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Research Article

Multiple Antibiotic Resistance Indices of Potential Pathogenic Bacteria Isolated from Street Vended Fruit and Sugarcane Juices, Malda Town, India

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Abstract

Street vended fruit juices have been reported worldwide to be contaminated with various bacterial pathogens causing several illnesses to humans. This study determines the bacterial contamination of fruit (pomegranate and sweet orange) and sugarcane juices available from street vendors in Malda town (West Bengal state, India) and explores antibiogram of the isolated bacteria. The pure bacteria culture obtained from the juice samples were identified by phenotypic characterization, as *Escherichia coli, Enterobacter cloacae, Micrococcus roseus, Staphylococcus aureus, Bacillus cereus, Bacillus licheniformis, Enterococcus faecalis, Staphylococcus saprophyticus* and *Vibrio cholerae*. The antibiotic susceptibility of the isolated bacteria was determined by disc diffusion method using 10 antibiotics; most of the isolates were resistant to three or more antibiotics tested. The calculated multiple antibiotic resistance (MAR) indices for the juice bacterial isolates varied from 0.1 (for *E. faecalis*) to 0.6 (for *V. cholerae*). The study of antibiogram to detect the drug resistant bacteria from fruit and sugarcane juices, which was not conducted previously from this part of the globe, added a new insight to the accessible information in the field.

Keywords: Pomegranate Juice; Sweet Orange Juice; Sugarcane Juice; Bacterial Contamination; Antibiogram; MAR Index

Introduction

The street vending foods and beverages, including fruit juices, pledge the food security for a large number of populations from various socioeconomic groups and the livelihood for many people in developing countries, like India [1] and contribute to the nutritional status of the consumers, as well. However, the street vending foods are ready-to-eat foods, prepared instantly, or at home for selling on the street, and chances of microbial contamination is high for such kind of foods [2-4] and therefore, microbial food poisoning as well as food-borne diseases, in association with consumption of street foods, remains a major health crisis [1,2]. The microbial contamination of fruit juices with *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* is of prime concern since these pathogenic bacteria have been recognized to cause a number of fruit juice related outbreaks [5]. Tambekar., *et al*. [6] isolated a number of potential bacterial pathogens: *E. coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp. and *S. aureus* from different types of street vended fruit juices. Potential pathogenic bacteria, such as *E. coli*, *Salmonella* spp., *Shigella* spp. and *S. aureus* have been isolated from grape, sweet lime, pineapple and sapota juices [7]. The presence of *Staphylococcus* spp. and fecal coliform bacteria has been found to contaminate the street vended coliform juices studied [8]. Reports have been made on food-borne illnesses associated with the consumption of fruit juices in India and elsewhere [9-11]. Moreover, the multiple antibiotic resistances among the food-borne bacteria intensely exacerbate the situa-

tion causing fruit juice associated outbreaks [5]. In our part of the globe (Malda town, West Bengal, India), street vended fruit juices available are cherished by the local consumers, not only for health benefits, but also for the taste, affordable cost and easy availability, even, in spite of the concern on safety and microbiological quality of freshly prepared ready-to-consume fruit juices, due to lack of scientific data on safety issues. Therefore, the current study has been undertaken to bacteriological profiling of locally available fruit and sugarcane juices from street vendors, and antibiotic susceptibility testing for the isolated bacteria.

Materials and Methods

Collection of juice samples

A total of three juice samples, one each of pomegranate fruit, mosambi fruit (sweet orange) and sugarcane, were collected (from April to June, 2016) from three different street vendors at three different sites within from Malda town (West Bengal, India), and were processed microbiologically, within an hour of collection, in the Laboratory of Microbiology and Experimental Medicine (Department of Zoology, University of Gour Banga).

Microbiological analysis

In order to enrich bacterial growth, each of the samples were inoculated into nutrient broth (Hi-Media, India) for 24 h at 35^0C, and thereafter subculture (of the broth cultures) was done, by streak-plate dilution, on selective/differential media (SRL, India): nutrient agar, MacConkey agar (bile salt concentration 1.4 g/liter), brilliant green bile agar and TCBS agar, in order to get pure bacteria cultures, which were stored and maintained in cystine tryptone agar stab, at 4^0C temperature. The haemolytic capacity of the isolated bacteria was determined using blood agar plate (Hi-Media, India). All incubation was done at 35^0C, for 18 - 24h.

