



Multidrug-Resistant Bacteria in Fresh Fruit Juices Sold in Benghazi, Libya.

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Highlights

- Fresh fruit juices in Benghazi showed high contamination with multidrug-resistant bacteria, mainly Gram-negative Enterobacteriaceae.
- Significant resistance to β -lactams and macrolides was observed, with emerging carbapenems resistance.
- Elevated MAR indices and detection of WHO priority pathogens highlight a potential community transmission risk.

ARTICLE INFO

Article history:

Received 24 March 2025

Revised 22 June 2026

Accepted 24 June 2026

Keywords:

antimicrobial resistance; multidrug resistance; MAR index; Gram-negative bacteria; Gram-positive bacteria; Enterobacteriaceae; non-Enterobacteriaceae; fresh juice contamination.

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ABSTRACT

Antimicrobial resistance (AMR) is a major global public health threat, particularly in ready-to-consume foods produced under informal urban conditions. Fresh fruit juices may serve as reservoirs and transmission vehicles for multidrug-resistant (MDR) bacteria, posing a significant potential risk to community health. A cross-sectional study was conducted on 128 fresh strawberry and mango juice samples, randomly collected from 16 cafés in Benghazi, Libya. Bacterial identification was performed using standard techniques described in Bergey's Manual of Systematic Bacteriology. Antimicrobial susceptibility testing and MDR prevalence were assessed using the Kirby-Bauer disk diffusion method according to CLSI M100, 31st edition (2021), against 11 commonly used antibiotics: amoxicillin, amikacin, clarithromycin, ciprofloxacin, imipenem, oxacillin, cefixime, cephalixin, doxycycline, cefuroxime, and sulfamethoxazole-trimethoprim. The evaluation of bacterial contamination revealed that 110(94.02%) isolates were Gram-negative, comprising 95 (86.4%) Enterobacteriaceae and 15(13.6%) non-Enterobacteriaceae, whereas 7(5.98%) isolates were Gram-positive bacteria. Antimicrobial susceptibility testing showed that Gram-negative isolates exhibited high resistance to amoxicillin-clavulanate (61%), oxacillin (61%), and clarithromycin (50%), complete susceptibility to doxycycline (0%) and ciprofloxacin (0%), and moderate resistance to cephalosporins (10–12%). Enterobacteriaceae displayed similar resistance patterns, with 61% resistant to amoxicillin-clavulanate, 61% to oxacillin, and 50.5% to clarithromycin. Non-Enterobacteriaceae showed 20% resistance to imipenem and amikacin, while remaining fully susceptible to ciprofloxacin, doxycycline, and sulfamethoxazole-trimethoprim. Gram-positive bacteria exhibited complete resistance to ciprofloxacin (100%), high resistance to clarithromycin (100%) and amoxicillin-clavulanate (57%), moderate resistance to cephalosporins (36%), and full susceptibility to doxycycline (0%). MDR prevalence and mean MAR indices were calculated for each group: Gram-positive bacteria exhibited 100% MDR with a mean MAR of 0.45; Gram-negative isolates showed 87.3% MDR with a mean MAR of 0.29; Enterobacteriaceae had 89% MDR with a mean MAR of 0.27; and non-Enterobacteriaceae displayed 100% MDR with a mean MAR of 0.55. Several isolates corresponded to WHO Critical Priority Pathogens, including carbapenem-resistant Enterobacteriaceae and non-Enterobacteriaceae, ESBL-producing Enterobacteriaceae, and methicillin-resistant Gram-positive bacteria (MRSA). These findings indicate that fresh juices in urban informal settings may act as reservoirs and transmission vehicles for MDR bacteria. The results emphasize the urgent need for improved food hygiene, routine microbiological surveillance, and One Health interventions to prevent the spread of multidrug-resistant pathogens in the community.

1. Introduction

Antimicrobial resistance (AMR) poses a critical threat to global public health and ecological stability, arising from selective pressures in clinical, agricultural, and environmental settings (Berendonk et al., 2015; Holmes et al., 2016). The World Health Organization (WHO) classifies AMR among the top global risks, with increasing resistance observed against key antimicrobial classes, including β -lactams, carbapenems, and fluoroquinolones (WHO, 2017; WHO, 2023).

