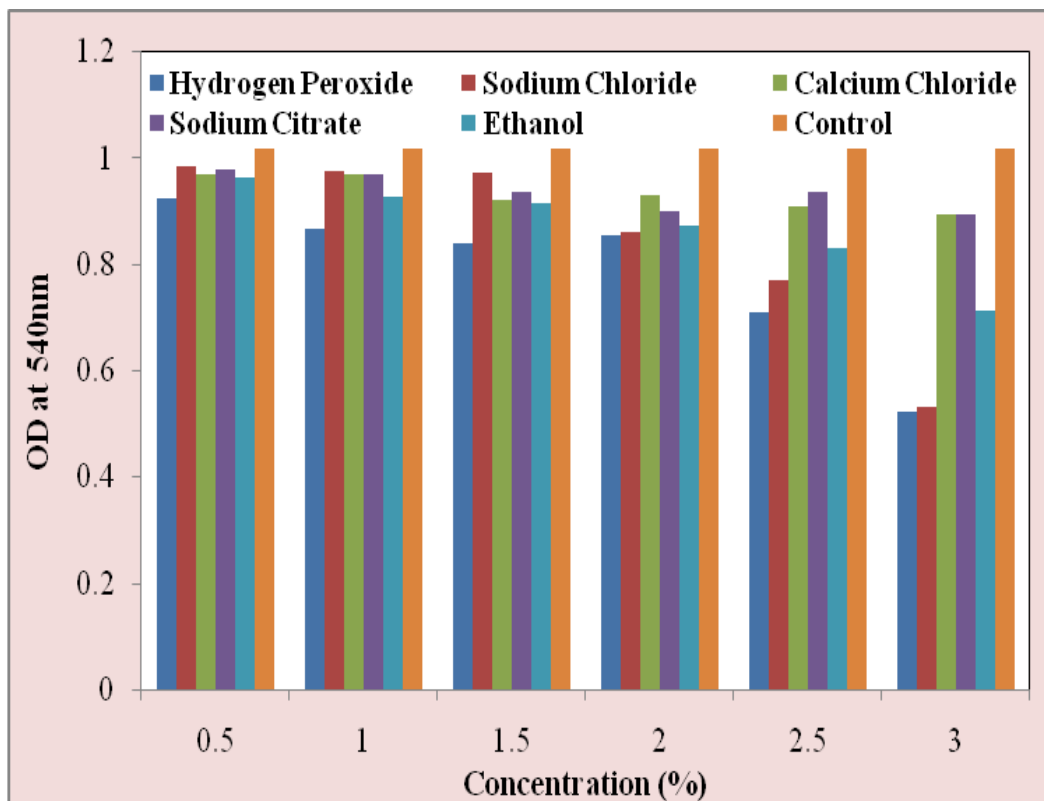**Fig.3** Citrate test   **Fig.4** Catalase test

Fig.5 Antibiotic susceptibility patterns of *E. coli* strains isolated in the present

Fig.6 Antibiotic susceptibility patterns among Gram positive strains isolated in the present study

Fig.7 Effect of antimicrobial agents on superficial bacterial population of carrot

Almost 99 per cent of the *E. coli* strains were resistant to all the antibiotics tested in the present study (Ciprofloxacin, Penicillin-G, Rifampycin, Amoxicillin) whereas, 75 per cent of the Gram positive strains showed resistance to all the four drugs (Clindamycin, Vancomycin, Erthromycin and Methicillin). Overall, 95% isolates were resistance to antibiotics that were used in this study (Fig. 5 and 6) Resistance of *E. coli* for Ciprofloxacin, Penicillin-G, Rifampycin and Amoxicillin has been reported earlier by Akond *et al.,* (2009).

Similar findings on multiple drug resistance of *E. coli* strains has been reported from in several parts of the world by several workers (Khan *et al.,* 2002; Guerra *et al.,* 2003; Zhao *et al.,* 2005; Rahman *et al.,* 2008). High resistance for Amoxycillin among *E. coli* have been also reported by Garba *et al.,* (2009).

Treatment with five antimicrobial agents namely (Hydrogen peroxide, Sodium chloride, Calcium chloride, Sodium citrate and Ethanol) was carried out. Varying concentrations of these agents ranging from 0.5% to 3.0% were used and it was observed that all of the agents reduced the cell count to some extent.

H_2O_2 was observed to be most effective antimicrobial agent because at its 3% highest cell reduction was observed (50%) Our findings draw support from the work of Patel and Joshi (2008) who reported H_2O_2 to be the most effective antimicrobial for the treatment of bacterial load. Saper (1997) has also suggested the efficacy of H_2O_2 in improving the microbiological quality and extending the shelf life of fruit and vegetable products. Similarly, Ukuku (2004) reported that treatments of fruits for 5 minutes with Hydrogen peroxide (2.5% and 5%) caused remarkable reduction in the indigenous surface micro flora of fruits and vegetables.

On the basis of above results it can be concluded that fresh vegetables and fruits mainly from local Market harbour many microbial contaminants and pathogens as compared to samples of super markets, indicating that these are protected from contamination while subsequent handling, packaging, storage and transit. As done by Ibrahim *et al.,* (2009), Patel and Joshi (2008) who observed high contamination and high number of pathogens on the surface of fresh vegetables and fruits in case of local market samples (log 9-10 cfu/ml) as compared to super market samples (log 5-6 cfu/ml). All the fruits and vegetables assessed bacteriologically had high viable counts even though within the tolerable limit. A good pre-decontamination of the vegetables as shown by this study could reduce the density of the microbial load by the antibacterial agents used. Similarly Patel and Joshi (2008) who reported H_2O_2 to be the most effective antimicrobial for the treatment of bacterial load. A combination of these agents can be used as rinsing agents to reduce the superficial contamination and improve the quality of these fresh fruits and vegetables. Saper (1997) has also suggested the efficacy of H_2O_2 in improving the microbiological quality and extending the shelf life of fruit and vegetable products. Similarly, Ukuku (2004) reported that treatments of fruits for 5 minutes with Hydrogen peroxide (2.5% and 5%) caused remarkable reduction in the indigenous surface micro flora of fruits and vegetables.

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