The isolated bacteria were identified by phenotypic characterization, following gram-staining, biochemical tests including sugar fermentation and TSI tests, following Bergey's manual [12] as explained earlier [13].

Antibiogram and MAR index

The antibiotic susceptibility, for the isolated juice bacteria, was determined according to disc diffusion method of Bauer., *et al*. [14] as described by Das., *et al.* [15], utilizing nalidixic acid (Nx: 30 µg/disc), ampicillin (Am: 10 µg/disc), cefpodoxime (Ce: 10 µg/disc), amikacin (Ak: 30 µg/disc), amoxyclav (Ac: 30 µg/disc), trimethoprim (Tm: 5 µg/disc), methicillin (Me: 5 µg/disc), piperacillin (Pc: 100 µg/disc), gentamicin (Gm: 30 µg/disc) and cefotaxime (Ct: 30 µg/disc), all procured from Hi-Media(India) . The results, in terms of zone diameter of inhibition (ZDI; nearest whole in mm) were interpreted following the CLSI criteria (CLAI, 2011). The MAR indices were calculated and interpreted according to the published protocol [13,16], as per the details mentioned earlier [13,15].

Results and Discussion

Various different kinds of potential human pathogenic bacteria: gram-positive (*S. aureus*, *Micrococcus* spp., *Bacillus subtilis*, *Streptococcus* spp., *B. cereus*) and gram-negative (*E. coli*, *Klebsiella pneumoniae*, *Serratia* spp., *Proteus mirabilis*, *Salmonella* spp.) have been isolated from different types of street vended fruit juices, mainly from developing parts of the globe [17,18]. In the current study, a total of 10 bacteria were isolated from 3 juices: 3 from sugarcane juice (strain code: SJ1, SJ2 and SJ3), 5 from mosambi juice (strain code: MJ1, MJ2, MJ3, MJ4 and MJ5), and 2 from pomegranate juice (strain code: PJ1 and PJ2). Among the isolated bacteria (n = 10), 3 were gram-negative (SJ1, SJ3 and MJ5); the 7 rests were gram-positive. All the gram-negative bacteria isolated were rod shaped, while the gram-positive bacteria were rod shaped (n = 2; MJ2 and PJ2) as well as coccus (n = 5; SJ2, MJ1, MJ3, MJ4 and PJ1). The sugar fermentation test results for the sugarcane juice and fruit juice bacteria are represented in Table 1. The biochemical properties of the sugarcane juice and fruit juice bacterial isolates are represented in Table 2. The growth properties in TSI agar slant for the juice bacteria are depicted in Table 3; the H_2S production was negative for all the isolated bacteria, while two were positive for gas (CO_2) production. Street vended fruit juices have been reported to contain gram-positive (*Staphylococcus aureus*) as well as gram-negative (*Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Pseudomonas* spp.) pathogenic bacteria [19]. Report has been made earlier on the contamination of pomegranate juice with *E. coli, P. aeruginosa* and *S. typhi* [20]. Sugarcane juices were reported to be contaminated with *E. coli, K. pneumonia, Proteus* spp., *P. aeruginosa*, *Enterococcus* sp. and *S. aureus* [21]. Bacteria, such as, *Bacillus subtilis*, *B. cereus*, *Escherichia coli*, *Micrococcus* sp., *Staphylococcus aureus* have been reported to be isolated from orange and sweet orange (mosambi) juices [22].

Bacterial Strain	Sugar							
	Glucose	Xylose	Sucrose	Mannitol	Melezitose	Cellobiose	Mannose	Raffinose
SJ1	+/G	+	+	+	-	-	+	+
SJ3	+	-	+	+	-	+	+	+
SJ2	+/G	+	+	+	-	+	+	+
MJ1	+	+	-	-	-	+	+	-
MJ2	+	-	+	+	-	-	-	-
MJ3	+	+	+	-	-	-	+	-
MJ4	+/G	+	+	+	-	+	-	+
MJ5	+	-	+	+	-	+	+	-
PJ1	+	+	+	+	-	+	+	-
PJ2	+	-	+	-	-	-	-	-

Table 1: Sugar fermentation test results for the isolated juice bacteria; n = 10.

