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Bacterial contamination and associated antibiotic resistance in Fresh Produce : A comparative study between retail outlets and local markets

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Abstract

The consumption of food is essential for human survival, but the presence of bacterial pathogens in fresh produce can pose significant risks to public health by causing severe foodborne and enteric diseases. With this concern in mind, our study aimed to assess the contamination levels of specific bacterial species, *E. coli*, *Salmonella* spp., and *Staphylococcus aureus*, in various fresh produce items (pear, guava, apple, carrot, turnip, and cucumber) sourced from both local markets and retail outlets. Additionally, we investigated the antibiotic resistance of these bacterial species.

Our findings revealed the presence of the selected bacterial species, *E. coli*, *Salmonella* spp., *S. aureus*, in all samples obtained from both retail outlets and local markets. Notably, from among the local markets, pear exhibited the highest bacterial load (4.29×10^5 CFU/gm) compared to guava (3.16×10^5 CFU/gm) and apple (2.83×10^5 CFU/gm). Among vegetables, carrots demonstrated the highest contamination (4.27×10^5 CFU/gm) compared to turnip (3.38×10^5 CFU/gm) and cucumber (2.45×10^5 CFU/gm). Similar pattern was also obtained from among the common retail outlets. Pear exhibited itself as the most contaminated fruit (2.13 × 10^5 CFU/gm), followed by guavas (1.43 × 10^5 CFU/gm) and apples (0.882 × 10^5 CFU/gm). Among vegetables, carrots exhibited the maximum contamination (2.31 × 10^5 CFU/gm) compared to turnip (1.05 × 10^5 CFU/gm) and cucumber (0.689 × 10^5 CFU/gm).

Comparing the presence of various microbes among the available fresh produce, it was observed that *E. coli* consistently showed the highest abundance amongst all bacterial species cultivated from the fresh produce of both local markets and retail outlets. Alarmingly, isolated bacterial species also displayed substantial resistance to commonly used broad-spectrum antibiotics, emphasizing the inherent health risks posed by bacterial pathogens present on raw fresh produce. This resistance underscores the potential threat to consumer health upon the consumption of such contaminated raw produce. We provide scientific evidence to prove that fresh produce may serve as a vehicle of foodborne exposure to antimicrobial-resistant bacteria. It's a global alarming situation, which needs immediate attentions and steps for control. Concerted efforts should be made to mitigate the resistant bacteria at all stages of the food chain, from production to consumption. To achieve this goal, good agricultural practices should be employed and regulatory authorities need to address this problem immediately.

Keywords: Fresh produce, bacterial contamination, antibiotic resistance, *E. coli*, *S. aureus*, *Salmonella* spp.

Introduction

Fresh produce is an important healthy food option consisting high amounts of vitamins B, C, and K, minerals like calcium, potassium, and magnesium, and also includes dietary fibre (Yahia et al., 2019). Developing countries like India rely on these fruits and vegetables as best source of micronutrients required for a healthy and balanced diet that prevents chronic diseases such as heart disease, cancer, diabetes, and obesity (Septembre-Malaterre et al., 2018). However, consuming the produce in raw state, might pose yet another health risk of acquiring food-borne illness associated with the contaminating microbes that might be present on them. Fresh produce becomes contaminated by microbes during its production, storage, packing, processing, and transportation stages which originates from municipal waste, manure soil amendments, irrigation water, and wild animal intrusion (Sapers and Doyle, 2014; He et al., 2020, Allende and Monaghan, 2014; Jian et al., 2015).

