World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

INCINDENCE OF ENTERIC HUMAN PATHOGENS IN FRUIT AND VEGETABLE JUICES VENDED AT WASHIM

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Article Received on 05/06/2017 **Article Revised on 20/06/2017** **Article Accepted on 05/07/2017**

ABSTRACT

The present investigation was carried out to enumerate total viable count of bacteria, *Salmonella, Shigella* and fecal coliforms from Wheat grass, Amla, Pudina, Ginger, Tulsi and Beet juices sold at sport complex in Washim. As well as the antibiotic sensitivity and resistance pattern of isolated pathogens were also analyzed against common antibiotics. The present research work carried out by employing standard techniques viz .SPC, MPN and Kirby Bour. Results revealed that the maximum number of bacteria recorded in Wheat grass followed by Beet, Pudina, Tulsi, Ginger and Amla juice. The highest *salmonella* count observed in wheat grass followed by pudina,Beet,Tulsi,Ginger and Amla. In case of *Shigella* the maximum count recorded in Pudina followed by Beet, Wheat grass, Tulsi, Ginger and Amla. However, all juice samples were found contaminated with *fecal coliform*. The MPN index recorded maximum in Wheat grass followed by Beet, Tulsi, Ginger, Pudina and Amla juice. The result on antibiotic sensitivity and resistance pattern indicates that *Salmonella*, *Shigella* and Fecal coliform (*E.coli*) were found sensitive against 2,3 and 3 tested antibiotic. Whereas, resistance recorded against 4,3 and 3antibiotisc respectively. The result showed the poor hygienic quality of juice sold at sport complex and create possible risk to the consumers.

KEYWORD: SPC (Standard Plate Count), MPN(Most Probable Number), CFU(Colony Forming Unit), Antibiotics, Fruit juice etc.

INTRODUCTION

Fruit juice is the non-fermented and non- sparkling beverage prepared from the extraction and pressing out the natural liquid contain in fruit and vegetable.[1] Juices contain a variety of beneficial micronutrients, including significant quantities of minerals like calcium ,magnesium, potassium, vitamins etc and hence, plays an important role in attaining and maintaining good health.[2] In some studies, juice intake was found to be associated with higher dietary status index in rural women.[3,4] The diet supplemented with full of fruits and vegetables reduce risk of some chronic diseases. Well balanced diet rich in fruits and vegetable should be taken to prevent vitamin deficiencies, developing blood lipid profile and detoxification of human body.[5,6] Eating sufficient amount of vegetables ,fruits and fruit juices also control blood pressure, lower the risk of heart diseases, reduces blood cholesterol levels and avoid some kinds of cancer.[7] The fruit and vegetable juice obtained from of medicinally important plants contain biologically active compound which possess antimicrobial, anticancer, antioxidanant and antidiabetic activities. It has been shown that the fruit and vegetables juices have many health benefits. In many tropical countries they are common man's beverages and are sold at all public places and roadside shops.[8] However, reports of food borne illness associated with the consumption of fruit juices at several places in India has recorded.[9] Improperly prepared fruits and vegetable juices are recognized as an emerging cause of food borne illness.[10] Food borne disease outbreaks from enteric pathogens, such as *E.coli Salmonella, Vibrio cholerae, Vibrio parahaemolyticus etc* are common cases of food borne infection through vegetable and fruit juices.[11] also isolates 250 microbes and parasites from fruit and vegetables salad responsible for food born illness. It has observed that Juices are the potential sources of bacterial pathogens such as *E. coli* 0157:H7, *Salmonella, Shigella* and *Staphylococcus aureus*.[12] The presence of *E.coli* and *S.aureus* in fruit and vegetable juices is due to the improper handling, use of contaminated water for preparation of juice and personal hygiene of man who related to the preparation of juices.[13] Inspite of frequent reports about the information on the analysis of pathogens in fruit and vegetable juices has been poorly investigated. Hence, the present investigation conducted

to analyzed the bacteriological quality of fruit and vegetable juice sold at Sport complex at Washim district.

MATERIALS AND METHODS

Collection of Juice Samples

The fresh vegetable juices of Wheat grass, Amla, Ginger, Tulsi, Pudina and, Beet were purchased from local seller at sport complex of Washim city and transported in BOD bottles separately at Microbiology research laboratory, Department of Microbiology, R.A. College Washim within 2 hours. All the juice samples were stored in refrigerator for further investigation.