+: Positive; - Negative; G: Gas.

Bacterial isolate	Biochemical (enzyme utilization) property								
	Citrate	Indole	Catalase	Oxidase	Urease	MR	VP	Gelatinase	Nitratase
SJ1	-	+	+	-	-	+	-	-	-
SJ3	+	-	+	-	-	-	+	+	+
SJ2	+	-	+	+	-	-	+	-	-
MJ1	+	-	+	-	-	+	+	+	-
MJ2	-	-	+	+	-	+	+	+	+
MJ3	-	-	-	-	-	+	+	-	+
MJ4	-	-	+	-	+	-	+	-	+
MJ5	+	+	+	+	-	+	-	+	+
PJ1	+	-	+	-	-	+	+	+	+
PJ2	-	-	+	+	-	+	+	+	+

Table 2: Biochemical features of the isolated juice bacteria; n = 10.

+: Positive; -: Negative.

Bacterial isolate	TSI			
	Slant	Butt	H_2S	Gas
SJ1	Yellow	Yellow	-	+
SJ3	Pink	Yellow	-	-
SJ2	Yellow	Yellow	-	+
MJ1	Yellow	Yellow	-	-
MJ2	Yellow	Yellow	-	-
MJ3	Pink	Yellow	-	-
MJ4	Yellow	Yellow	-	-
MJ5	Yellow	Yellow	-	-
PJ1	Yellow	Yellow	-	-
PJ2	Pink	Yellow	-	-

Table 3: Growth properties in TSI (triple sugar iron) agar slant for the juice bacteria; n = 10.

In the current study, the identity of various bacteria isolated from three different juices has been represented in Table 4. All the juice samples tested were found to be contaminated with bacteria; the bacterial isolates from sugarcane juice were *Escherichia coli*, *Enterobacter cloacae* and *Micrococcus roseus* and bacteria isolated from mosambi juice were *Staphylococcus aureus*, *Bacillus licheniformis*, *Enterococcus faecalis*, *Staphylococcus saprophyticus* and *Vibrio cholerae*, while the pomegranate juice was contaminated with *Staphylococcus aureus* and *Bacillus cereus*. Therefore, the highest bacterial diversity was recorded in mosambi juice, followed by sugarcane and pomegranate juice; the bacterial load estimated in the test juices ranged from 1.988×10^3 cfu/ml ($3.298 \log_{10}$ CFU/ml of mosambi juice) to 2.792×10^3 cfu/ml ($3.446 \log_{10}$ CFU/ml of pomegranate juice). It has been reported that water used for juice preparation might be the source of contamination of juices with bacteria,