In addition to the microbial contamination, agricultural use of antibiotics as growth supplements, further contributes towards contaminating fresh produce. These antibiotics enter the food chain by pressurizing resistant bacteria to grow and eventually contaminate the fresh produce (Roe and Pillai, 2003). Food contaminated with the antibiotic resistant bacteria may serve as a reservoir of resistant genes which may be horizontally transferred to the microbiota of the gut upon its consumption (Larsson and Flach, 2022). Studies have indicated the presence of not only antibiotic resistant bacteria (ARBs), but also antibiotic-resistant genes (ARGs), and antimicrobial residues in produce which might eventually lead to non-availability of useful antibiotics against dreadful bacteria in near future (Holzel et al., 2018; Ulger et al., 2018; Founou et al., 2021, Chinemerem et al., 2022, Abatcha et al., 2018; Freitag et al., 2018; Janalikova et al., 2018; Usui et al., 2019). Research indicates that the indiscriminate use of antibiotics used during the agricultural practices have led to the emergence of resistant bacteria, particularly Enterococcus spp., Campylobacter spp., Salmonella spp., Escherichia coli O157:H7, Stapholococcus aureus, Bacillus cereus etc. (Tueber, 1999; Samtiya et. al. 2022). It has become extremely important to conduct systematic studies of microbial load on fresh produce and associated antibiotic resistance so as to ensure that the food borne illness are treated well by the physicians and we are not heading towards an era of no antibiotics. Gathered information would be helpful to mitigate the transfer of ARB/ARGs through fresh produce to human being. Moreover, foodborne infection risk can be minimized by empowering consumers with information about bacterial contamination and antibiotic resistance in fresh produce.

The present study is an attempt to assess the bacterial contamination of fresh produce available from the local and retail outlets of New Delhi, India. A comparative analysis of bacteria present on fresh produce was also undertaken in order to ascertain the predominant bacterial load among the selected retail outlets and local markets. It was observed that E. coli was more prevalent as compared to the other bacteria. Further, antimicrobial resistance profiling of the isolated bacteria from available fruits (pear, guava, apple) and vegetables (carrot, turnip, and cucumber) was also performed. From the collected samples, E. coli, Salmonella spp., and S. aureus bacteria were chosen for antimicrobial resistance profiling assay and nearly all the samples tested positive for known antibiotics. The present study provides a preliminary data for presence of antimicrobial resistance among the bacteria harboring the fresh produce. The exact source of this resistance needs to be further investigated. The data so collected and published can reach food handlers and consumers so that they maintain hygienic practices while handling fresh fruits and vegetables..

Materials and methods

Sample Collection

Three samples from each fresh fruits (Apples, Pear, Guava) and fresh vegetables (Carrot, Turnip, Cucumber) were selected from retail outlets and local markets of Delhi, India, according to their seasonal availability in the market at the time of the study. A total of three retail outlets and three local markets were considered. A total of 108 samples were purchased from retail outlets (54) and local markets (54) between the period of January 2018-December 2021.

Transportation of Samples

Each sample was collected with a sterile glove and kept in a sterile poly bag. A sterile environment was maintained during transport and bacterial analysis was done within six hours of collection.

Samples processing

25 ml of 2% sterile peptone water (Himedia RM001) was added into the sterile bag containing the peel and was vortexed for 5 minutes at 5000 rpm. The resulting solution was examined for the presence of different bacterial population and bacterial load.

Isolation of bacterial species

Three bacterial species namely E. coli, Salmonella spp. and S. aureus, most commonly associated with food-borne diseases, were selected for the study and isolated using different selective media:, Eosin methylene blue agar (E. coli), MacConkey agar (Salmonella spp) and Mannitol salt agar (S. aureus). 100 µl of the dislodged peptone water (in which the required sample was submerged for 20min) was inoculated on the surface of each selective media plate and spreading was performed as per the standard procedures. The plates were incubated for 16-17 hours at 37°C and later number of colonies were counted.

