

RESEARCH PAPER

Microorganisms in Cut Cucumber and their Public Health Significance

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Abstract

To assess the microbiological quality of cut cucumbers (*Cucumis sativus*) sold by itinerant roadside vendors in and around Kolkata, 25 such samples were collected over a two-months period during summer, and tested for total aerobic plate counts (TPCs), coliform and fecal-coliform (*Escherichia coli*) counts, and other food-borne pathogens. TPCs ranged from 5.093 to 7.477 log cfu/g, 18 samples out of which (72%) had counts >7 log cfu/g. Coliforms were detected in all 25 samples (100%). *E. coli* was detected in 23 of them (92%), having an average count of 140.80 MPN/g, with 18 samples (78.26%) showing counts >7 log cfu/g. *Salmonella* and *Vibrio cholerae* were detected in 12 (48%) and 1 (4%) of the total samples respectively, with 7 of the 12 samples of the former (58.33%), and the latter (100%) showing counts >7 log cfu/g respectively.

Keywords: Coliform and fecal-coliform counts, cucumber, food-borne pathogens, roadside vendors, TPCs

Fresh vegetables have always been an important food group in the human diet, particularly in a hot, tropical place like Kolkata and its adjoining areas, where cut cucumbers (*Cucumis sativus*) are a popular summer delicacy for the exhausted and weary people out in the sun for long. Cucumbers have a unique moist and cooling taste, besides being naturally hydrating (Country Food and Dining Ltd., 2017) and having high quantities of silica, vitamin C, caffeic acid and several essential phytonutrients (Country Food and Dining Ltd., 2017; Lins *et al.* 2017; The George Mateljan Foundation, 2017; SkipThePie.org, 2012).

Cucumbers have an epidermal layer of cells which provides a barrier to penetration by most microorganisms. However, cutting and slicing, a

practice often practised, removes this protection, and as a result, microbes can invade its inner tissues. Therefore, cucumbers may be viewed as a fertile niche in which the normal flora microorganisms, as also the contaminating pathogens, vie for the rich nutrients, multiply and predominate. By the time passers-by purchase and eat raw cucumbers sold openly by the street-vendors, those already have become laden with microorganisms.

The food-industry recognizes cucumbers as a 'perishable food', as it has a pH in the range of around 6, a high moisture-content (a_w) of about 0.96, a high oxidation-reduction (O-R) potential (Eh) favoring the growth of aerobic, anaerobic and facultative microorganisms, and all the necessary nutrients in

the right kind and proportion required for an effective establishment of a chemoorganotrophic microbial flora (Frazier *et al.* 1995; Greenblender, 2017).

Cucumbers are easily contaminated by pathogenic bacteria, such as *Salmonella*, *Shigella* and *Vibrio*, in a multitude of ways that are generally harmful to the quality of the food. Potential ways of contamination include physiological (abiotic) stress generated during their harvest (Frazier *et al.* 1995; Shanker *et al.* 2011; Garcia *et al.* 2017), and several post-harvest routes, including inoculations from infected roadside-vendors. All these necessitates improved scientific methods in maintaining the microbiological quality and shelf-life of cucumbers, as role of microbes in spoilage and safety of this fresh produce is still a much limiting factor.

Thus, a number of mild to severe health hazards like upset stomach, abdominal cramps, nausea and vomiting, diarrhea, fever and dehydration may result from the consumption of these street-vended cucumbers (U.S. Department of Health & Human Services, 2017).

