Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Prevalence, characterization, and transmissible factors of foodborne pathogens in the Al-Qassim Region, Saudi Arabia

Abdulrahman Almujaidel, Adil Abalkhail, Ayman Elbehiry*

Department of Public Health, College of Applied Medical Sciences, Qassim University, Buraydah, 51452 P.O. Box 6666, Saudi Arabia

Article Info

Article history:

Received: April 12, 2025
Accepted: June 09, 2025
Published: August 31, 2025

Use your device to scan and read the article online

Abstract

Foodborne illnesses pose a significant public health threat globally, particularly in Saudi Arabia, where the rapid growth of the food service sector has increased the risk of exposure to multidrug-resistant (MDR) bacteria. Traditional microbiological methods are often time-consuming and may lack precision, highlighting the need for faster and more accurate diagnostic alternatives. In this study, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) was employed for the rapid and precise identification of bacterial contaminants in ready-to-eat (RTE) foods, alongside an assessment of their antibiotic resistance profiles. A total of 80 RTE food samples—including chicken shawarma, shish tawook, chicken burgers, and falafel sandwiches—were collected from restaurants across the Al-Qassim region between September and November 2024. Bacterial identification was performed using standard culturing techniques in combination with MALDI-TOF MS, while antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method in accordance with CLSI guidelines. The predominant pathogens identified were Escherichia coli (E. coli) (32.5%), Staphylococcus aureus (S. aureus) (27.5%), and Acinetobacter baumannii (A. baumannii) (18.75%), with the highest contamination observed in chicken shawarma samples. MALDI-TOF MS provided high-confidence species-level identifications, with score ranges between 2.00 and 2.49. E. coli isolates exhibited complete resistance (100%) to ampicillin, amoxicillin-clavulanic acid, cefoxitin, and cephalothin. High resistance rates were also observed for norfloxacin (80.8%) and tetracycline (73.1%). Similar MDR patterns were detected in S. aureus and A. baumannii. Imipenem remained the most effective antibiotic, showing 100% susceptibility among E. coli and S. aureus isolates and 93.3% effectiveness against A. baumannii. These findings underscore the critical need for routine microbial surveillance, implementation of rapid diagnostic tools, and enforcement of stringent food safety regulations to curb the spread of antimicrobial-resistant pathogens through the food supply chain.

Keywords: Foodborne pathogens, MALDI-TOF MS, Ready-to-eat foods, Antibiotic resistance, Saudi Arabia, Public health.

1. Introduction

Foodborne pathogens remain a major global public health challenge, leading to significant health and economic burdens—particularly in the Kingdom of Saudi Arabia [1]. Food safety is shaped by various factors, including microbiological, chemical, and nutritional changes; biodiversity; water activity; climate variability; and environmental hygiene [2]. Consumption of food contaminated with viruses, parasites, or bacteria can result in illnesses such as hemorrhagic colitis, typhoid fever, acute gastroenteritis, and diarrhea [3].

Among foodborne pathogens, bacteria are the most prevalent, causing a wide range of diseases in both humans and animals [4]. Common bacterial pathogens include Salmonella, Shigella, Listeria monocytogenes, Bacillus spp., Yersinia spp., Clostridium botulinum, Clostridium perfringens (C. perfringens), Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Campylobacter spp., and Vibrio cholerae [5]. The increasing number of foodborne illness outbreaks worldwide highlights the urgent need for effective public health interventions [6]. According to the World Health Organization (WHO), 31 foodborne pathogens account for an estimated 600 million illnesses and 420,000 deaths globally each year [7]. In Saudi Arabia, microbial contamination of food leads to considerable morbidity and mortality annually [8]. Adults account for most outbreaks (68.1%), with Salmonella causing 81% of cases and S. aureus 19.1% [9].

Food contamination can occur at various stages, including animal farming, crop production, food transport, processing, and handling [10]. ross-contamination during food preparation and consumption also presents a significant risk [11]. Public awareness of foodborne pathogens is closely linked to safe food handling practices [12], as informed consumers are more likely to follow safety protocols [13]. To address these challenges, Saudi Arabia has

* Corresponding author.
E-mail address: ar.elbehiry@qu.edu.sa (A. Elbehiry).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.8.8

implemented a comprehensive food safety policy, including the establishment of a national food and drug authority and the enforcement of updated regulations. Offenders face strict penalties, and the Ministry of Health collaborates with the Ministry of Municipal and Rural Affairs to investigate foodborne disease outbreaks [9].

The growth of the fast food industry, driven by increasingly fast-paced lifestyles, has added complexity to food safety concerns. In Saudi Arabia, commonly consumed fast foods include shawarma, falafel, vegetable salads, and kibtha, with chicken serving as the primary protein source [1]. Red meats, such as lamb, goat, and camel, are also staples in Gulf Cooperation Council countries. Poor hygiene practices and limited knowledge of food safety principles contribute to the high incidence of foodborne illnesses in low-income regions, where up to 70% of diarrheal cases are linked to contaminated food [14]. Several cities in Saudi Arabia have reported foodborne outbreaks, including Salmonella-induced gastroenteritis in Sulyyel, Riyadh, following a wedding [15], and outbreaks in Hail and Abha involving 39 cases of S. aureus and 26 cases of Salmonella enteritidis [15].