Antibiotic susceptibility testing

Each of the selected bacterial group was screened for antibiotic susceptibility profiling using the Kirby Bauer disk diffusion method. From each of the fresh produce sample, one isolate of each bacterial species, viz. E. coli, Salmonella spp. and S. aureus was considered in the group and was subjected to the Kirby Bauer disk diffusion method. As per the performance standards of the Clinical and Laboratory Standards Institute 2018 (CLSI) for the determination of the resistance of bacteria against selected antibiotics (as mentioned in the Table 1), the turbidity of all bacterial cultures was maintained at 0.5 McFarland units. Accordingly, an isolated single colony of each of the bacterial species was picked from the selective media plate and immersed for few minutes in Mueller Hinton Broth for further processing (MHB, Hi-Media Laboratories Pvt. Ltd) so that the cultures reached a turbidity of 0.5 McFarland units. A cotton swab was suspended in the respective culture tube of bacterial species and excess broth was removed from the swab by pressing the swab against the test tube wall. Swab was applied uniformly on the Mueller Hinton Agar (MHA) plate and the plates were kept in an upright position for 3 min. Using a sterile forcep selected antibiotic disks were placed on the surface of cultured MHA plates. The plates were incubated for 16-18 hours at 37°C. Diameter of the zone formed on the MHA plate was analyzed after incubation according to the guidelines of CLSI performance standards (https://file.qums.ac.ir/repository/mmrc/CLSI-2018-M100-S28.pdf).

Escherichia coli	*Salmonella* spp.	*Staphylococcus aureus*
AMP (10 µg)	AMP (10 µg)	PEN (10 µg)
PIT (10 µg)	PIT (10 µg)	ERY (15 µg)
CAZ (30 µg)	CAZ (30 µg)	CD (2 µg)
MRP (10 µg)	MRP (10 µg)	NIT (300 µg)
TET (30 µg)	TET (30 µg)	TET (30 µg)
CIP (5 µg)	CIP (5 µg)	CIP (5 µg)
IMP (10 µg)	IMP (10 µg)	CX (30 µg)
CHL (30 µg)	CHL (30 µg)	LZ (30 µg)

Table 1: List of antibiotics used for determining resistance of selected bacterial species from different fresh produce samples.

Abbreviations used : AMP: Ampicillin, CHL: Chloroamphenicol, CAZ: Ceftazidime, CD: Clindamycin, CIP: Ciprofloxacin, CX: Cefoxitin, ERY: Erythromycin, IMP: Imipenam, MRP: Meropenam, LZ: Linezolid, NIT: Nitrofurantoin, PEN: Penicillin, PIT: Piperacillin+Tazobactum, TET: Tetracycline.

Statistical analysis

SPSS software program (SPSS 16.0 version available for Windows) was used, to analyse correlation between different fresh produce and bacterial load,. The comparative assessment of mean bacterial counts from fresh produce collected from different outlets was performed using Tukey's range test ($p < 0.05$). One-way and two-way ANOVA was used to determine significant difference ($p<0.05$) of different bacterial species within the same fruit and significant difference ($p<0.05$) of the same bacterial species between different fruits.

Result

Abundance of selected bacterial species on fresh produce

All the samples were found to be contaminated with the selected bacterial species E. coli, Salmonella spp., S. aureus. The mean bacterial load on different fresh produce (pear, guava, apple, carrot, turnip, and cucumber) procured from local markets and retail outlets have been represented in Figure 1. In local markets, pear was found to contain higher bacterial load (4.28×105 CFU/gm) than guava (3.16×105 CFU/gm), and apple (2.83×105 CFU/gm) (Figure 1). Among vegetables in local markets, carrot showed higher bacterial load (4.27 ×105 CFU/gm) than turnip (3.38×105 CFU/gm), and cucumber (2.45×105 CFU/gm). In retail outlet, pear showed maximum bacterial count (2.13×105 CFU/gm), followed by guava (1.43 ×105 CFU/gm), and apple (0.882×105 CFU/gm) (Figure 2). This showed that local markets contain more bacterial contamination than retail outlets. Among vegetables, carrot showed higher bacterial count (2.31×105 CFU/gm) than turnip (1.05×105 CFU/gm), and cucumbers (0.689×105 CFU/gm). Among the selected bacterial species, E. coli contamination was found to be highest on pears, guavas, apples, carrots, turnips and cucumbers than Salmonella spp., and S. aureus, respectively in both local markets and retail outlets (Figure 3). Two-way ANOVA test revealed the significant difference among selected bacterial species within the same fruit and significant difference ($p<0.05$) of the same bacterial species between different fruits (Figure 1). However, the mean count comparison of selected bacterial species (E. coli, Salmonella spp., and S. aureus) between different fresh produce (pear, guava, apple, carrot, turnip, and cucumber) of local market and retail outlet showed no statistical significance (Figure 3).