Presently, reports are available on the microbiological quality of different other cut fruits and vegetables, like papaya (Mukhopadhyay *et al.* 2002). However, although many researchers have pointed out to the contamination of cut cucumbers by pathogenic microorganisms like *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Campylobacter* spp., *Enterobacter aerogenes* B199A, *Lactobacillus* spp. (including *L. plantarum*, *Leuconostoc* spp.), *Aeromonas* spp. (including *A. caviae* and *A. hydrophila*), and *Pythium* spp. (including *P. debaryanum* and *P. aphanidermatum*) (Jordan, 2010; Pommerville, 2004), no investigative reports are currently available on their contamination by the highly notorious food-borne pathogens of public-health significance, like *Salmonella* and *Vibrio*. Also a regular monitoring of these street-vended cut cucumbers is necessary due to constantly changing climatic conditions and food habits in different parts of the city and its surroundings over the years, which may have, in the mean time, altered their usual contamination profiles.

So, the purpose of the present work was to investigate the microbiological quality of cut cucumbers sold in Kolkata and its outskirts, and to assess its potential as a vehicle for the transmission of several harmful food-borne pathogens of enormous public health concern. The results obtained are described in this communication.

MATERIALS AND METHODS

Chemicals, reagents, dehydrated media, media base and supplements

All chemicals and reagents used in this study were purchased from Merck Specialities Private Limited, India and Sisco Research Laboratories Private Limited, India. All dehydrated media, media base and supplements were procured from HiMedia Laboratories, India.

Collection of samples

Twenty-five 100 g (approx.) samples of cut cucumbers were collected over a period of two months during summer (8th April – 3rd June, 2016) from itinerant roadside vendors in and around Kolkata (Fig. 1) (Mukhopadhyay *et al.* 2002). At the beginning of analysis, the pH of all the samples was checked.

Total plate counts (TPCs), coliform and fecal coliform (*E. coli*) counts

Total plate counts (TPCs), and coliform and fecal coliform (*E. coli*) counts were determined by the standard methods cited by APHA, 1992. Characteristic colonies, typical of *E. coli*, were isolated from the Eosin Methylene Blue (EMB) agar-plates following incubation, and identification carried out on the basis of their characteristic growth on Nutrient Agar (NA) slant, standard Gram-staining reaction, and routine biochemical tests as described by Barrow *et al.* 1993.

Salmonella

Detection of *Salmonella* was carried out by the standard methods given by APHA, 1992 and Mukhopadhyay *et al.* 2002. Characteristic colonies, typical of *Salmonella*, were isolated from the *Salmonella-Shigella*



Fig. 1: (a) Cucumbers sold by road-side vendors from pavements of Kolkata; and (b) a cut cucumber sample collected from the same site

(SS) agar-plates and Hektoen Enteric Agar (HEA)-plates following incubation, and identification done on the basis of their characteristic growth on NA slant, standard Gram-staining reaction, and routine biochemical tests as prescribed by APHA, 1992.

Vibrio cholerae and *Vibrio parahaemolyticus*

Vibrio cholerae and *Vibrio parahaemolyticus* were detected by the standard methods of APHA, 1992 and Mukhopadhyay *et al.* 2002. Characteristic colonies, typical of *Vibrio cholerae*, were isolated from the thiosulphate citrate bile salts sucrose (TCBS) agar-plates following incubation, and identification carried out on the basis of their characteristic growth on NA slant, standard Gram-staining reaction, and routine biochemical tests as specified by APHA, 1992. The same steps were followed for the isolation and identification of *Vibrio parahaemolyticus*.

Statistical analysis

All results were expressed as mean \pm SEM for individual experiment. Each experiment was performed three times (n=3), and the mean value from all set of those experiments was presented. Student's t-test was performed as applicable in each case, and the values were found to be significant at 5% probability level.

RESULTS AND DISCUSSION

pH of the cut cucumber samples

The pH of all twenty-five samples studied was

found to lie between 6.4-6.8. This range of pH makes cucumber susceptible to the growth of bacteria, including the pathogenic ones.

Total plate counts (TPCs), coliform and fecal coliform (*E. coli*) counts

An alarmingly high contamination profile in the cut cucumber samples was found, as indicated by the TPCs, along with coliform and fecal coliform (*E. coli*) counts (Table 1). The observed average total TPCs (7.000 log cfu/g), average coliform count (165.76 MPN/g) and average fecal coliform (*E. coli*) count (140.80 MPN/g) indicated that cucumbers have been cut and stored under less than ideal hygienic conditions.

