

Microbiological assessment and detection of drug resistant bacterial isolates in some vended fresh fruit juice samples in Dhaka city, Bangladesh

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Abstract

The consumption of fruit juices may affect both positively and negatively the health status of the consumers. When processed under a hygienic condition it could improve consumers' health by preventing various types of diseases. On the contrary, in absence of good manufacturing practices considering the nutritional affluence of fruit juices makes the product good middling for microbial growth and vehicle of foodborne pathogens. The current study was undertaken to determine the microbiological traits of the vended fruit juices collected from different areas of Dhaka city, Bangladesh. A total of twenty juice samples particularly of four categories such as lemon, sugarcane, malta and watermelon were analyzed for the detection of total viable bacterial load, coliforms, and some other pathogenic bacteria. In these samples, total viable bacteria were within the array between 10^4 - 10^7 CFU/mL. Total coliforms (both *Escherichia coli* and *Klebsiella* spp.) were found in 50% of the samples, alarmingly all exceeding the standard bacteriological limits (1.0×10^4 CFU/mL) recommended for fruit juices. Among the pathogenic bacteria *Salmonella* spp. and *Vibrio* spp. were detected. All these bacterial isolates were detected through standard cultural, microscopic and biochemical tests. A varying degree of drug resistance among the isolates was observed against Amoxicillin, Ampicillin, Azithromycin, Erythromycin, Imipenem and Vancomycin. Overall, the study indicated that the quality of vendor fresh juices was not up to the mark. That's why there is a continuous need for the microbiological assessment of these popular ready-to-drink products otherwise they may create potential health hazards.

1. Introduction

Fruit juice is a well-liked soft drink prepared from the pulp of different types of fresh fruits (Ahmed *et al.*, 2018). The natural flavour in the juice comes from the fruit. Fruit juices have numerous constituents which are valuable for maintaining sound health. In spite of the variation in components, in general, fruits include flavonoid glycosides, dietary fibre, calcium, vitamin C, carotenoids, carotene, phenolic acids, amino acids, aromatic compounds, polyphenols, potassium, vitamin D, a small amount of sodium, and fat (Amarowicz *et al.*, 2009). These ingredients in fruit juices have been found helpful in preventing heart disease, some specific types of cancers, diabetes, cataracts, Alzheimer's disease, and asthma and contribute to forming collagen, cartilage, blood vessels and muscles (Amarowicz *et al.*, 2009; Liu, 2013). Because of their positive effects on human health besides the feelings of getting energized upon consumption, fruit juices have turned out to be popular in

the whole world.

There has been a remarkable increase in consuming both street-based foods and drinks mostly in the low and medium-income countries (LMIC) as very small initial investments are needed by the vendors. Ultimately this makes the price of these types of foods within the reach of people having low socioeconomic status (Sharma *et al.*, 2020). Unfortunately, there have been numerous reports of high morbidity and mortality around the globe each year because of the intake of such contaminated fruit and vegetable juices (Callejón *et al.*, 2015; Kechero *et al.*, 2019).

Vendor-based fruit juices and drinks are obtainable mostly in urban areas of Bangladesh. Here various carbonated soft drinks, tea, coffee, fruit juices and sherbets are enjoyed on the streets or by the roadside shops. Particularly in the summer season, a large part of the population of all income and age groups drinks these

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fresh squashed juices (Ahmed *et al.*, 2009). Nevertheless, deficient education among the vendors on food safety and hygiene practices generates scopes of elevated microbial contamination of the fruit juices. Frequently detected microorganisms in street juice comprise both Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Salmonella enterica* serovar Typhi, *Pseudomonas* spp., *Staphylococcus aureus* and *Vibrio cholerae*. These pathogens are responsible for producing various diseases such as typhoid fever, food poisoning, gastroenteritis, enteric fever and diarrhea, which may lead to life-threatening conditions and such situations are observed worldwide (Aneja *et al.*, 2014; Verma and Gaur, 2017).

