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Bacterial Evaluation of Fresh Juices Sold in Cafes and Restaurants in the City of Benghazi, Libya

Ismaeel H. Bozakouk[1*], Monia Ahmed H. BaLshikh[2], Mohamed M. Bumadian[1]
[1]*Department of Microbiology, Faculty of Science, University of Benghazi, Libya.*
[2]*Department of Botany, Faculty of Science, University of Benghazi, Libya.*

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Abstract: Food safety is a big challenge that concern all human communities and food production factories. The information on the bacterial evaluations of fresh natural juices in the city of Benghazi remained very limited and unclear. This study was carried out to evaluate the bacterial contamination for two of the most popular selling freshly made juices, including mango and strawberry juices. The results of screening showed bacterial growth in 117 (91.41%) out of the total samples 128. The evaluation during the second season showed a higher bacterial growth rate of 96.55 %. The most predominant bacteria contaminating the targeted juices was *Klebsiella pneumoniae*, with an average of (36.21%), followed by fecal *Escherichia coli* (31.03%) for both juices. All the contaminated juices represented bacteria count out of the accepted value. Gram-negative pathogens showed resistance to limited antibiotics, including Amoxicillin, Amikacin, and Clarithromycin, whereas the Gram-positive bacteria showed higher resistance rates. This study concluded that, increase in bacterial diversity and bacterial count out of the accepted standard as well as the presence of fecal *E. coli* indicating a poor level of hygiene during the process of preparing juices, leading to the possibility of causing diseases and exposing people to food poisoning.

التقييم البكتيري للعصائر الطبيعية الطازجة المباعة في مقاهي ومطاعم مدينة بنغازي، ليبيا

الكلمات المفتاحية:
الامن الغذائي
تلـ .وث العصـ .ائر الطبيعية
بكتيريـ .ا *E. coli* البرازية
التعداد الميكروبي

المستخلص: تعتبر سلامة الغذاء من التحديات الكبيرة التي تشغل بال كل المجتمعات البشرية ومصانع إنتاج الغذاء بشكل مستمر. ظلت المعلومات الخاصة بالتقييمات البكتيرية للعصائر الطبيعية الطازجة في مدينة بنغازي محدودة للغاية وغير واضحة. أجريت هذه الدراسة لتقييم التلوث البكتيري لاثنين من أكثر العصائر الطازجة متضمنة كلا من عصائر المانجو والفراولة. أظهرت نتائج الفحص نمو بكتيري في 117 (91.41٪) من مجموع العينات 128. أظهر التقييم خلال الموسم الثاني معدل نمو بكتيري أعلى بلغ 96.55٪. كانت البكتيريا الأكثر انتشارا هي بكتيريا *Klebsiella pneumoniae* بمتوسط (36.21٪)، تليها بكتريا *Escherichia coli* البرازية (31.03٪) لكلا نوعي العصائر. أظهرت النتائج أن جميع العصائر الملوثة بالبكتيريا هي خارج قيمة التعداد الميكروبي المقبول. أظهرت نتائج اختبار حساسية المضادات الحيوية أن البكتيريا سالبة الجرام مقاومة للمضادات الحيوية المحدودة، تتضمن كلا من Amoxicillin، Amikacin، Clarithromycin، بينما أظهرت البكتيريا موجبة الجرام معدلات مقاومة أعلى. وخلصت هذه الدراسة إلى أن زيادة التنوع البكتيري والعدد البكتيري هو خارج عن المعيار المقبول وكذلك وجود بكتيريا *E. coli* البرازية مما يشير إلى ضعف مستوى النظافة أثناء عملية تحضير العصائر مما يؤدي إلى إمكانية التسبب في الأمراض وتعريض الناس للتسمم الغذائي.

INTRODUCTION

Food safety is an important issue alarmingly concerned by all human communities and food production factories. Lack of the food hygiene and food sanitary quality direct of these products to be contaminated with several pathogenic bacteria leading to risk of foodborne illness

***Corresponding author:** Ismaeel H. Bozakouk: *Ismaeel.bozakouk@uob.edu.ly*, Department of Microbiology, Faculty of Science, Benghazi University, Libya.