Enumeration of total viable count in juice samples

The enumeration of bacterial count in all fruit juices were carried out by Standard plate count method described by.[14,15] 1 ml of each juice sample was diluted in 10 ml sterile distilled water and further serially diluted in 9 ml sterile distilled water up10^{-5} separately. 0.1 ml sample from each dilution from all juice samples were aseptically transfer in respective sterile petri plates and approximately 20 ml nutrient agar plates (45^{o}C) poured in to petriplate. The sample and agar mixed thoroughly rotating the plates in eight directions several time. The viable count of *Salmonella* and *Shigella* was determined by same method by substituting Bismuth sulfite agar to nutrient agar .Thereafter all the sets of petriplate allowed to solidify and incubated at 37^{o} C for 24 hours. After incubation colony count of all the samples was done using digital colony counter. Colony count was determined by considering plates contain 30-300 colonies. Black and brown colored colonies on Bismuth sulfite agar were count to enumerate the *Salmonela* and *Shigella* respectively. The colony count of each sample was expressed as cfu/ml (colony forming unit). The number of bacteria per ml of sample was enumerated by using following formula.

$$\text{Number of bacteria /ml} = \frac{\text{No .of colony counted} \times \text{Dilution factor}}{\text{volume of sample taken.}}$$

Enumeration of Fecal coliforms in juice samples

The enumeration of fecal coliforms in all collected juice samples were estimated adopting most probable number (MPN) method. [16] Six sets of nine test tube containing Durham's tube were prepared. Three tubes of each set filled with 10 ml double strength Macconkey's broth and remaining six were filled with 5 ml of single strength Macconkey's broth. All the sets were sterilized in autoclave at 121^{0} C for 20 min at 15 lbs Pressure. After sterilization 10 ml, 1ml and 0.1ml sample of each fruit juice aseptically transfer in double and single strength Macconkey's broth respectively. All the sets were incubated 37^{o} C for 24 hours. All the tubes were observed for the acid and gas production. Count the tubes of each set of each fruit sample for acid and gas production and MPN of the sample calculated by using following formula.

$$\text{Formula MPN/100 ml} = \frac{\text{Number of positive tube} \times 100}{\sqrt{\text{ml of sample in negative tube} \times \text{ml of sample in all tube}}}$$

Identification of Salmonella, Shigella and Fecal coliform

The black and brown colored colonies from SPC plates and 1ml sample from positive tube of MPN aseptically transfer in to 50 ml of nutrient broth separately and incubated at 37^{o} C for 24 hours. for enrichment. The enriched cultures were used for identification. The identification of isolates was done on the basis of Morphological characters, Cultural characters and Biochemical characters. The morphological characters were studied microscopic method by performing Grams staining and Motility and Cultural characters viz, size, shape, color, margin and opacity were assessed by macroscopically by observing growth on ordinary medium. Whereas, the biochemical characters of the isolates were studies by sugar fermentations and IMVIC test. The observations of above performed tests were compared with standard literature for the confirmation of *Salmonella*, *Shigella* and Fecal coliform.[17]

Studies on antibiotic sensitivity and resistance pattern

The antibiotic sensitivity and resistance pattern of *Salmonella*, *Shigella* and *E.coli* against Ampicillin, Azithromycine, Chloramphinicol Ceftriaxone, Ofloxacin, and Tetracycline was analyzed by employing the Kirby Bauer's disc diffusion method.[18] The loop full pure culture of *Salmonella*, *Shigella* and *E.coli* were separately inoculated in 50 ml of nutrient broth and incubated at 37°C for 24 hours. The broth cultures of *Salmonella*, *Shigella* and *E.coli* were aseptically spread by sterile swab on separate sterile Mueller Hintons agar plates. The antibiotic discs were aseptically placed on *Salmonella*, *Shigella* and *E.coli*. seeded Mueller Hinton agar plates. All the plates were incubated 37°C for 24 hours. After incubation the sensitivity and resistance pattern was determined by measuring the diameter of zone inhibition in millimeters by antibiotic zone reader scale and compared with the standard diameters that installed in the standard scales.