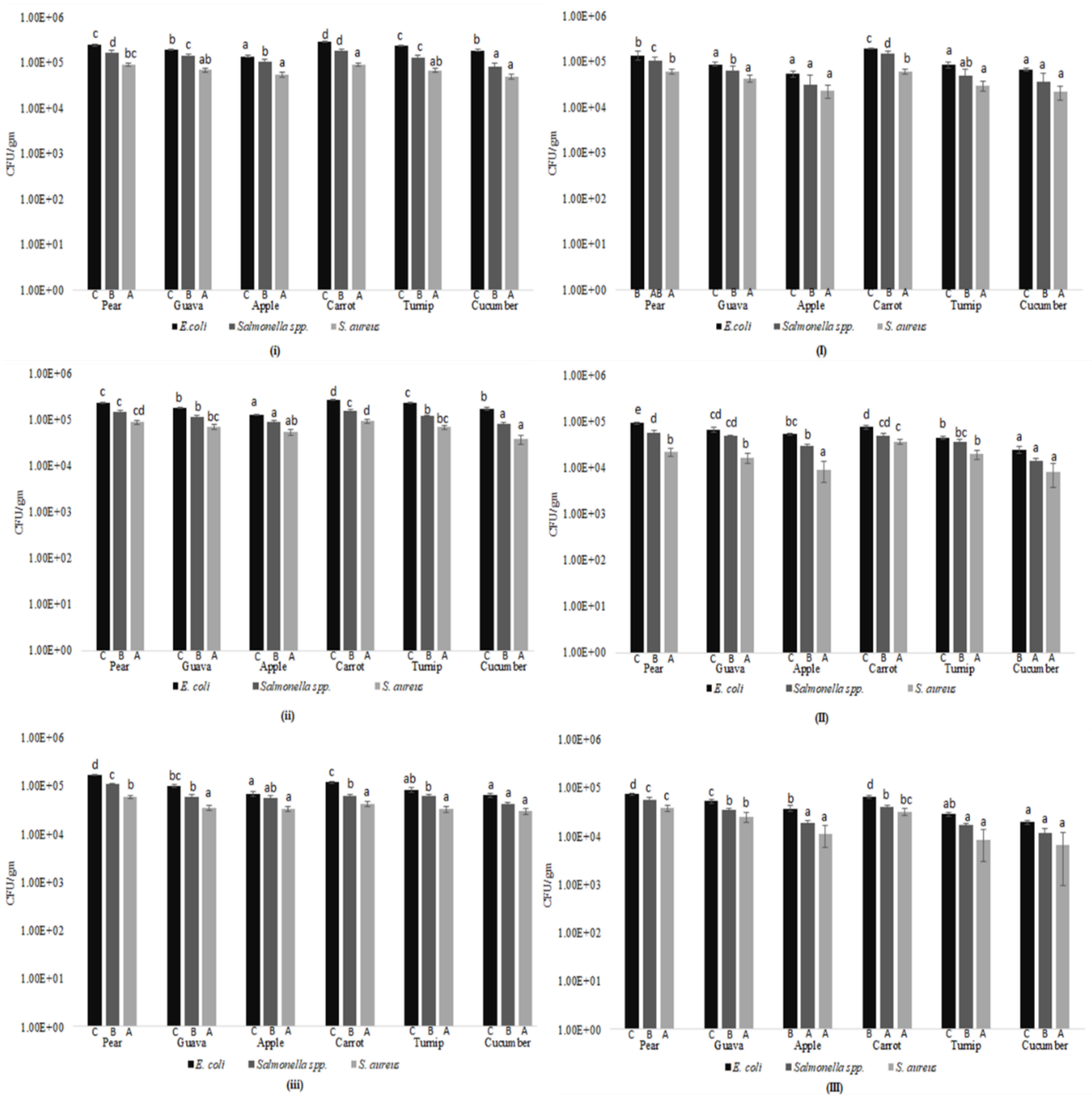

Figure 1: Mean bacterial load (CFU/gm) on different fresh produce (pear, guava, apple, carrot, turnip, and cucumber) procured from local markets (i, ii, iii) and retail outlets (I, II, III). Error bars represent standard deviation (*n*=3) and two-way ANOVA was used to evaluate the statistical significance. A significant difference ($p<0.05$) of different bacterial species within the same fruit is represented by capital letters (e.g., A, B etc.), while a significant difference ($p<0.05$) of the same bacterial species between different fruits are represented by small letters (e.g., a, b).