(Gizaw, 2019; Ncama et al., 2021). Natural juices are among the food products that people drink a lot specially during the summer, as they are refreshing and rich in nutrients, however they are exposed to many pollutants, especially microbial contamination during the preparation process. Many of people working in the field of juices preparation do not have enough knowledge on the food safety and some do not follow the safety roles to avoid potentially severe health hazards, therefore the possibility of microbial contamination is very likely to occur (Ho et al., 2020; Ruxton et al., 2021). Several pathogenic bacteria are more frequent that combined with the juice contamination including *Escherichia coli*, *Staphylococcus aureus*, *Cryptosporidium*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, *Salmonella sp.* and *Acinetobacter sp*. (Callejón et al., 2015; Hossen et al., 2020; Kader et al., 2014; Reddy et al., 2009; Tambekar et al., 2009).

The information on the microbial evaluations of fresh natural juices in the City of Benghazi are remained very limited and unclear. This project was carried out to evaluate the bacterial contamination into two of the most popular selling of freshly made juices including mango juice and strawberry juice in order to encourage people to spread awareness on food safety management and ways to combat foodborne illness.

MATERIALS AND METHODS

Study area and samples collection: The study was performed to evaluate the bacterial contamination of natural juices sold in the city of Benghazi. The samples collection targeted the most popular sale points including local fresh juice shops, cafes and restaurants and for the most popular sale products including mango and strawberry juices. In order to make sure the study cover the whole city areas, the collection of samples was designed to be including North, South, East, and the City Center within two seasons of collection. The investigation was carried out for 128 of fresh fruit juice samples served into plastic containers collected within two seasons as 64 sample of each. Within each season the microbial isolations were applied for 32 strawberry juice and 32 mango juice samples. After each collection, the samples were preserved into cold conditions and immediately transported to the laboratory for microbiological investigation.

Samples processing and culture conditions: In order to perform initial isolation and screening for bacterial contamination, all the fruit samples were first homogenized and under aseptic conditions one milliliter from each sample was introduced on Nutrient agar plate and MacConkey agar the plates were then incubated aerobically at 37 °C for overnight. Next day, all the plated were checked for bacterial growth and determine the diversity of bacterial types that involved the contamination. The bacterial cultures that showed more than one type of bacteria were processed for sub-culture step in order to obtain pure microbial cultures. All the well-isolated bacteria were preserved refrigerated at 4 °C using a slant Nutrient agar tubes prior bacterial identification and their susceptibility to the antibiotics.

Determination of the bacterial load and the most probable number that contaminate the juice samples: Serial dilutions using a sterile normal saline were made from each type of juice to make appropriate juice dilution concentrations of (10^{-1} up to 10^{-3}), one milliliter from each dilution was introduced and spread on Nutrient agar plate, the plates were then incubated aerobically at 37 °C for overnight. Next day, all the plated were checked to determine the bacterial colony forming unite (cfu/ml) according to the following formula: Number of bacteria colonies in the original sample = Number of colonies counted x 1/df x V (ml). Where: df, Dilution factor; V, Volume in ml and compared with the standard bacterial count (Asghar et al., 2018). For the MPN method, the analysis was performed in three steps including presumptive test, confirmatory test and completed test.

Identification of the isolated bacteria: After bacterial purification, the bacterial cultures were directed for morphological characterization including colonies differences on the agar plates such as shape, size and color of the colonies, lactose fermentation, and blood hemolysis, followed by microscopic morphology examination to investigate the differences between bacterial cells by using Gram staining techniques. Further assessments were used for bacterial identification including several traditional biochemical tests by using lactose fermentation, glucose fermentation, sucrose fermentation, gas production and using different culture media including MacConkey agar, Blood agar, Eosin methylene blue agar (EMB), Lactose Broth, Selenite Broth, Salmonella Shigella agar (SS), Triple sugar iron agar (TSI), Simmon`s citrate agar, Urease agar, DNase agar and Mannitol salt agar (MSA).