Ready-to-consume foods, particularly fresh fruit juices, may serve as reservoirs and transmission routes for resistant bacteria

(Raybaudi-Massilia et al., 2009; Tambekar et al., 2009). Contamination can occur at multiple stages from farm to consumer, including irrigation water, soil, handling surfaces, and processing equipment. Exposure to human-derived antibiotics further enhances the survival and spread of resistant organisms in these products (Hernando-Amado et al., 2019; FAO & WHO, 2022). Gram-negative enteric bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, are capable of surviving acidic juice environments through adaptive mechanisms, highlighting their role in potential community-level dissemination of multidrug-resistant

(MDR) pathogens (Martínez, 2009; Berendonk et al., 2015). MDR, defined as resistance to three or more antimicrobial classes, is often linked to mobile genetic elements that facilitate horizontal gene transfer and rapid spread within microbial populations (Magiorakos et al., 2012; Bush & Bradford, 2020). The Multiple Antibiotic Resistance (MAR) index is a quantitative measure of contamination risk, with values above 0.2 indicating frequent or uncontrolled antibiotic exposure (Krumperman, 1983). High MAR indices in food-borne bacteria reflect public health risks, particularly in informal urban food sectors where Good Hygienic Practices (GHP) and Hazard Analysis and Critical Control Points (HACCP) may not be consistently applied (Tambekar et al., 2009; Grace, 2015).

Despite growing awareness of foodborne AMR, there is limited information from North African urban settings, which hinders regional risk assessment and the integration of food-sector data into national AMR surveillance programs. In Libya, data on antimicrobial resistance in ready-to-consume fruit juices are scarce, representing a critical knowledge gap.

This study therefore aimed to evaluate bacterial contamination, antimicrobial resistance patterns, MDR prevalence, and MAR indices in 128 fresh strawberry and mango juice samples collected from 16 cafés in Benghazi, Libya. By providing detailed information on the prevalence and distribution of resistant bacteria in these beverages, this research informs community-level exposure pathways to AMR within a One Health framework while addressing a regional data gap.

2. Materials and Methods

2.1 Study Design and Sample Collection

A cross-sectional study was conducted on 128 fresh juice samples, including strawberry (n=64) and mango (n=64), collected from 16 cafés across Benghazi, Libya. From each café, four samples of each juice type were collected to ensure representative sampling. Samples (~250 mL) were aseptically collected, transported at 4 °C, and processed within 2 hours.

2.2 Microbiological Analysis

Samples were homogenized in buffered peptone water under aseptic conditions, followed by serial decimal dilution. Aliquots of appropriate dilutions were plated onto selective and non-selective media, including MacConkey agar, Eosin Methylene Blue (EMB) agar, Mannitol Salt Agar, and Nutrient agar, to facilitate the recovery of diverse bacterial groups. Plates were incubated aerobically at 37 °C for 24–48 h. Distinct colonies were repeatedly subcultured to obtain pure isolates. Preliminary characterization was performed based on colony morphology and Gram staining. Based on the observed morphological characteristics and Gram reaction, bacterial isolates were classified at the family level and grouped into Enterobacteriaceae, non-Enterobacteriaceae Gram-negative bacterial families, and Gram-positive bacterial families. The classification procedures followed standard microbiological methods described in Bergey's Manual of Systematic Bacteriology and other established laboratory protocols.

2.3 Antimicrobial Susceptibility Testing (AST)

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing (M100, 31st edition, 2021). Eleven antibiotics representing diverse and commonly used antimicrobial classes were selected to assess multidrug resistance and facilitate comparison with previous studies. The antibiotics and their disc potencies were as follows: ciprofloxacin (CIP, 5 µg), clarithromycin (CLR, 15 µg), imipenem (IPM, 10 µg), amikacin (AK, 30 µg), amoxicillin–clavulanate (AMC, 30 µg), oxacillin (OX, 5 µg), cefixime (CFM, 5 µg), cephalexin (CL, 30 µg), sulfamethoxazole–trimethoprim (SXT, 25 µg), doxycycline (DO, 30 µg), and cefuroxime (CFM, 30 µg).

Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial classes according to the criteria proposed by Magiorakos et al. (2012). The multiple antibiotic resistance (MAR) index was calculated by dividing the number of antibiotics to which an isolate was resistant by the total number of antibiotics tested (11), as described by Krumperman (1983). ESBL production in Enterobacteriaceae was detected using the double-disk synergy test (DDST) with cefotaxime, ceftazidime, and amoxicillin–clavulanate. Carbapenem resistance was screened using imipenem disks and confirmed using carbapenemase confirmatory discs containing boronic acid and EDTA.