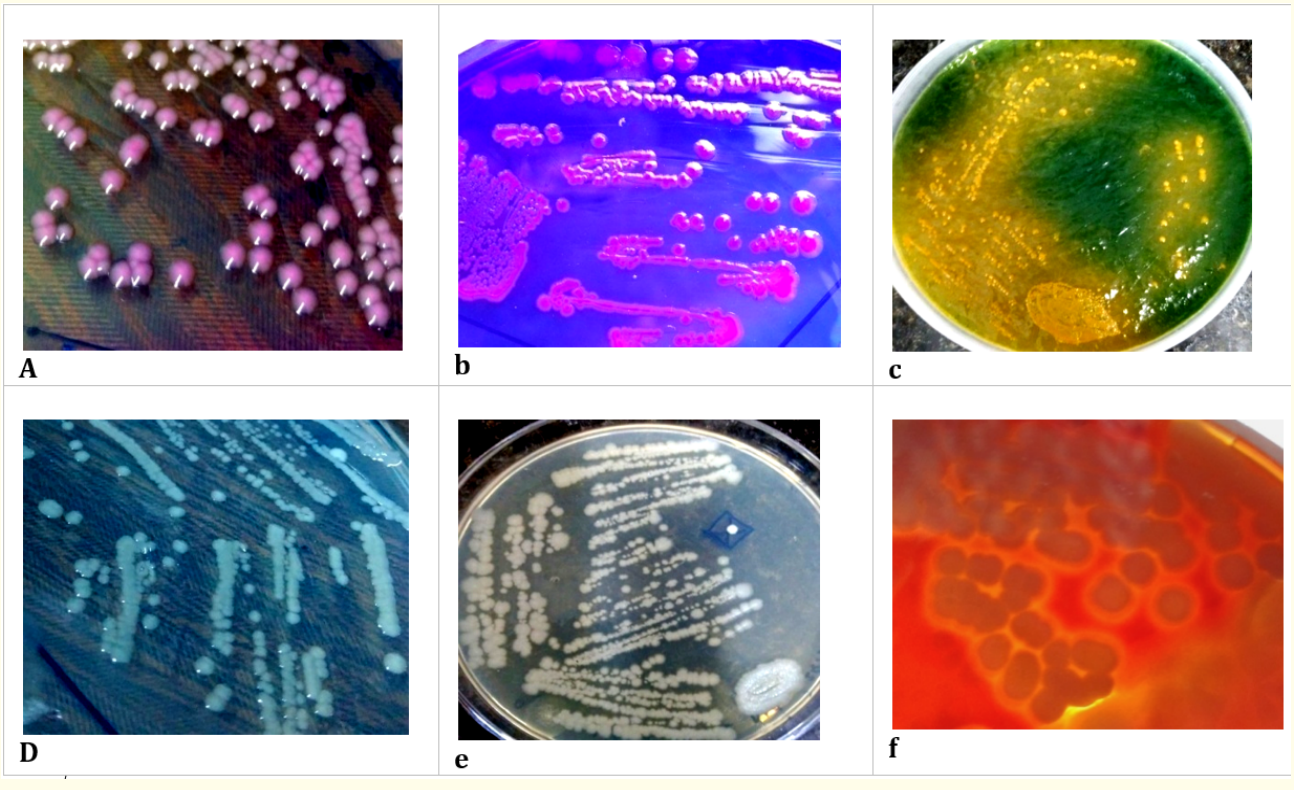

Figure 1: Colony characteristics of bacterial isolates; a: *Escherichia coli* SJ1 on MacConkey agar; b: *Escherichia coli* SJ1 on brilliant green bile agar; c: *Vibrio cholerae* MJ5 on TCBS agar; d: *Vibrio cholerae* MJ5 on nutrient agar; e: *Bacillus cereus* PJ2 on nutrient agar; f: *Bacillus cereus* PJ2 on blood agar. Note the characteristic β-haemolysis caused by *Bacillus cereus* PJ2 on blood agar.

such as coliform bacteria, fecal coliform bacteria and fecal streptococci [23]. Thus, the street vending juices are not entitled for human consumption. The poor hygienic practices by the food handlers, during juice preparation, poor method of storage of fruits as well as application of non-potable water in juice preparation, and site selection of stalling alongside the streets, sometimes near to drainages, might play role in the bacterial contamination of freshly prepared juices by street vendors. The presence of total coliform bacteria, *E. coli* and *S. aureus* from street vended fruit juices and absence of any microbial pathogens in the branded pasteurized fruit juices suggest that good hygienic practices in juice preparation as well as scientific preservation of fruits are mandatory to prepare and supply fruit and other herbal juices, in order to provide health benefits to the consumers [24,25].

Juice	Bacterial isolate	Bacterial identity
Sugarcane	SJ1	*Escherichia coli*
	SJ3	*Enterobacter cloacae*
	SJ2	*Micrococcus roseus*
Mosambi	MJ1	*Staphylococcus aureus*
	MJ2	*Bacillus licheniformis*
	MJ3	*Enterococcus faecalis*
	MJ4	*Staphylococcus saprophyticus*
	MJ5	*Vibrio cholerae*
Pomegranate	PJ1	*Staphylococcus aureus*
	PJ2	*Bacillus cereus*

Table 4: Identity of sugarcane juice and fruit juice bacterial isolates; n = 10.