Table 1: Microbial counts* of 25 cut cucumber samples

Parameter	Minimum	Maximum	Average
TPC (log cfu/g)	5.093	7.477	7.000
Coliform count (MPN/g)	24	240	165.76
Fecal coliform (<i>E. coli</i>) count (MPN/g)	0	240	140.80

* Each value represents the average of 3 determinations (n=3).

Among the 25 cut cucumber samples examined, 3 (12%), 4 (16%) and 18 (72%) were found to have

Table 2: Relationship between TPCs* and the presence of coliforms, fecal coliforms (*E. coli*) and selected food-borne pathogens in 25 cut cucumber samples

TPCs (log cfu/g)	Number of samples within the range	Number of samples positive for				
		Coliforms	Fecal-coliforms (<i>E. coli</i>)	<i>Salmonella</i>	<i>Vibrio cholerae</i>	<i>Vibrio parahaemolyticus</i>
<6	3 (12%)	3 (100%)	1 (33.33%)	2 (66.66%)	N.D.	N.D.
6-7	4 (16%)	4 (100%)	4 (100%)	3 (75%)	N.D.	N.D.
>7	18 (72%)	18 (100%)	18 (100%)	7 (38.88%)	1 (5.55%)	N.D.

N.D. - Not detected in any sample; values in parentheses indicate percent positive for coliform, fecal-coliform or food-borne pathogens of the number of samples of the particular range of TPC; *each value represents the average of 3 determinations (n=3).

TPCs (log cfu/g) <6, 6-7 and >7 respectively (Table 2). Coliforms were detected in all 25 samples (100%), and fecal coliforms (*E. coli*) in 23 (92%) (Table 2). The number of samples positive for fecal coliforms (*E. coli*) increased with an increase in the TPCs, from 1 (33.33%) in cases of samples with aerobic TPCs < 6 log cfu/g to 4 (100%) in cases of samples with aerobic TPCs between 6-7 log cfu/g, and 18 (100%) in cases of samples with aerobic TPCs > 7 log cfu/g (Table 2).

Small, discrete, flat, smooth, non-mucoid, nucleated (black centre), purple colonies, most with a very prominent greenish metallic sheen typical of fecal coliform (*E. coli*), were isolated from the EMB-agar plates. *E. coli* was confirmed by its pale-white, translucent growth on a NA slant, reddish-pink (Gram-negative), short, thin rod appearance after Gram-staining, and typical results in tests including acid production from glucose, standard IMViC reactions, production of H₂S, gluconate and malonate utilization, L-arginine dihydrolase, and L-Lysine and L-ornithine decarboxylase activities (Table 3).

Furthermore, *Salmonella* and *Vibrio cholerae* were detected in 12 (48%) and 1 (4%) out of the 25 cut-cucumber samples examined, with 7 samples (58.33%) and 1 sample (100%) having aerobic plate counts > 7 log cfu/g respectively (Table 2).

Salmonella

Characteristic colonies, typical of *Salmonella*, were isolated from the SS agar-plates and HEA- plates. Small, discrete, slightly-raised, smooth, translucent colonies, most with a very prominent black centre, were observed on the SS agar-plates (Table 4), whereas small, discrete, slightly-raised, smooth, transparent colonies, most with a very prominent black centre, were observed on the HEA-plates, both suggesting the presence of *Salmonella*. *Salmonella* was confirmed by its pale-white, translucent growth on a NA slant, reddish-pink (Gram-negative), short, thin rod appearance after Gram-staining, and typical results in tests including growth in potassium cyanide (KCN), urease activity, utilization of glucose, lactose,