2.4 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize bacterial prevalence, antimicrobial resistance rates, multidrug resistance (MDR) frequencies, and mean Multiple Antibiotic Resistance (MAR) indices. Categorical variables were compared using the chi-square (χ^2) test to assess differences in resistance distribution and high-MAR (≥ 0.5) proportions between bacterial groups (Gram-positive vs Gram-negative; Enterobacteriaceae vs Non-Enterobacteriaceae). A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Bacterial Prevalence

A total of 117 bacterial isolates were recovered from 128 fresh juice samples. Gram-negative bacteria predominated, accounting for 110 isolates (94.02%), whereas Gram-positive bacteria represented 7 isolates (5.98%). Among Gram-negative isolates, Enterobacteriaceae comprised 95 (86.4%), while Non-Enterobacteriaceae accounted for 15 (13.6%).

3.2 Antimicrobial Resistance Patterns

The antimicrobial resistance profiles across bacterial groups are summarized in Table 1.

Overall, the highest resistance rates were observed for β -lactam antibiotics, particularly amoxicillin–clavulanate and oxacillin (61%) among Gram-negative isolates. High resistance to clarithromycin (50%) was also detected in Gram-negative bacteria, while Gram-positive isolates exhibited extremely high resistance to clarithromycin (97.6%) and complete resistance to ciprofloxacin (100%). In contrast, doxycycline demonstrated complete susceptibility across all bacterial groups (0% resistance), highlighting its potential efficacy. These patterns indicate that β -lactams and macrolides are the most compromised antibiotic classes in the studied fresh juice isolates, whereas tetracyclines and certain aminoglycosides remain largely effective.

3.3 Multidrug Resistance and MAR Index

The distribution of multidrug resistance (MDR) and multiple antibiotic resistance (MAR) indices among bacterial groups isolated from fresh fruit juices is presented in Table 2. Overall, MDR was highly prevalent among the isolates, particularly in Gram-positive bacteria (7/7, 100%) and non-Enterobacteriaceae (15/15, 100%). Gram-negative isolates exhibited an MDR prevalence of 96/110 (87.3%), while Enterobacteriaceae showed 85/95 (89%) MDR. The mean MAR index varied across bacterial groups, with Gram-positive isolates exhibiting the highest value (0.45), followed by non-Enterobacteriaceae (0.55), Gram-negative isolates overall (0.29), and Enterobacteriaceae (0.27). According to Krumperman (1983), MAR values greater than 0.2 indicate contamination from sources exposed to frequent or indiscriminate antibiotic use. All groups in this study exceeded this threshold, strongly suggesting that fresh juice contamination originates from environments subjected to sustained antimicrobial pressure, including contaminated irrigation water, inadequately sanitized preparation equipment, and food handlers exposed to widespread community antibiotic misuse (Fig. 1).

Table 1

Antibiotic resistance profiles (n/N, %) among bacterial groups isolated from fresh fruit juices in Benghazi.

Antibiotic class	Antibiotic	Gram-negative		Enterobacteriaceae		Non-Enterobacteriaceae		Gram-positive	
		n/N	%	n/N	%	n/N	%	n/N	%
Carbapenem	Imipenem (IPM)	19/110	17.3	15/95	16.0	3/15	20.0	1/7	14.3
Fluoroquinolone	Ciprofloxacin (CIP)	0/110	0.00	0/95	0.00	0/15	0.00	7/7	100
Aminoglycoside	Amikacin (AK)	17/110	15.5	10/95	10.5	3/15	20.0	0/7	0.00
Macrolide	Clarithromycin (CLR)	55/110	50.0	48/95	50.5	1/15	6.70	7/7	100
β-lactam	Amoxicillin-clavulanate (AMC)	67/110	61.0	58/95	61.1	2/15	13.3	4/7	57.1
	Oxacillin (OX)	67/110	61.0	58/95	61.1	2/15	13.3	4/7	57.1
	Cefixime (CFM)	11/110	10.0	10/95	10.5	2/15	13.3	3/7	42.9
	Cephalexin (CL)	11/110	10.0	10/95	10.5	2/15	13.3	3/7	42.9
	Cefuroxime (CXM)	11/110	10.0	10/95	10.5	2/15	13.3	3/7	42.9
Sulfonamide	Trimethoprim-sulfamethoxazole (SXT)	4/110	3.60	4/95	4.20	0/15	0.00	1/7	14.3
Tetracycline	Doxycycline (DO)	0/110	0.00	0/95	0.00	0/15	0.00	0/7	0.00

Note: n/N (%) indicates the number of resistant isolates (n) out of the total number of isolates tested in each group (N), expressed as a percentage.