Some enzymatic chemical test also used to determine and differences between microbes including oxidase test, catalase test, and coagulate test. In order to investigate presence of fecal coliform, the samples that showed presence of *E. coli* growth were checked for positivity to the gas production and then incubated at 44.5 °C for 24–48 hrs. For conformation of the bacterial identification, Automated Phoenix 100 System for bacterial identification was used.

Antibiotic susceptibility testing of the bacteria contaminating fresh juices: All the identified bacteria tested for their sensitivity to the antibiotics in order to determine the antibiogram profile that contributing the multidrug resistant pathogens. This test was performed using the Kirby–Bauer disc diffusion method using Muller Hinton agar (MHA) plates as described by Clinical and Laboratory Standards Institute (CLSI, 2006) as follow: a bacterial suspension was prepared according to the 0.5 McFarland standard using a sterile normal saline and the turbidity was adjusted such that it contained approximately 1×10^6 cfu/ml.

The prepared bacterial suspension was smeared onto the Muller Hinton agar (MHA) plates using a sterile cotton swab followed by selection of known concentration of antibiotic-disks purchased from (Oxoid) including Amikacin (5 µg), Amoxicillin (10 µg), Cefixime (5 µg), Cefuroxime (30 µg), Cephalexin (30 µg), Ciprofloxacin (5 µg), Clarithromycin (15 µg), Doxycycline (30 µg), Imipenem (10 µg), Oxacillin (1 µg) and Sulfamethoxazole-Trimethoprim (25 µg) were placed onto the plates, after that the plates were incubated aerobically at 37 °C for overnight. Next day, the effect of the antibiotics was evaluated as a clear inhibition zone formed arrowed the antibiotic discs measured in millimeter of diameter.

RESULTS

The assessment was carried out according to increase the sources and the risk factors that represent a possible source of contamination with bacterial pathogens leading to health problems. The bacterial screening results for strawberry and mango juices showed that 117 (91.41%) of the total samples were contaminated with bacteria, the Gram-negative bacteria were representing the most predominant 110 (94%) out of 117 selected isolates.

The distribution of isolated bacteria according to the season and time of isolation was showing microbial growth at first isolation 58 (90.62%) out of 64 samples and 56 (96.55%) out of the 58 samples, were showing Gram-negative bacteria. During the second season, the results of bacterial screening showed 59 (92.19%) out of 64 samples were contaminated with bacteria, as well as 54(91.53%) of the isolated pathogens were classified belong to Gram-negative bacteria, figure (1).

Frequency of bacterial contamination according to the area: In order to cover the whole study area, the samples were collected from the four geographic areas of the City of Benghazi, including North, South, East and city Center. Within each geographic area, four targeted collection areas were included. From

each collection area, four collection points were targeted. From each collection point, two samples were collected during the two seasons.

The results showed high bacterial growth into the four geographic areas. For the North area, the bacterial growth was into 93.75% and 100% of the samples during the first and second seasons respectively. For the South area, the bacterial growth was into 93.75% and 81.25% of the samples during the first and second seasons respectively. For the East area, the bacterial growth was into 100% and 87.50% of the samples during the first and second seasons respectively. For the City Center area, the bacterial growth was into 87.50% of the samples for both the first and the second seasons, table (1).

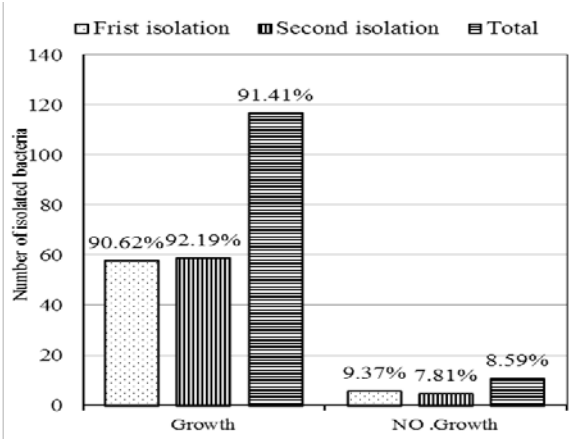

Figure (1): Shows the general bacterial investigation during the two seasons of collection representing total bacterial growth**.**