RESULT AND DISCUSSION

Total viable count in fruit and vegetable juice sample

The results on total viable count in fruit and vegetable juices were presented in table 1.The results revealed that the maximum numbers of bacteria were recorded in wheat grass juice followed by, Pudina, Beet, Tulsi juice, Ginger and Amla juice where the bacterial count recorded as $5.7x10^{6}$cfu/ml, 5.3×10^{6}, 5.0×10^{6}cfu/ml, 3.8×10^{6}cfu/ml, 4.5×10^{6}cfu/ml and 1.4×10^{6}cfu/ml. The total viable count of *salmonella* was found maximum in Wheat grass juice ($2x10^{3}$cfu/ml) followed by Pudina juice($1.5x10^{3}$cfu/ml),Beet juice ($1.2x10^{3}$cfu/ml),Tulsi juice($1x10^{3}$cfu/ml),Ginger juice(($0.95x10^{3}$cfu/ml) and Aamla juice($0.8x10^{3}$cfu/ml) respectively. However, the

maximum viable count of S*higella* was observed in Pudina followed by Beet, wheat grass, Tulsi,Ginger juice and Amla juice respectively. The colony forming units were recorded as $1.5x10^3$cfu/ml,1.4×10^3 cfu/ml,1.2×10^3cfu/ml, 1.1×10^3cfu/ml,0.7×10^3cfu/ml and 0.5×10^6 cfu/ml. These values are quite higher than the microbiological limits for fruit juices and nacters. According to Development and use of Microbiological criteria for foods, 1997, the maximum acceptable limit is 10^6 CFU/ml. by comparing the results of present work with this standard limit proved that the microbial load of the juice samples analysed are quite high except amla juice. It might be due to the acidic pH of the Amla fruit that greatly limits the numbers of bacteria. The increase in microbial load in juices is due to the improper washing of fruits leading to contamination. In addition to this use of unsterilized extractors, homogenizers and other equipments used in the process line, unavailability of treated running water for dilution and washing, prolonged preservation without refrigeration, unhygienic surroundings with swarming flies and airborne dust.[19,20]

Most Probable Number (MPN)

The fecal coliforms in all the sample of juices were estimated adopting the method most probable number described by A.M.Deshmukh.[21] The data presented in Table 1 explained the results on probable numbers of fecal coliforms per 100ml of juice sample .It indicates that the highest MPN recorded in Wheat grass (2400) followed by Beet (1100), Tulsi (210). Ginger(150), Pudina(120)and amla (3) respectively. The results of our investigation are accordance with findings of Reddy [21] who reported the presence of fecal coliforms in fruit and vegetable juice as a indicator of fecal contamination. According to the UNBS [standard, fecal coliforms should not be detected in the fruit juices and nectars. The normal habitat of faecal coliforms is the intestinal tracts of man and animals and they are not known to be found in nature in the absence of faecal contamination. They are excluded out of animal body through excretion process, in the form of faeces. This indicates the poor quality of fruit and vegetable juices due the preparation of the juices without following good manufacturing practice. The present study also indicates that Wheat grass, Ginger, Tulsi, Pudina and Beet juice was found unsafe for the consumer. Where as Amla juice was found safe. It might be due to either the acidic P^H or antimicrobial effect of Amla on fecal coliform. The minimum MPN recorded in Amla juice might be either due to acidic P^H or antimicrobial activity of Amla juice.

Identification of *Salmonella, Shigella* and *Fecal Coliforms*

The identification of *salmonella, Shigella* and Fecal Coliforms confirmed by comparing the obtained observation of morphology, Biochemical character and IMVIC test with with standard literature. The results presented in table 2 reaveled the results on identification. The black and brown colored bacteria were identified as S*almonella,* and *Shigella* respectively. Whereas, the enriched culture from positive MPN sample identified as *E.coli*. Similar findings were also recorded by.[22,23] in street vended fruit and vegetable juices that indicate the risk of food born infection for consumer.

Studies on antibiotic sensitivity and resistance pattern

The sensitivity and resistance of isolated *Salmonella, Shigella* and *E.coli* against common prescribed antibiotics were determine by Kirby Bour's disc diffusion method .The results presented in table 3 indicates that *Salmonella* found highly sensitive against Azitromycine and Ofloxacin, intemidiate sensitive against Tetracyclineate and Ceftxiaxone and resistance observed against Ampicillin and Choromphenicol. Whereas *Shigella* found sensitive against Azythromycine, Ceftxiaxone, Ofloxacine and tetracycline. *Shigella* showed resistance against Ampicillin and Choromphenicol. It is also observed that *Salmonella* and *shigella* were highly sensitive to Azithromycine and Ofloxacin and resistant Ampicillin and Chloromphenicol. It is also observed that the *E.coli* isolate showed maximum sensitivity against Azithromycine, Ampicillin and Tetracycline however, it displayed resistance against Choromphenicol, Ceftxiaxone and Of loxacine. From the results it was also observed that multi drug resistance (MDR) strains of *Salmonella*, *Shigella* and *E.coli* was not recorded in all juice samples. The result on antibiotic sensitivity of present investigation was found accordance with the experimental findings of Jain [24] who studied the Antibiotic Resistance of Different Bacterial Strain Isolated from orange juice. Their study observed 80% bacterial isolates were found multidrug Resistance (MDR). However, in Present investigation no multidrug resistance strains found in all juices samples.