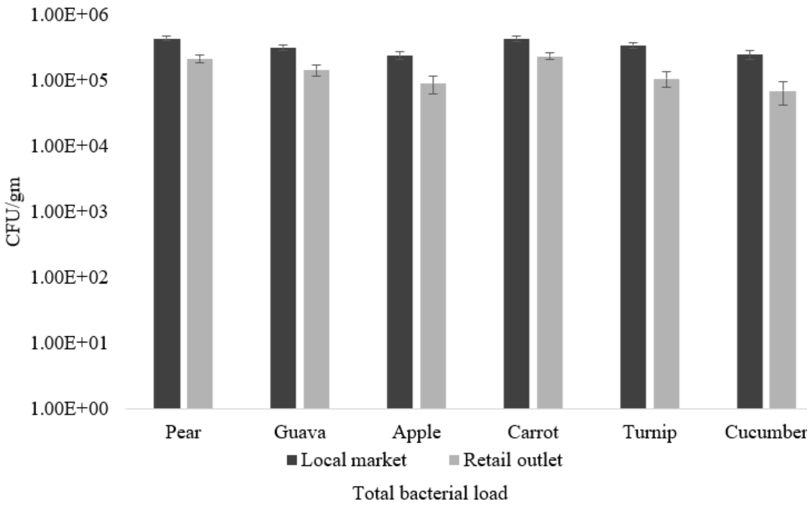

Figure 2: Total bacterial load (CFU/gm) between different fresh produce (pear, guava, apple, carrot, turnip, and cucumber) of local market and retail outlet.