Diversity of the bacterial pathogens associated with the four studied areas: In order to determine the most predominant microbial contaminant in each geographic studied area, the isolated bacteria were assessed for identification, the results showed that *Klebsiella pneumoniae* represented the most predominant pathogen among all species, where it isolated from 42 (35.89%) juice sample, followed by fecal *E. coli* 36 (30.76 %), *Pseudomonas aeruginosa* 15 (12.82 %), *E. coli* 9 (7.69 %), *Enterobacter aerogenes* 8 (6.84 %), *Staphylococcus aureus* 5 (4.27 %) and *Staphylococcus schleiferi* 2 (1.71%).

The distribution of the isolated bacteria according to the geographical area separately showed that, in the North area, the most frequent isolated contaminant was fecal *E. coli* 12 (37.50%) followed by *K. pneumoniae* 8 (25%). In the South area, *P. aeruginosa* 10 (31.25 %) represented the most frequent bacteria followed by *K. pneumoniae* 9 (28.13%). In the East area, the most frequent isolated contaminant was fecal *E. coli* 12 (37.50%) followed by *K. pneumoniae* 9 (28.13%). In the City Center area, the most frequent isolated contaminant was *K. pneumoniae* 16 (50%) followed by fecal *E. coli* 6 (18.75%), table (2).

Distribution of bacterial species according to the types of juices: The results showed the contamination with *K. pneumoniae* represented the most predominant microbe contaminating the mango juice 23 (54.76%) and 19 (45.24%) for strawberry juice. The juice contamination with fecal *E. coli* showed 22 (61.11%) isolates into the mango juice and 14 (38.89%) isolates for the strawberry juice. The juice contamination with *P. aeruginosa* showed 8 (53.33%) for the strawberry juice and 7 (46.67%) mango juice. The results also showed the both juices have contaminated with some other different microorganisms at low rate of bacterial percentage as shown in the figure (2).

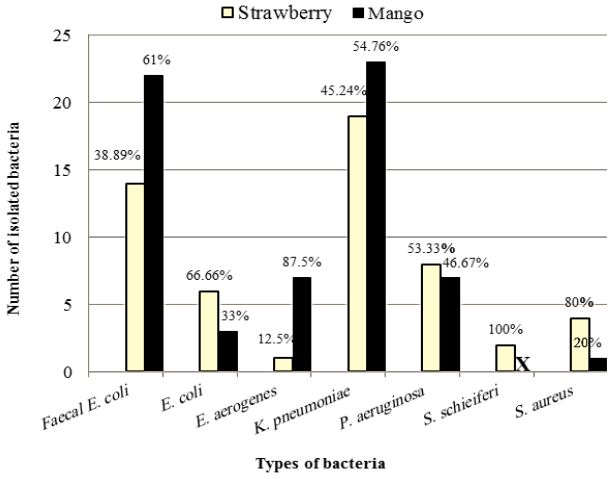

Figure (2): Distribution of the bacterial species according to the types of juices

Direct measurement of microbial growth through determination of most probable number: In order to investigate the most probable number for the bacteria that contributing the bacterial infection risk factor, a direct measurement of bacterial count on plate agar was performed through serial dilutions were made from the two type of juices; the results of growth were compared with the standard bacterial count chart provided by the recommended Gulf standard[11] that instructed the mean total viable count (microbial load) should be in all the freshly prepared fruit juices in the range of $0.1x10^2$ to $1.48x10^3$ cfu/ml, where the results showed that, *P. aeruginosa* revealed the highest rate of bacterial average count of $1.484x10^3$ cfu/ml, followed by fecal *E. coli* that showed average of count of $1.228x10^3$ cfu/ml, *K. pneumoniae* with an average of count $9.70x10^2$ cfu/ml, *E. aerogenes* showed $5.74x10^2$ cfu/ml, *E. coli* showed $2.7x10^2$ cfu/ml, and the contamination with *S. aureus* and *S. schleiferi* showed low bacterial count of $0.7x10^2$ cfu/ml and $0.1x10^2$ cfu/ml, figure (3).