A common problem regarding medication failure due to the rise of antibiotic resistance has recently gone far, posing severe public health threats in developing countries like Bangladesh (Tabassum and Uddin, 2016). The reason for such drug resistance has already been well known, contributed by the natural transfer of the drug-resistance genes accompanied by non-prescribed antibiotics (Molton, 2013). A number of studies have highlighted the incidence of antibiotic-resistant bacteria in the samples of apple and orange juice (Tadesse *et al.*, 2018; Sarker *et al.*, 2018; Mandal, 2018; Kebede *et al.*, 2018).

Based on these facts, the present study first assessed the microbiological quality of these refreshing drinks available within the Dhaka metropolis and further chalked out the antibiotic resistance pattern of pathogens isolated from these samples.

2. Materials and methods

2.1 Sample collection and processing

A total of twenty vended fruit juice samples of four categories such as lemon, sugarcane, malta and watermelon were collected from street-side shops located in various areas of Dhaka city such as Malibagh, Shantinagar, Khilgaon, Farmgate and Badda areas. Samples were collected from November 2020 to February 2020 and brought to the laboratory as soon as possible according to the method suggested by FDA (2013). Samples were subjected to serial dilutions up to 10^{-4} prior to the calculation of bacterial load.

2.2 Quantification of total viable bacteria and coliforms

The spread plate method was used for the determination of the total viable count using nutrient agar. This media is unique and nonselective to count bacteria and fungi. The samples were diluted at 10-fold dilution up to 10^{-4} according to American Public Health Association (APHA) sample dilution guidelines (APHA,

1998). For the enumeration of total viable bacterial count (TVBC), 0.1 mL of each sample from the dilutions 10^{-2} and 10^{-4} were spread onto the nutrient agar (NA). The NA plates were incubated at 37°C for 24 hrs. For the estimation of total coliforms, 0.1 mL from each of the 10^{-2} and 10^{-4} dilutions of all samples were spread onto the MacConkey agar followed by incubation at 37°C for 24 hrs (APHA, 1998; Cappuccino and Sherman, 2014).

2.3 Enrichment methods

For the isolation and quantification of *Salmonella*, *Shigella* and *Vibrio* spp., the enrichment procedure was applied. For this purpose, 1 mL of samples were transferred into 9 mL of selenite cysteine broth (SCB) and alkaline peptone water (APW) for the enrichment of *Salmonella*, *Shigella* and *Vibrio* spp., consecutively and incubated at 37°C for 4-6 hrs. An aliquot of 0.1 mL of each of the samples from 10^{-2} and 10^{-4} dilutions was spread onto Salmonella-Shigella (SS) agar and thiosulfate citrate bile salt sucrose (TCBS) agar for the isolation of *Salmonella* spp. and *Shigella* spp. and *Vibrio* spp., consecutively. Followed by incubation at 37°C, the appearance of typical colonies was noticed within 24-48 hrs (Alfrad, 2007).

2.4 Confirmative biochemical identification

Finally, a series of biochemical tests were conducted to confirm the identity of all the isolates. Some biochemical tests such as Methyl Red-Voges-Proskauer (MR-VP) test, Citrate test, Indole test, Urea test and Triple sugar iron (TSI) tests were performed for the identification of bacterial isolates following standard protocols (Cappuccino and Sherman, 2014).

2.5 Study of antibiogram

Isolates were tested against ten common antibacterial drugs by disc diffusion assay (Bauer *et al.*, 1966) on Mueller-Hinton Agar (Difco, Detroit, MI) with antibiotic discs (Neo-Sensitabs, Rosco, Denmark). Briefly, a single colony of each isolate was introduced into 2 mL of Mueller-Hinton broth, incubated for 4 hrs, and the culture turbidity was then adjusted to a 0.5 McFarland standard. Sterile cotton swabs were dipped into the suspensions and were spread evenly over the entire agar surface. Antibiotics impregnated discs (Amoxicillin 30 µg, Ampicillin 10 µg, Azithromycin 30 µg, Chloramphenicol 30 µg, Ciprofloxacin 5 µg, Erythromycin 30 µg, Gentamycin 30 µg, Imipenem 30 µg, Tetracyclin 30 µg, Vancomycin 30 µg) were then placed onto the surface of the inoculated plates. After incubation, diameters of the zones of inhibition were measured and interpreted as susceptible, intermediate, and resistant.