Statistical analysis confirmed that high MAR (≥ 0.5) was significantly more frequent among Gram-positive compared to Gram-negative isolates ($\chi^2 = 10.5$, $p = 0.001$). Additionally, a significant difference was observed between *Enterobacteriaceae* and non-*Enterobacteriaceae* ($\chi^2 = 6.53$, $p = 0.011$), indicating heterogeneity in resistance accumulation within Gram-negative populations.

From a broader perspective, the elevated MAR indices highlight the role of ready-to-consume juices as potential nodes within One Health transmission pathways, linking environmental reservoirs, food systems, and human exposure. The detection of high-risk resistance profiles in a community food setting underscores the interconnected dynamics of antimicrobial use across clinical and non-clinical sectors.

Table 2

Distribution of multidrug resistance (MDR) and multiple antibiotic resistance (MAR) indices among bacterial groups isolated from fresh fruit juices.

Group	MAR ≥ 0.5 (n/N)	MAR ≥ 0.5 (%)	Mean MAR	MDR (n/N)	MDR (%)
Gram-positive	7/7	100	0.45	7/7	100
Gram-negative	16/110	14.5	0.29	96/110	87.3
<i>Enterobacteriaceae</i>	6/95	6.7	0.27	85/95	89.0
Non- <i>Enterobacteriaceae</i>	15/15	100	0.55	15/15	100

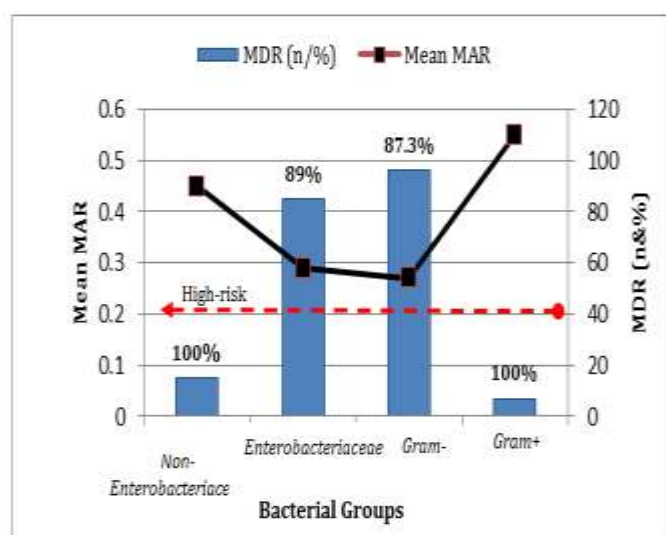


Fig. 1. MDR (n/N) (blue columns) and mean MAR (line) among bacterial groups isolated from fresh fruit juices in Benghazi. The dashed line at MAR = 0.2 indicates the high-risk threshold.

4. Discussion

Fresh fruit juices sold in informal cafés in Benghazi were frequently contaminated with multidrug-resistant (MDR) bacteria, predominantly Gram-negative members of the *Enterobacteriaceae* family. High levels of resistance to β-lactams and macrolides, together with emerging carbapenem resistance, likely reflect multiple contamination pathways, including contaminated irrigation or preparation water, inadequate hygiene during juice preparation, and excessive or unregulated antibiotic use within the community (Elbasha *et al.*, 2018). Interestingly, fluoroquinolone resistance was absent among Gram-negative isolates but universal among Gram-positive bacteria, suggesting differential antibiotic exposure patterns between bacterial groups.

The mean Multiple Antibiotic Resistance (MAR) index was markedly elevated among Gram-positive bacteria (0.45), indicating resistance to approximately five of the eleven tested antibiotics. *Enterobacteriaceae* showed slightly lower MAR values compared to overall Gram-negative isolates (0.27 vs. 0.29), yet still exceeded the high-risk threshold. These findings confirm the widespread distribution of multidrug resistance across all bacterial groups isolated from fresh juices.

The MAR index provides important insight into potential contamination sources. The elevated MAR values observed across all bacterial groups provide strong evidence of substantial antimicrobial selective pressure within environmental reservoirs. MAR values exceeding the threshold of 0.2 are widely recognized as indicators of high-risk environments associated with frequent or indiscriminate antibiotic use (Krumperman, 1983). In the present study, all bacterial groups exceeded this threshold, suggesting that contamination likely originated from environments subjected to sustained antimicrobial exposure rather than a single point source.