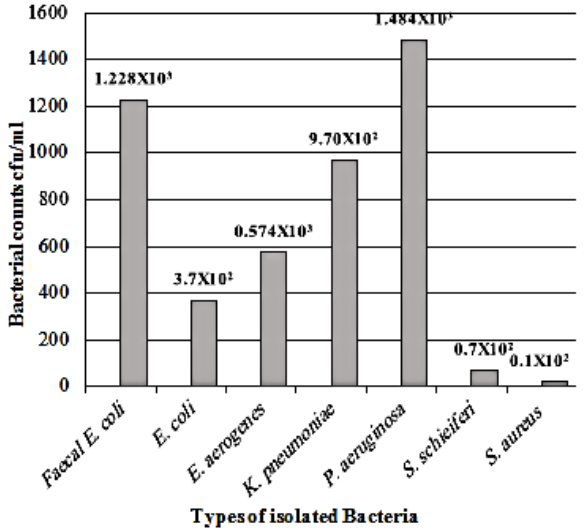

Figure (3): Bacterial counts (cfu/ml) into the both fresh juices

Antibiotic susceptibility testing of the bacteria contaminating fresh juices: Antibiotic susceptibility testing was performed according to the Kirby Power disc diffusion method. The results showed that, fecal *E. coli* demonstrated complete resistance to antibiotic Clarithromycin (100%), whereas it was less resistant to Sulfamethoxazole-Trimethoprim (3.85%) and sensitive to the rest of tested antibiotics including Ciprofloxacin, Imipenem and Oxacillin, Cefixime and Doxycycline and Cefuroxime (100%), whereas Sulfamethoxazole - trimethoprim (96.15%), followed by Amikacin (84.6%) and Amoxicillin, Cephalexin (76.9%). *E. coli* and *E. aerogenes* showed resistance to Clarithromycin and Amoxicillin (100%); alone *E. coli* recorded a partial resistant to Amikacin (89%), whereas *E. aerogenes* showed a complete resistance to the same antibiotic (100%). In contrast, the two isolates showed sensitivity to rest of antibiotics including Ciprofloxacin, Imipenem and Oxacillin, Cefixime and Doxycycline, Sulfamethoxazole-trimethoprim Cefuroxime and Cephalexin with percentage of sensitivity reached to (100%). *K. pneumoniae* showed resistance to Clarithromycin and Amoxicillin, Sulfamethoxazole - trimethoprim (95%), however, the isolate showed sensitivity to rest antibiotics including Ciprofloxacin, Cefixime, Doxycycline, Cefuroxime, and Cephalexin (100%), Amikacin (95%), Imipenem and Oxacillin (90%). *P. aeruginosa* has recorded resistance to Imipenem, Amikacin and Amoxicillin (100%). In contrast, it showed sensitivity to rest tested antibiotics including Ciprofloxacin, Cefixime and Doxycycline, Clarithromycin, Cefuroxime, and Cephalexin, Sulfamethoxazole trimethoprim (100%). *Staphylococcus schleiferi* showed resistance to Ciprofloxacin and Clarithromycin, Amikacin, Amoxicillin, Oxacillin, Cefixime and Cephalexin (100%), however, the contaminant showed a partial sensitivity to Sulfamethoxazole -trimethoprim and Imipenem (50%) and complete sensitive to the other tested antibiotics including Doxycycline, Cefuroxime (100%). *Staphylococcus aureus* showed full resistance to the most tested antibiotics (100%) with the exception of Imipenem and Sulfamethoxazole-trimethoprim, Doxycycline, Cefuroxime showed (50%) of resistance, table (3).