Table 1: Total Bacterial, *salmonella, shigella* and Fecal coliforms in Fruit and Vegetable Juice.

Sr.No	Name of juice sample	TVC cfuX10^6	VCS cfuX10^3	VCSh cfuX10^3	MPN/100ml
1	Wheat grass juice	5.7	2.0	1.2	2400
2	Aamla juice	1.4	0.8	0.5	3
3	Ginger juice	3.8	0.95	0.7	150
4	Tulsi juice	4.5	1.0	1.1	210
5	Pudina juice	5.0	1.5	1.5	120
6	Beet juice	5.3	1.2	1.4	1100

TVC=Total viable count, VCS=Viable count of salmonella, VCSh=Viable count of shigella MPN=Fecal coliform

Table 2: Identification of *Salmonella, Shigella* and Fecal *Coliforms*.

A. Morphological character				
Sr. no	**Character**	**Black color colony**	**Brown color colony**	**Fecal Coliforms**
1	Grams character	Gram negative bacillus	Gram negative bacillus	Gram negative
2	Motility	Motile	Non motile	Motile
B. Cultural Characteristics				
1	Size	3mm	2mm	0.2µm
2	Shape	Circular	Circular	Rod
3	Colour	White	White	Transparnt
4	Margin	Regular	Irregular	Round
5	Opacity	Translucent	Translucent	Translucent
6	Consistence	Smooth	Rough	Smooth
7	Elevation	Convex	Convex	Convex
C. Biochemical character				
1	Indol	Negative	Positive	Positive
2	Methyl Red	Positive	Positive	Positive
3	Vogues proskuor	Negative	Negative	Negative
4	Citrate	Positive	Negative	Negative
D Fermentation of sugars				
1	Glucose	Acid positive and Gas positive	Acid positive and Gas negative	Acid positive and Gas negative
2	Lactose	Acid Negative and Gas Negative	Acid negative and Gas negative	Acid positive and Gas negative
3	Mannitol	Acid positive and Gas positive	Acid positive and Gas negative	Acid positive and Gas positive

Table 3: Antibiotic Sensitivity and Resistance pattern of enteric pathogens isolated from Fruit and Vegetable Juice.

Sr.No	Name of antibiotics	Zone of Inhibition in mm		
		Salmonella	***Shigella***	***E coli***
1	Azithromycin	22 (S)	22 (S)	20 (S)
2	Ampicilline	15 (R)	14.5 (R)	18 (S)
3	Chloromphinicol	15 (R)	16 (R)	10.5 (R)
4	Ceftxiaxone	18 (R)	18 (R)	10 (R)
5	Ofloxocine	20 (S)	17 (S)	16 (R)
6	Tetracylline	19 (R)	14 (S)	15 (S)

S=Sesitivity, R=Resistance

CONCLUSIONS

The present study concluded that all investigated juices had high bacterial load except Amla juice in comparison to the standards. All the juice samples were contaminated with, *Salmonella*, *Shigella* and fecal coliform(*E.coli*). The results also showed that the isolated pathogens viz *Salmonella*, *Shigella* and fecal coliform (*E.coli*) were found sensitive and resistance against some Antibiotics tested. No isolated Strain generate Multidrug resistance. The contamination of juices is mainly due to poor quality of water used for preparation of juice as well as unhygienic conditions related to washing of utensils such as mixer and juicer. The higher bacterial count and presence of *Salmonella, Shigella* and *E. coli* in fruit and vegetable juices is frightening enough for an instant action by the suitable agency. These pathogenic organisms have also shown resistant to few common antibiotics tested, indicating a possible reason of public health hazards. Hence, Regular inspection of the quality of fruit and vegetable juices for human consumption must be introduced to avoid any future pathogen outbreaks.

ACKNOWLEDGEMENT

The authors are thankful to Microbiology Research Laboratory, Rajasthan Education Society Washim, M.S. India for providing the necessary facilities during the tenure of research work.

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