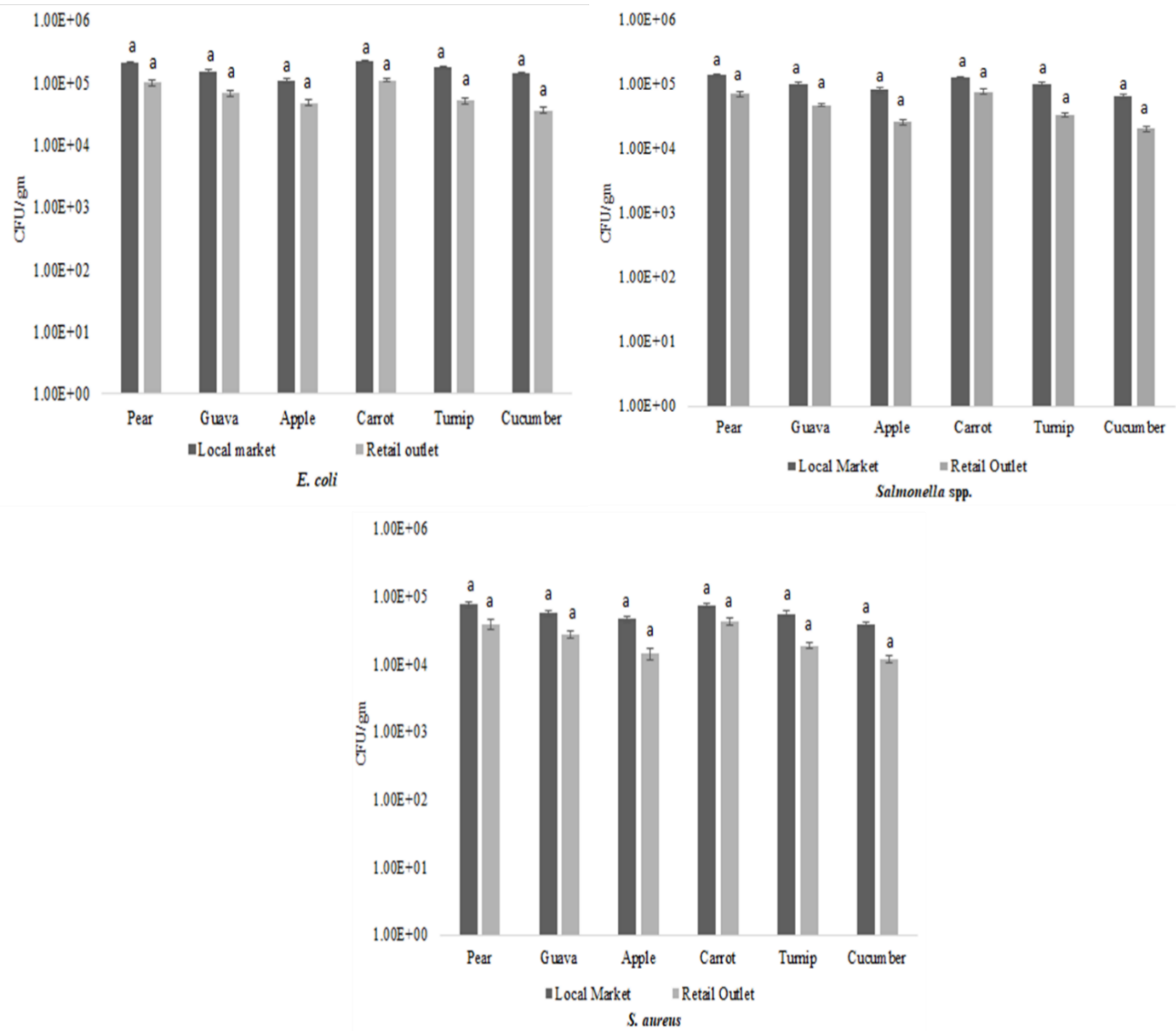

Figure 3: Mean count (CFU/gm) comparison of selected bacterial species (*E. coli*, *Salmonella* spp., and *S. aureus*) between different fresh produce (pear, guava, apple, carrot, turnip, and cucumber) of local market and retail outlet.

Antibiotic Susceptibility Profiling

Each selected bacterial group (n = 108) was tested for antibiotic susceptibility using selected antibiotics. (Table 2, Figure 4). All the selected bacterial species showed resistance to broad spectrum antibiotics to a great extent. E. coli showed resistance (%) to ampicillin (72.2), tetracycline (12.9), ciprofloxacin (6.4), chloramphenicol (4.6), and ceftazidime (3.7). Salmonella spp. was found to be resistant (%) to ampicillin (76.8), tetracycline (22.2), ciprofloxacin (6.4), chloramphenicol (6.4), and ceftazidime (1.8). In the case of S. aureus, the resistance (%) of penicillin was maximum (62), followed by cefoxitin (50), erythromycin (36.1), tetracycline (11.1), ciprofloxacin (7.4), ceftazidime (3.7). Methicillin Resistant Staphylococcus aureus (MRSA) (54/108) was also found to be present on fresh produce. Hence among the selected broad-spectrum antibiotics, E. coli and Salmonella spp. showed the highest rate of ampicillin resistance (%) and S. aureus showed the highest rate of penicillin resistance (%)

Table 2. Number of bacterial isolates resistant against selected antibiotics

(n=108: Retail outlets-54 and Local markets-54)

Bacterial spp.	AMP	TET	CIP	CHL	CX	CAZ	PEN	ERY	CD	IMP	MRP	LZ
E. coli	78	14	7	5	-	4	-	-	-	0	0	-
S. aureus	-	12	8	-	54	-	67	39	4	-	-	0
***Salmonella* spp.**	83	24	7	7	-	2	-	-	-	0	0	-

Please refer Table 1 for the abbreviations.