DISCUSSION

Drinking fresh juices are popular with people all times especially in summer. We often find juice street sellers. People drink these types of juices that overlook microbiology as well as the health standard, therefore high load of distributors and lack of hygienic practicing, so people often get sick. In this study, two main types of juice are used from different area in the city of Benghazi. Most juice samples showed high level of bacterial contamination. The resources of the contamination may be due to contaminated water or the use of ice to dilute juices.

It could be because contamination by unsterilized container, place, air, and preparing bare, hands. Microorganisms can spoil or decompose fruits through damaged surfaces, such as holes, cuts and cracks that occur during growth or harvesting (Mahale et al., 2008). Contamination from raw materials and equipment, additional processing conditions, improper handling, and the spread of unsanitary conditions contribute significantly to the entry of bacterial pathogens into juices prepared from these fruits (Nicolas et al., 2007; Ogodo et al., 2016; Oliveira et al., 2006).This study has revealed that, the most predominant microbes that contaminated the studied juices were *K. pneumoniae* and fecal *E. coli*, according to another study performed by (Fatema et al., 2016)on the microbiological quality assessment of hand-made juice in Dhaka City Street, where they also found that *E. coli* and *K. pneumoniae* have represented the most frequent isolated pathogens. A similar study was performed by (Berhanu et al., 2020) on the microbial quality spectrum of fresh and packaged fruit juices (pineapple and mango) Juices sold in supermarkets and cafes in Gondar city, Northwest Ethiopia, they have showed that the

bacteria that were the source of juice contamination were included *Salmonella sp.*, *Shigella sp.*, *E. coli*, *Pseudomonas sp*., *Klebsiella sp*. and *S. aureus*; whereas (Wedajo & Kadire, 2019) found less frequent bacterial diversity, the juice contamination included just of *Salmonella sp*., *E. coli*, and *S. aureus*. Plate count technique was performed in order to determine the most probable number (MPN) and to estimate the actual bacterial count (cfu/ml) into the testes juices as a food poisoning risk factor probability. According to the standard juice microbial quality, the contamination with fecal *E. coli* was max higher than exceeded limit (0cfu/ 10 ml and 100 ml) of sample, our results showed average contamination of fecal *E. coli* $1.23x10^5$ cfu/ 100 ml of juice, *E. coli* $3.7x10^4$ cfu/ 100 ml, *E. aerogenes* $5.74x10^4$ cfu/ 100 ml, *K. pneumoniae* $9.70x10^4$ cfu/100 ml. The contamination with *Staphylococcus sp.* was within the acceptable bacterial limitation count (less than $5x10^3$cfu/10 ml) compare to the Recommended Gulf Standard. Other similar results obtained by (Reddy et al., 2009), they have shown in the first bacterial isolation from fresh juices a level of bacterial contamination higher than our results even to the acceptable bacterial count, where the coliforms count reached to $1.4x10^5$ cfu/ 100 ml. In contrast, (Babiye, 2017) performed another study demonstrated that, the studied fresh mango and avocado juice samples were contaminated with 150 and 120 coliforms per 100 ml, this is still within the unacceptable bacterial count and probably this conflict with the level of hygiene safety during the juice preparation. (Reda et al., 2017) reported that the presence of thermotolerant fecal *coliform* can be attributed to fecal contamination of the water used to wash utensils, fruits, or transferred directly from the vendors, as well as the environment in which the juice is prepared, and leaving food at room temperature, at this case of these juices, and these can multiply to reach high concentrations, (Andrés et al., 2004) noticed that presence of coliform in fruit juice is not permitted by safe food consumption standard. This result is also in agreement with some other research works performed by (Ahmed et al., 2010; Mahale et al., 2008), where they showed the studied fruit juices were heavily contaminated by *E. coli.* A few reports have shown the prevalence of *staphylococci* in fruit

juice samples (Ahmed et al., 2010; Tambekar et al., 2009) about our study, *staphylococci* we isolated seven samples. Consequently confirmed the presence of *E. coli* and *Salmonella sp*. (Koneman & Allen, 2008) indicative of recent fecal contamination and unsanitary processing (Maturin & Peeler, 2001). An important task of the diagnostic microbiology is the performance of antimicrobial susceptibility testing in order to detect of the significant bacterial isolates that accumulate to the drug resistance pattern.