The antibiotic resistance patterns of the fruit juice and sugarcane juice are depicted in Table 5. The mosambi juice bacteria showed lowest (*Enterococcus faecalis* MJ3) to highest (*Vibrio cholerae* MJ5) resistance patterns; all the sugarcane juice bacterial isolates had 3-drug resistances of varied patterns, while the pomegranate juice bacteria had 3- to 4-drug resistances. *Klebsiella pneumoniae* isolated from sugarcane juice had resistance to Am, Gm, Km, neomycin and penicillin, as has been reported by Amin., *et al* [26]. The *Vibrio cholerae* isolates from sugarcane juice was resistant to polymyxin B, and sensitivity to Gm and Cp [27]. Rashed., *et al.* [24] reported that high rate of antibiotic resistance was seen among *Staphylococcus* spp. isolates from street vended fruit juices to Am (93), Pc (75%), Ax (92%) and vancomycin (Vm; 63%), while for the overall juice bacterial isolates had resistance to Am, Cp, amoxicillin, erythromycin, chloramphenicol, Ct, Pc, trimethoprim-sulfamethoxazole, Nx and Vm. The street vended unpasteurized juices (apple, banana, mango, orange, lemon and sugar cane) were found to be contaminated with *Escherichia coli* O157:H7 having resistance to Ax, Am and Cp [25]. The sugarcane juice isolate of *Staphylococcus aureus* was resistant to Am and Vm, as has been reported by Lucky., *et al* [19].

Drug resistance	Bacterial isolate	Resistance pattern	MAR index
1-drug resistant	*Enterococcus faecalis* MJ3	Me	0.1
3-drug resistant	*Escherichia coli* SJ1	Am-Ce-Me	0.3
	Enterobacter cloacae SJ3	Am-Ac-Me	0.3
	Micrococcus roseus SJ2	Am-Ac-Me	0.3
	Bacillus licheniformis MJ2	Am-Ac-Ce	0.3
4-drug resistant	*Staphylococcus aureus* MJ1	Am-Ac-Ce-Me	0.4
	Staphylococcus aureus PJ1	Ac-Ce-Tm-Me	0.4
	Bacillus cereus PJ2	Am- Ac-Ce-Tm-Me	0.4
5-drug resistant	*Staphylococcus saprophyticus* MJ4	Am-Ac-Ce-Pc-Me	0.5
6-drug resistant	*Vibrio cholerae* MJ5	Am-Ac-Ce-Nx-Tm-Me	0.6

Table 5: Antibiotic resistance pattern and MAR indices for juice bacterial isolates; n = 10.

Am: Ampicillin; Ac: Amoxyclav; Ce: Cefpodoxime; Me: Methicillin; Nx: Nalidixic Acid; Pc: Pipercillin; Tm: Trimethoprim.

The bacterial MAR indices are useful tools in determining the source of origin of bacterial contamination, whether it could be from human-fecal or animal-fecal sources, or from the antibiotic contaminated niches [28,29]. The MAR indices for the isolated juice bacteria ranged 0.1 - 0.6 (Table 5). The all sugarcane juice bacteria, and one mosambi juice bacterial isolate (*Bacillus licheniformis* MJ2) had MAR index of 0.3, while all the bacteria isolated from pomegranate juice and one from mosambi juice (*Staphylococcus aureus* MJ1) displayed MAR index of 0.4; the two mosambi juice bacteria: *Staphylococcus saprophyticus* MJ4 and *Vibrio cholerae* MJ5 had MAR indices 0.5 and 0.6, respectively. Therefore, the mosambi juice isolates of *Staphylococcus saprophyticus* MJ4 and *Vibrio cholerae* MJ5 might be originated from human-fecal contaminated sources, and all (but one, *Enterococcus faecalis* MJ3, from mosambi juice) isolates having MAR indices > 0.2 possibly disseminated from antibiotic polluted niches [13,15,16]. Believes from the authors' knowledge that this was first to report on the issues of MAR indices of fruit juice bacteria within from Malda town, West Bengal, India.

Conclusion

The fruit juices (pomegranate juice and mosambi juice) and sugarcane juice, from street vendors in Malda town (West Bengal state, India) were found to be contaminated with gram-positive as well as gram-negative bacteria, which possess the capacity to cause human illnesses. The bacteria displayed resistance to single to multiple antibiotics, having MAR indices 0.1 - 0.6. Thus, consumption of such beverages might be associated to different health hazards. On the contrary, the vending as well as consuming the juices from street stalls cannot be bunged considering the nutritional justification of local community and livelihood of street vendors. This study, thus, reflects an intervention in minimizing the risk of human health hazards, through the awareness of the street vendors, from the consumption of unpasteurized fruit and sugarcane juices available from them; still, further studies are warranted in mitigating the emerging paramount burden of microbial food-borne human illnesses from street vended juice consumption.

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