3. Results and discussion

There is a preference for choosing fresh fruit juices consumers because of possessing numerous vitamins and natural minerals and are handy to all. However, the presence of a profound load of microorganisms in these types of juices may become the reason for producing severe illnesses (Asghar *et al.*, 2018). In the present study, the objective was to investigate the microbiological quality of four categories of vended fresh fruit juice samples consumed by the inhabitants of Dhaka city, Bangladesh. Therefore, the current study endeavoured to assess the existence of microbes in such types of food items particularly by detecting total viable bacterial count and total coliform count as well as monitoring the presence of various pathogenic organisms such as *Vibrio* spp., *Salmonella* spp. and *Shigella* spp. Besides the drug resistance profile of the presumptive bacterial isolates was also analyzed.

3.1 Isolation and enumeration of microorganisms

3.1.1 Total viable bacterial count

In this study, the total viable bacterial counts varied from 2.5×10^4 CFU/mL to 1.8×10^7 CFU/mL which was observed in watermelon-17 and Lemon-1 samples respectively (Table 1). This finding was quite similar to the study done by Noor *et al.* (2013). According to the

Gulf Standard No. 1016/2000 (Emirates Authority for Standardization and Metrology (ESMA) United Arab Emirates, 2000), the maximum bacterial load permitted is 1.0×10^4 CFU/mL in the case of total viable bacteria (Table 2). From Figures 1a-1d it was evident that most of the fruit juices from all the four categories failed to meet this criterion. Similarly, Asghar *et al.*, (2018), demonstrated that nearly 80% of fresh vended fruit juice samples had a higher value and 20% of samples contained less TVBC of maximum bacterial load as per the Gulf Standard No. 1016/2000. In addition, a higher value of TVBC might be an indication that the fresh juices were prepared in an unhealthy environment (Emirates Authority for Standardization and Metrology (ESMA) United Arab Emirates, 2000).

3.1.2 Coliform, *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. count.

Coliform was detected in ten out of twenty samples of which the highest count was 1.76×10^5 CFU/mL in lemon-1 whereas the lowest count was 1.5×10^3 CFU/mL in sugarcane-7 (Table 1). The detection of coliform bacteria, especially *E. coli* and *Klebsiella* spp. in these samples showed the possibility of the presence of fecally contaminated microorganisms. Alarmingly *Salmonella* spp. was present in two and *Vibrio* spp. in 5 samples

Table 1. Microbiological assessment of different types of fruit juice samples

Sample no.	TVBC (CFU/mL)	TCC (CFU/mL)	<i>Salmonella</i> spp. (CFU/mL)	<i>Vibrio</i> spp. (CFU/mL)
Lemon-1	1.8×10^7	1.76×10^5	0	0
Lemon-2	8.16×10^6	2.5×10^3	1.2×10^4	0
Lemon-3	2.72×10^6	0	0	1.5×10^4
Lemon-4	4.9×10^4	0	0	0
Lemon-5	3.9×10^5	0	0	0
Sugarcane-6	8.99×10^6	6.8×10^4	0	8×10^3
Sugarcane-7	5.8×10^5	1.5×10^3	0	0
Sugarcane-8	3.94×10^6	3.7×10^3	7×10^3	0
Sugarcane-9	1.56×10^6	5.9×10^4	0	1.9×10^4
Sugarcane-10	8.9×10^5	0	0	0
Malta-11	3×10^4	0	0	0
Malta-12	4×10^4	0	0	0
Malta-13	2.8×10^5	0	0	0
Malta-14	6.8×10^5	7×10^3	0	0
Malta-15	5×10^5	2×10^3	0	8×10^3
Watermelon-16	2.4×10^5	0	0	0
Watermelon-17	2.5×10^4	0	0	0
Watermelon-18	3.2×10^4	0	0	0
Watermelon-19	4.8×10^5	4×10^3	0	0
Watermelon-20	4.4×10^5	6×10^3	0	4×10^4

Each experiment of this study was performed three times and the results were reproducible. One representative data was shown here. TVBC: Total viable bacterial count, TCC: Total coliform count, *Shigella* spp. and fecal coliforms were completely absent in all the samples being studied.