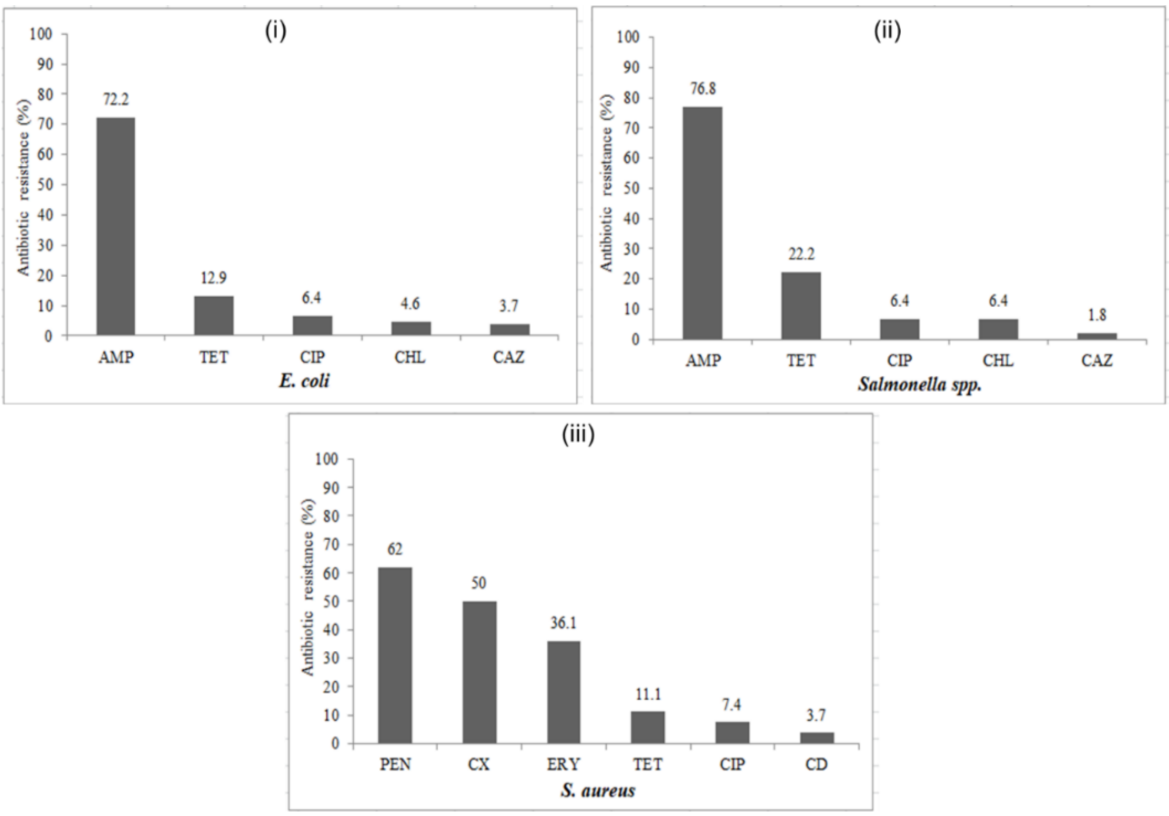

Figure 4. The percentage (%) of antibiotic resistant bacterial colonies (*n*=108) isolated from fresh produce of different outlets and local markets. Please refer Table 1 for the abbreviations.

Discussion

The investigation highlights the recognition of fresh produce as potential carriers of foodborne pathogens, displaying varying degrees of drug resistance (Holzel et al., 2018; Iseppi et al., 2018). The bacterial contamination observed on these raw fruits and vegetables is multifaceted and can be attributed to several factors, including water quality, hygiene practices, transportation conditions, market environment, storage methods, processing techniques, and preparation levels. The ramifications of such contamination are substantial, as raw produce contaminated with microbial pathogens can lead to food poisoning and other enteric diseases (Johnston et al., 2005; Saksena et al., 2019).

Moreover, the study draws attention to the widespread use of antibiotics in agriculture, a practice that may heighten the risk of bacteria on plant surfaces developing resistance to antibiotics (Zalewska et al., 2021). Antibiotics, in addition to controlling bacterial diseases in plants, are employed as soil fertilizers, particularly through the use of animal manure. This introduces the potential for the soil to become a reservoir for bacteria and genes resistant to antibiotics (Iseppi et al., 2018).