From an environmental perspective, such elevated MAR indices are commonly associated with antibiotic-impacted ecosystems. Potential contamination pathways include irrigation water contaminated with wastewater effluents, agricultural soils exposed to animal manure or untreated waste, and inadequately sanitized food-contact surfaces (Hernando-Amado *et al.*, 2019; FAO & WHO, 2022). These environments act as reservoirs of resistant bacteria and resistance genes, facilitating their persistence and subsequent transmission into the food chain.

Furthermore, the informal nature of juice preparation in urban settings may amplify this risk. Limited adherence to hygienic practices, combined with repeated exposure to contaminated water sources and handling surfaces, can promote the accumulation and dissemination of resistant microorganisms. Therefore, the consistently high MAR indices observed in this study reflect not only microbial contamination but also the broader influence of environmental antimicrobial pollution and community-level antibiotic misuse.

The observed resistance patterns may also be explained by the biological adaptability of *Enterobacteriaceae*, which can survive in

diverse environmental niches, colonize the gastrointestinal tracts of humans and animals, and acquire resistance determinants through horizontal gene transfer (Martínez, 2009; Magiorakos *et al.*, 2012). These characteristics facilitate the accumulation of resistance genes, including extended-spectrum β -lactamase (ESBL) and carbapenemase determinants, often carried on mobile genetic elements such as plasmids, which play a key role in horizontal gene transfer and rapid dissemination of antimicrobial resistance (Carattoli, 2013). The detection of ESBL- and carbapenemase-producing isolates further supports the circulation of mobile genetic elements capable of rapid dissemination among bacterial populations.

The findings of this study are consistent with reports from several regions worldwide. In India, street-vended fruit juices have been shown to harbor enteric pathogens such as *Escherichia coli*, *Salmonella spp.*, and *Klebsiella spp.*, many of which exhibit resistance to commonly used antibiotics (Tambekar *et al.*, 2009; Raybaudi-Massilia *et al.*, 2009; Singh *et al.*, 2020; Abdel-Rahman *et al.*, 2019). Similar patterns have been documented in Sub-Saharan Africa, where fresh produce frequently contains multidrug-resistant bacteria (Mensah *et al.*, 2002; Abakari *et al.*, 2018). Comparable resistance trends have also been reported in Sudan and the Middle East (Elbagory *et al.*, 2015; Ibrahim *et al.*, 2021; Salloum *et al.*, 2018; Al-Jasser, 2012; Al-Harbi & Uddin, 2016), as well as in more recent studies from Ethiopia, Ecuador, and South Africa (Osei *et al.*, 2021; Mekonnen *et al.*, 2023; De La Cruz *et al.*, 2023).

Numerically, the MDR prevalence and MAR indices observed in this study are comparable to or slightly higher than those reported globally. Previous studies have documented MDR rates of 60–80% in street-vended juices in India and MAR values ranging from 0.20 to 0.45 in fresh produce from Sub-Saharan Africa (Tambekar *et al.*, 2009; Abakari *et al.*, 2018). In comparison, the elevated MAR indices observed in this study further reinforce the influence of sustained antimicrobial exposure within the local food environment.

Despite the strength of these findings, several limitations should be acknowledged. The cross-sectional design limits the ability to establish causal relationships between contamination sources and antimicrobial resistance patterns. In addition, environmental sampling was not performed, preventing the direct identification of potential contamination pathways. Future studies should incorporate environmental surveillance and molecular characterization of antimicrobial resistance genes to better understand transmission dynamics (Berendonk *et al.*, 2015; Hernando-Amado *et al.*, 2019).

From a public health perspective, the detection of MDR bacteria in ready-to-consume fruit juices represents a significant concern, as these products may serve as potential vehicles for the dissemination of difficult-to-treat infections within the community. This issue is particularly critical in low- and middle-income settings, where regulatory frameworks and antimicrobial resistance surveillance systems may be limited (WHO, 2015; WHO, 2023). Furthermore, antimicrobial resistance is associated with increased healthcare costs, prolonged illness, and a substantial economic burden.

Addressing these risks requires integrated interventions, including enforcement of Good Hygienic Practices (GHP), routine microbiological monitoring of ready-to-consume foods, training of food handlers, and stricter regulation of antibiotic use. Within the Libyan context, practical measures may include implementing hygiene standards for juice preparation, conducting regular microbiological testing, raising public awareness about antibiotic misuse, and integrating informal food vendors into national antimicrobial resistance surveillance systems.

These findings highlight the urgent need to integrate food safety monitoring into national antimicrobial resistance surveillance frameworks in Libya.

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