Table 2. Biochemical results of *Salmonella* spp., *Klebsiella* spp., *E. coli* and *Vibrio* spp. according to the Gulf Standard No. 1016/2000 (Emirates Authority for Standardization and Metrology (ESMA) United Arab Emirates, 2000)

Identified Microorganisms	TSI				MIU			NO ₃	MR	VP	Citrate	Catalase	Oxidase
	Slant	Butt	H ₂ S	Gas	Motility	Indole	Urea						
<i>Salmonella</i> spp.	R	Y	-	-	+	+	-	+	+	-	+	+	-
<i>Klebsiella</i> spp.	Y	Y	+	-	-	-	+	+	-	+	+	+	-
<i>E. coli</i>	Y	Y	+	-	+	+	-	+	+	-	-	+	-
<i>Vibrio</i> spp.	R	Y	-	-	+	+	-	+	+	-	-	+	+

TSI: Triple Sugar Iron Test, Y: Yellow (Acid), R: Red (Alkaline), MR: Methyl red and, VP: Voges-Proskauer

(Table 1), wherein both cases crossed the standard limit as recommended by FDA, (2013) (Figure 1a-1d). Standard protocols were followed for the presumptive detection of the bacterial isolates (Table 3). A high load of such pathogens was also observed in the studies conducted by Sharma *et al.* (2020) in India and Islam *et al.* (2014) in Dhaka, Bangladesh. Fortunately, *Shigella* spp. and fecal coliforms were totally absent in the samples being investigated. The presence of *E. coli*, *Klebsiella* spp., *Salmonella* spp. and *Vibrio* spp. in a number of samples was an indication of looming health hazards as these organisms have pathogenic potential

and have drawn in foodborne diseases (Uddin *et al.*, 2020). The possible reasons for these conditions were largely due to the fact that street vendors were generally unaware of good hygienic practices (GHP), food regulations and the factors responsible for producing diarrheal diseases. All these parameters increase the possibility of contamination in street foods. Besides the vendors are also in short of supportive services such as good and sufficient quality water supply and waste dumping facilities (Malik *et al.*, 2020).