The results of the antimicrobial susceptibility tests revealed that all the Gram-negative pathogens were resistant to Clarithromycin, Amoxicillin, and Amikacin. For Gram *Staphylococcus spp.* showed the high rate of resistance to Ciprofloxacin, Clarithromycin, Amikacin, Amoxicillin, Oxacillin, Cefixime, and Cephalexin. (Uddin et al., 2017) in report on microbial safety of fruit juices, results from antibiogram test of the isolated microorganisms were *Klebsiella sp*. found to be less sensitive against Sulfamethoxazole -trimethoprim and Ciprofloxacin having 10% and 22% sensitivity, respectively whereas highest susceptibility was found against Nalidixic acid that was 90%. Most potent fecal *coliform*, *E. coli* showed moderate level of sensitivity against Sulfamethoxazole trimethoprim (55%). Additionally, Ampicillin was found to be less effective against *E. coli* as it showed about 95% resistance against it. Pathogenic *Staphylococcus sp.* showed highest resistance against Netilmicin (90%) followed by Ampicillin (84%). Antibiotic sensitivity of *K. pneumoniae* and *S. aureus* were found against Ciprofloxacin, Imipenem, gentamicin, levofloxacin with the exception of Amoxicillin (Sultana et al., 2019).

Table (1): Frequency of bacterial growth according to the areas

Geo-graphic areas	Areas of col-lection	Frist isolation				Second isolation			
		Growth		No Growth		Growth		No Growth	
		No. of collection points	% of growth	No. of collection points	% of no growth	No. of collection points	% of growth	No. of collection points	% of no growth
North	N1	4	100	0	0	4	100	0	0
	N2	4	100	0	0	4	100	0	0
	N3	3	75	1	25	4	100	0	0
	N4	4	100	0	0	4	100	0	0
Total	4	15	93.75	1	6.25	16	100	0	0
South	S1	3	75	1	25	4	100	0	0
	S2	4	100	0	0	1	25	3	75
	S3	4	100	0	0	4	100	0	0
	S4	4	100	0	0	4	100	0	0
Total	4	15	93.75	1	6.25	13	81.25	3	18.75
East	E1	4	100	0	0	4	100	0	0
	E2	4	100	0	0	4	100	0	0
	E3	3	75	1	25	4	100	0	0
	E4	3	75	1	25	4	100	0	0
Total	4	14	87.50	2	12.50	16	100	0	0
Center	C1	4	100	0	0	4	100	0	0
	C2	4	100	0	0	3	75	1	25
	C3	3	75	1	25	4	100	0	0
	C4	3	75	1	25	3	75	1	25
Total	4	14	87.50	2	12.50	14	87.50	2	12.50
Sum	16	58	90.62	6	9.37	59	92.19	5	7.81

Table (2): Diversity of the bacterial growth at different selected cafes and restaurants in the four studied areas