Table 3: Results of the standard tests for *E. coli*

Acid production from glucose	IMViC				H ₂ S production	Gluconate utilization	Malonate utilization	L-arginine dihydrolase	L-Lysine decarboxylase	L-ornithine decarboxylase
	Indole	Methyl Red	Voges Prosk-auer	Citrate utilization						
+	+	+	-	-	-	+	-	±	+	±

Table 4: Results of the standard tests for *Salmonella*

Growth in potassium cyanide (KCN)	Urease activity	TSI				MIO			LIA				Indole production in PW
		Slant	Butt	Gas	H ₂ S	Indole production/ Ornithine Decarboxylation/ Motility	Slant	Butt	Gas	H ₂ S			
-	-	purple (alkaline)	yellow (acidic)	+	+	/-/+	violet (alkaline)	violet (alkaline)	+	+	-		

Table 5: Results of the standard tests for *Vibrio cholerae*

Slant	KIA				Oxidase	Acid production from glucose	Acid production from D-mannitol	Acid production from meso-inositol	L-Lysine decarboxylase	L-ornithine decarboxylase	L-arginine dihydrolase
	Butt	Gas	H ₂ S production								
Red (alkaline)	Yellow (acidic)	-	-	+	+	+	-	+	+	-	

sucrose and H₂S production in triple-sugar-iron (TSI) agar, indole production, ornithine decarboxylation and motility in motility-indole-ornithine (MIO) agar, L-Lysine decarboxylation and H₂S production in lysine-iron agar (LIA), and indole production in peptone water (PW) (Table 4).

Vibrio cholerae* and *Vibrio parahaemolyticus

Large (2-4 mm in diameter), discrete, slightly flattened, smooth, yellow colonies with opaque centres and translucent peripheries were observed on TCBS-agar plates, suggesting the presence of *Vibrio cholerae*, which fermented sucrose to produce the yellow color. *Vibrio cholerae* was confirmed by its pale-white, translucent growth on a NA slant, reddish-pink (Gram-negative), short, curved ('comma') rod appearance after Gram-staining, and typical results in tests including Kligler iron agar (KIA) reaction, oxidase reaction, acid production from glucose, D-mannitol and meso-

inositol, L-Lysine and L-ornithine decarboxylase, and L-arginine dihydrolase activities (Table 5). Colonies typical of *Vibrio parahaemolyticus* were not observed on any of the TCBS agar-plates.

CONCLUSION

In conclusion, the authors would like to point that the results of the present study revealed a strong positive correlation between high TPCs, detection of coliforms including fecal coliforms (*E. coli*), and the presence of enteric pathogens (*Salmonella* and *V. cholerae*) in the cut cucumber samples sold by itinerant vendors, indicating that the problem of contamination exists in full vigor in and around Kolkata.

In the present study, *E. coli*, a recognized indicator of fecal contamination, was confirmed to be present in all of the coliform-positive samples. This in turn, indicated the possibilities of the presence

of potentially hazardous enteropathogenic *E. coli* (EPEC) and/or enterotoxigenic *E. coli* (ETEC) in the cut cucumber samples, which may cause large outbreaks of gastro-enteritis and food poisoning in infants and young children. It is not surprising that fecal coliforms were detected, considering that these vegetables were first washed with water, peeled, re-washed, cut and sold in road pavements from containers without cover, getting contaminated as a result with water-borne *E. coli*. So, these observations do raise questions regarding public health, as *E. coli*-mediated severe diarrhea and vomiting may be the ultimate fate the consumers would be found to suffer from. Fecal coliforms are not part of the natural microflora of such vegetables, but the relationship between the number of samples positive for fecal coliforms (*E. coli*) and TPCs indicated that these were not processed or stored hygienically before selling.

Hence, it is of utmost necessity to improve the microbiological quality of cut cucumbers for consumer health protection. For this, an immediate betterment of the prevailing sanitary practices must be performed. Since street food and itinerant vendors have come to be accepted in Kolkata, the health authorities may consider if these vendors can be trained in the relevant hygienic practices as well.

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