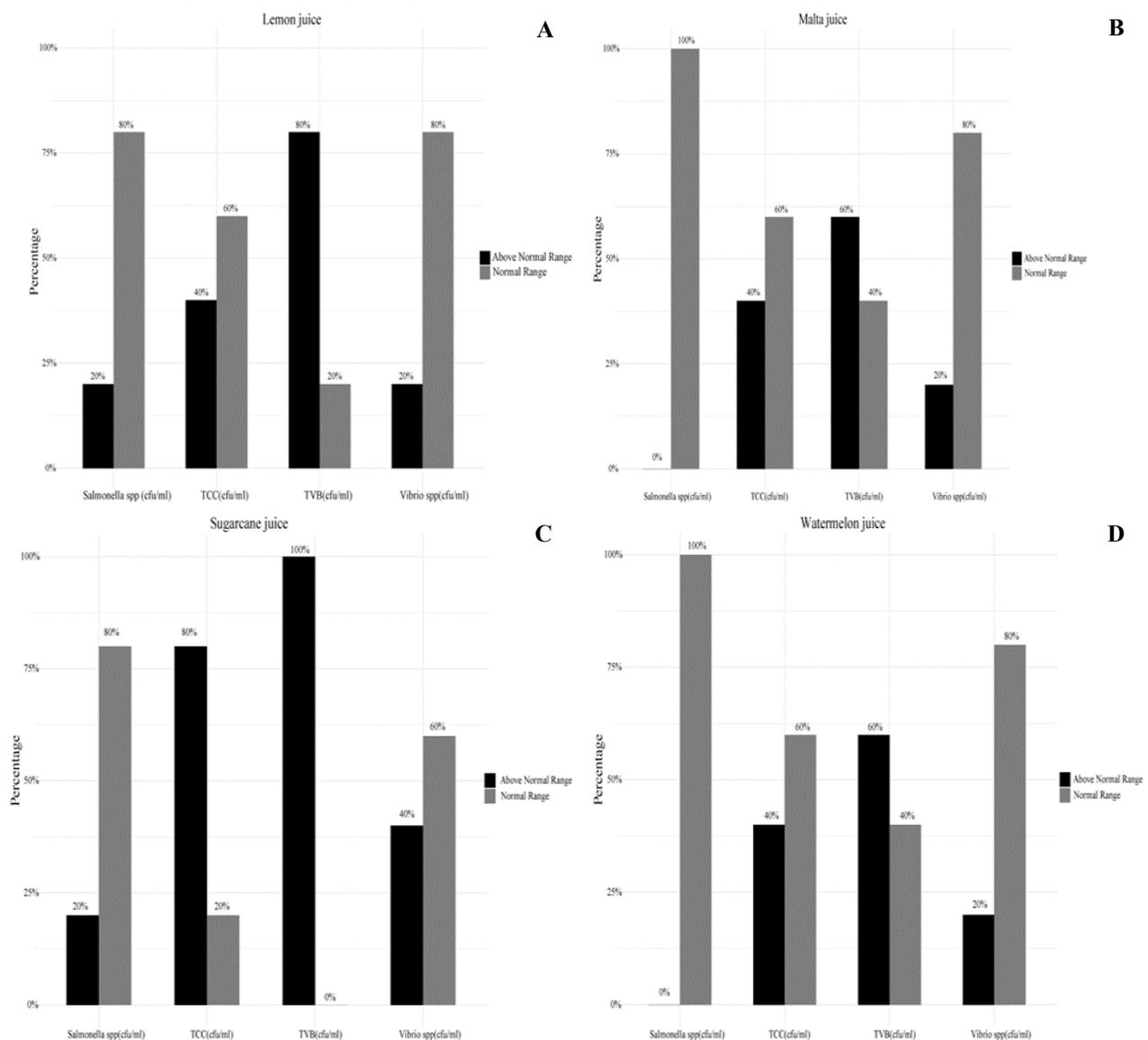


Figure 1. Microbial load in different juices A. lemon B. Malta C. Sugarcane D. Watermelon

3.2 Antibiogram of the isolates

Food can be contaminated with antibiotic-resistant bacteria. It is one of the foremost intimidations to public health (Tabassum and Uddin, 2016). Nowadays one of the major global concerns is the development of antimicrobial resistance by pathogenic bacteria which is ultimately causing a problem in the treatment procedure (Uddin et al., 2011). In this study, the Kirby-Bauer disk diffusion test was performed to resolve the antibiogram profile of the presumptively identified bacterial isolates towards some frequently advised antibiotics. The pattern of antibiotic resistance of *Vibrio* spp., *Salmonella* spp., *E. coli* and *Klebsiella* spp. has been depicted in Figures 2a-2d. Both the isolates of *Salmonella* spp. showed

100% resistance against Amoxycillin, Ampicillin, Azithromycin, Erythromycin, Imipenem and Vancomycin. *E. coli* displayed a variable degree of resistance against Amoxycillin, Ampicillin and Azithromycin. On the other hand, both *Vibrio* spp. and *Klebsiella* spp. were found to be almost sensitive against all the studied antibiotics except Tetracycline (33%) and Azithromycin (50%), respectively. Similar types of results were observed in some other studies conducted by Noor et al. (2013) in Dhaka, Bangladesh and Sharma et al. (2020) in India.