Area	fecal *E. coli*		*E. coli*		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*S. schleiferi*		*S. aureus*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
N1	4	50	1	12.5	0	0	2	25	1	12.5	0	0	0	0
N2	2	25	2	25	1	12.5	0	0	0	0	0	0	2	25
N3	3	37.5	0	0	0	0	5	62.5	0	0	0	0	0	0
N4	3	37.5	1	12.5	1	12.5	1	12.5	0	0	1	12.5	1	12.5
Total	12	37.5	4	12.5	2	6.25	8	25	1	3.13	1	3.13	3	9.37
S1	2	25	0	0	0	0	2	25	3	37.5	0	0	0	0
S2	3	37.5	0	0	1	12.5	1	12.5	0	0	0	0	0	0
S3	1	12.5	0	0	1	12.5	3	37.5	3	37.5	0	0	0	0
S4	0	0	1	12.5	0	0	3	37.5	4	50	0	0	0	0
Total	6	18.75	1	3.13	2	6.25	9	28.13	10	31.25	0	0	0	0
E1	5	62.5	1	12.5	1	12.5	1	12.5	0	0	0	0	0	0
E2	2	25	0	0	0	0	4	50	1	12.5	0	0	1	12.5
E3	3	37.5	2	25	1	12.5	1	12.5	0	0	0	0	0	0
E4	2	25	0	0	0	0	3	37.5	1	12.5	0	0	1	12.5
Total	12	37.5	3	9.38	2	6.25	9	28.13	2	6.25	0	0	2	6.25
C1	2	25	1	12.5	0	0	3	37.5	2	25	0	0	0	0
C2	3	37.5	0	0	1	12.5	3	37.5	0	0	0	0	0	0
C3	1	12.5	0	0	0	0	5	62.5	0	0	1	12.5	0	0
C4	0	0	0	0	1	12.5	5	62.5	0	0	0	0	0	0
Total	6	18.75	1	3.15	2	6.25	16	50	2	6.25	1	3.13	0	0
Sum	36	30.76	9	7.69	8	6.84	42	35.89	15	12.82	2	1.71	5	4.27

Table: (3) Characterization of Antibiotic sensitivity to the isolated bacteria contaminating the natural fresh juices

Antibiotics *vs* Bacteria	fecal *E. coli*		*E. coli*		*E. aerogenes*		*K. pneumoniae*		*P. aeruginosa*		*S. schleiferi*		*S. aureus*	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S)
	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %	(N) %
CIP	(0)0	100	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
CLA	(36)100	(0)0	(9)100	(0)0	(8)100	(0)0	(40)95.2	(2)4.8	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
IMP	(0)0	100	(0)0	(9)100	(0)0	(8)100	(4)9.5	(38)90.5	(15)100	(0)0	(1)50	(1)50	(0)0	(5)100
AK	(5)15.4	(31)84.6	(8)88.8	(1)11.2	(8)100	(0)0	(2)4.8	(40)95.2	(15)100	(0)0	(2)100	(0)0	(5)100	(0)0
AX	(8)23.1	(28)76.9	(9)100	(0)0	(8)100	(0)0	(40)95.2	(2)4.8	(15)100	(0)0	(2)100	(0)0	(5)100	(0)0
OX	(0)0	(36)100	(0)0	(9)100	-	-	(0)0	(42)100	-	-	(2)100	(0)0	(5)100	(0)0
CFX	(0)0	(36)100	(0)0	(9)100	(0)0	(8)100	(4)9.5	(38)90.5	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
CL	(8)23.1	(28)76.9	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(2)100	(0)0	(5)100	(0)0
SXT	(1)3.9	(35)96.1	(0)0	(9)100	(0)0	(8)100	(40)95.2	(2)4.8	(0)0	(15)100	(1)50	(1)50	(0)0	(5)100
DO	(0)0	(36)100	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(0)0	(2)100	(0)0	(5)100
CXM	(0)0	(36)100	(0)0	(9)100	(0)0	(8)100	(0)0	(42)100	(0)0	(15)100	(0)0	(2)100	(0)0	(5)100

CONCLUSION

This study has concluded that, most of the studied fresh juice samples showed bacterial growth, interestingly, this is revealed increase the bacterial contamination that indicating risk factor leading to possibility of illness, therefore, control microbial contamination through the application of the restricted hygiene strategies which needed to be considered in order to provide the markets with safe fresh juice products. Increase the educational awareness on the microbial hazards for the people who working on the juice preparations including food safety and sanitization which assist them to gain responsibility for quality control. Routine microbiological evaluation gives enough information on the expected microbial risk factors.

Duality of interest: The authors declare that they have no duality of interest associated with this manuscript.

Author contributions: Dr. Bozakouk developed the theoretical formalism, Mrs BaLshikh performed the analytic calculations and performed the numerical simulations. Both Dr Bozakouk, Mrs BaLshikh and Dr. Bumadian contributed to the final version of the manuscript. Dr. Bozakouk supervised the project.

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