This study revealed that, all examined fresh produce samples, sourced from both local markets and retail outlets in the Delhi-NCR region, were found to be contaminated with E. coli, Salmonella sp. and S. aureus. Notably, the contamination levels were higher in local markets compared to retail outlets (Berger et al., 2010; Holzel et al., 2018). The prevalence of these bacterial species on fresh fruits and vegetables surpasses the safety standards set by the Food Safety and Standards Authority of India (FSSAI, 2006). Moreover, the prevalence of E. coli contamination was substantially higher than other pathogens.

Further analysis revealed that pear exhibited the highest contamination among the various fruits and vegetables, displaying contamination with E. coli, Salmonella spp., and S. aureus (Figure 2). These findings align with previous studies that have reported contamination of pear, apple, guava, carrot, and cucumber with similar bacterial species (Afroz et al., 2015; Liao et al., 2000; Savic et al., 2021; Sarker et al., 2018; Lee et al., 2021; Rahman et al., 2022).

The study also delves into the observed differences in bacterial load between local markets and retail outlets, echoing findings from a similar study comparing contamination levels among roadside vendors, retail shops, and wholesale markets (Saksena et al., 2019). Notably, the practices of small vendors, including inadequate knowledge about proper food handling methods and the use of non-potable water for washing, contribute to cross-contamination, emphasizing the need for enhanced food safety education among such vendors (Nkere et al., 2011; Berger et al., 2010).

The investigation further revealed a concerning trend in antibiotic resistance among the isolated bacterial species. E. coli, Salmonella sp. and S. aureus exhibited resistance against a significant number of broad-spectrum antibiotics. This finding underscores the gravity of the situation, as these common foodborne pathogens, prevalent on fresh produce, are developing resistance to multiple antibiotics. Salmonella, in particular, demonstrated a notable resistance profile, indicating a potential challenge in treating infections caused by this pathogen. The coexistence of antibiotic resistance with bacterial contamination on fresh produce magnifies the health risks associated with the consumption of such contaminated items, as it limits the effectiveness of standard medical treatments and raises the spectre of more severe and challenging infections for consumers. This aspect adds a critical dimension to the overall public health implications elucidated by the study.

In conclusion, the study underscores the escalating risk of contamination in fresh produce and its correlation with the concurrent rise in antibiotic resistance among associated bacterial strains. These findings pose a significant threat to public health, necessitating a comprehensive approach that encompasses improved agricultural practices, heightened surveillance, and public awareness to mitigate the growing health risks associated with the contamination of fresh produce and the emergence of antibiotic resistance.

Conclusion

This study highlights the contamination, load, and antibiotic resistance of selected bacterial species on fresh fruit and vegetables taken from local markets and retail outlets in the Delhi-NCR region. The findings indicated a higher level of bacterial contamination in local markets than in retail outlets of fresh produce. The abundance of bacterial pathogens in raw fresh produce might pose a significant risk to human health by causing several foodborne diseases and enteric diseases. Raw vegetables and fruits have been recognized as a potential link for foodborne diseases. Several factors may affect the contamination of fresh produce, such as type of water, hygiene practices, transportation, storage methods, the surrounding environment of the market, processing, and preparation levels. Moreover, antibiotic resistance profiles reveal the potential transfer of resistance from fresh produce to humans. E. coli was found to contaminate all fresh produce, with pears showing higher contamination levels than Salmonella spp. and S. aureus in both local markets and retail outlets. The degree of contamination varied amid different fruits and vegetables. Local markets showed a higher bacterial load than retail outlets. The transmission of contamination may arise from improper washing of fresh produce and the type of water used. Overall, this study emphasizes the importance of implementing strict measures to ensure the safety, quality, and prevention of foodborne pathogen transmission of fresh produce. Monitoring the microbial load of produce at different levels of production and sale is essential to reach FSSAI-required levels.

Declaration

There is no potential conflict of interest

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Author contribution statement:

Conceptualization and methodology: UC and RK; writing: RK, SV, AK.; Data analysis: SV; Review of study plan: AP, AM, AK, UC. All authors have read and agreed to the published version of the manuscript.

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