4. Conclusion

The current study was demonstrated to chalk out the

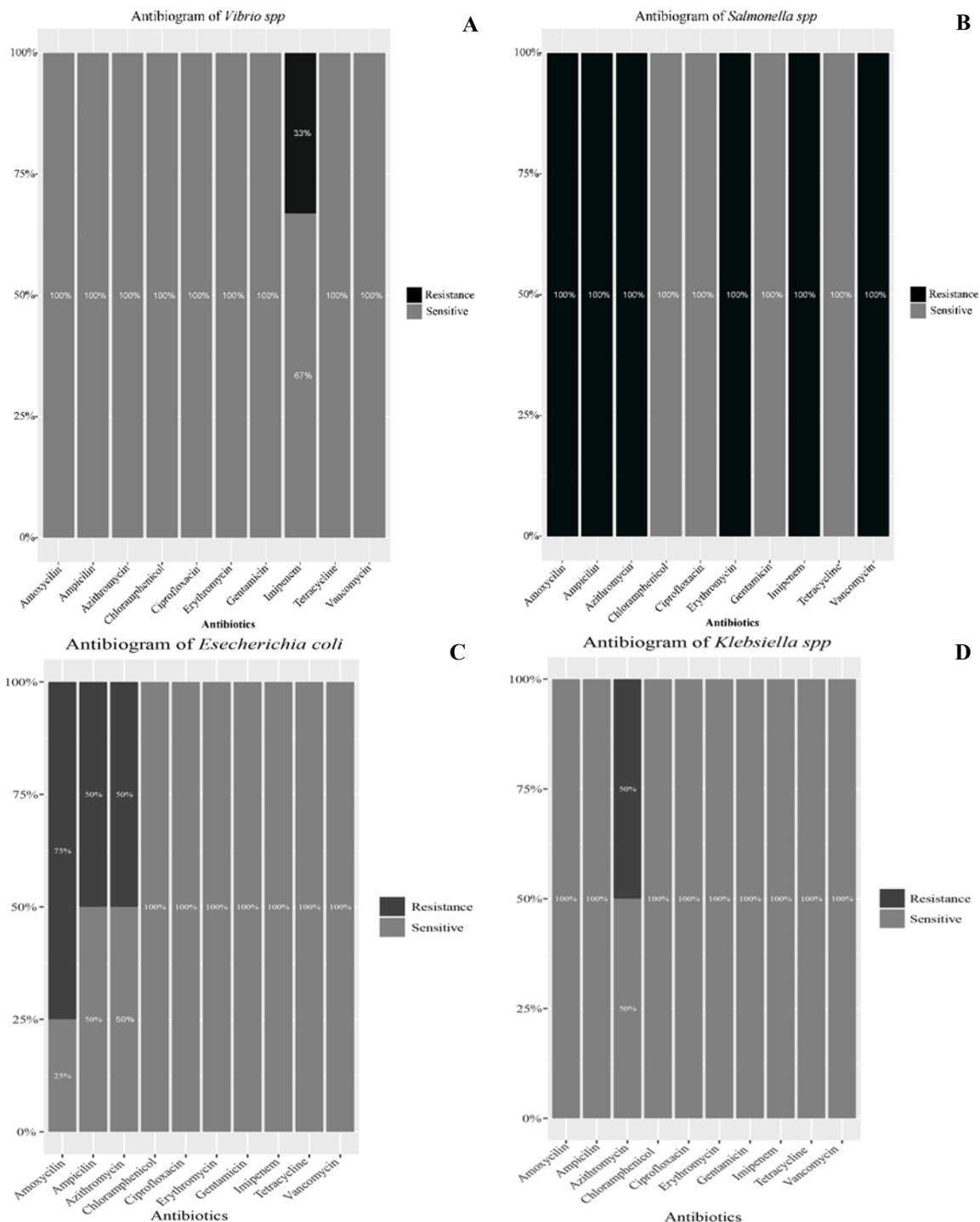


Figure 2. Antibiogram profile of A. *Vibrio* spp. B. *Salmonella* spp. C. *E. coli*. D. *Klebsiella* spp.

microbiological attribute of fresh fruit juice collected from the vendors of different important locations in Dhaka, Bangladesh. The results revealed the presence of a high microbiological load in the local fruit juice samples. Based on the Gulf Standard No. 1016/2000 (Emirates Authority for Standardization and Metrology (ESMA) United Arab Emirates, 2000) and FDA guidelines (2013), most of the samples harbored excessive microbial load and were unfit for consumption. In addition, antibiotic resistance in the strains of *E. coli*, *Salmonella*, *Klebsiella* and *Vibrio* spp. have been isolated from fruit juice samples against some clinically essential antibiotics such as Amoxicillin, Ampicillin, Azithromycin, Erythromycin, Imipenem and Vancomycin. Thus, there is an imperative necessity for all the concerned governmental and non-governmental organizations to adopt protective measures as soon as possible and devise a committed strategy to develop the microbiological safety outline for producing healthy and pathogen-free street-vended fruit juices.

Conflict of interest

The authors have declared no conflict of interest.

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