Timely detection of foodborne pathogens is essential for ensuring food safety, regulatory compliance, and outbreak prevention. Numerous technologies have been developed to support this effort [3]. Accurate identification and monitoring of pathogenic microorganisms are critical for effective control strategies [16]. By detecting pathogens, food producers and retailers can implement safety measures, such as proper preservation and handling, to reduce contamination risks. However, conventional microbial testing methods are often labor-intensive and may fail to detect pathogens at critical points, such as during food preparation [17].

Phenotypic methods, although traditional, are often imprecise, time-consuming, and impractical for routine screening [18, 19]. Consequently, recent studies have focused on alternative techniques aimed at improving accuracy and reducing manual labor [20]. Detecting trace levels of pathogens in food requires advanced diagnostic tools. Nucleic acid-based, immunological, and biosensor technologies have shown promise for rapid and accurate detection, though confirmatory testing may still be needed in cases of false positives or negatives [21].

One such advanced technology is matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), which has significantly advanced the detection of foodborne pathogens [22]. MALDI-TOF MS is recognized for its accuracy in identifying harmful microorganisms in processed foods [23] and is gaining popularity in food microbiology due to its speed and ease of use. Principal component analysis (PCA) further enhances the application of MALDI-TOF MS by identifying patterns within complex datasets [24]. Despite its advantages, the use of MALDI-TOF MS for food pathogen detection is still underreported in the literature [25]. The U.S. Food and Drug Administration has approved its use for identifying pathogens in cell culture extracts [26], valuing its speed, precision, cost-effectiveness, and minimal technical requirements—qualities critical for food safety laboratories [27]. MALDI-TOF MS has successfully identified pathogens such as Acinetobacter baumannii (A. baumannii), Campylobacter spp., E. coli, and S. aureus [25].

The misuse of antimicrobial agents in veterinary practice has contributed to the rise of antibiotic-resistant bacteria capable of transmission through the food chain [28]. There is an urgent need for stricter regulations governing antibiotic use in animal farming [29]. Many antimicrobials present in food from animal sources are believed to facilitate the emergence and spread of resistant microorganisms [30]. These pathogens pose a serious threat to human health and can result in infections that are difficult to treat [31, 32]. Resistance genes can be transferred between microorganisms through the food chain, compounding the public health challenge [33]. Once established in a host, antibiotic-resistant strains may share resistance traits with other microbes, exacerbating the problem [34].

Evidence indicates that meat and meat products can serve as carriers of antibiotic-resistant bacteria [35]. Studies have confirmed the presence of resistant bacteria in meat and related products [36], underscoring the need for ongoing surveillance and evaluation of regulatory measures. However, limited data exist on the prevalence of antibiotic-resistant pathogens in ready-to-eat (RTE) foods in Saudi Arabia. Moreover, the correlation between antibiotic resistance phenotypes and genetic determinants in bacteria isolated from RTE meat products in the Al-Qassim region remains poorly characterized.

This study aimed to enable the rapid and accurate identification of pathogenic bacteria isolated from fast food samples collected from restaurants and food establishments in the Al-Qassim region using MALDI-TOF MS. It also assessed the antibiotic resistance profiles of the most commonly detected bacterial strains. The findings of this research are expected to provide valuable insights into the potential health risks posed by antibiotic-resistant bacteria in the fast food sector.

2. Materials and methods

2.1. Collection and handling of food samples

A total of 80 RTE food samples were collected, comprising 20 samples each of chicken shawarma, shish tawook, chicken burgers, and falafel sandwiches. Samples were obtained from various restaurants across the Al-Qassim region between September and November 2024. Restaurants were selected using a convenience sampling approach from diverse urban and suburban locations to reflect typical consumer exposure; randomization or stratification was not applied. Each sample, weighing approximately 250 grams, was collected using aseptic techniques to prevent contamination. Samples were placed in sterile stomacher bags labeled with restaurant codes, sample identifiers, and food categories, sealed, and transported in coolers with ice packs at temperatures below 4°C for immediate analysis in the microbiology laboratory. Strict inclusion and exclusion criteria were applied, focusing exclusively on commonly available RTE sandwiches purchased directly from restaurants; unavailable items or leftovers were excluded.

2.2. Bacteriological indicators and quality standards for ensuring food safety

Traditional bacteriological indicators were used to evaluate the microbiological safety of the food samples, including aerobic plate count (APC), total coliforms (TC), E. coli, and S. aureus. APC reflected the total microbial load; TC indicated general hygiene status; E. coli signified fecal contamination; and S. aureus suggested improper handling due to its toxigenic potential. For analysis, 25 g of each

sample was placed in a sterile bag with 225 mL of 0.1% peptone water [37]. The mixture was homogenized using a stomacher (Thomas Scientific, USA). Serial dilutions were prepared in 1% peptone water, and 1 mL aliquots were plated on Count Agar and incubated at 37°C for 48 hours [38].

2.3. Quality control and data management

Standardized protocols were followed to maintain data integrity and prevent contamination during sample collection. Recorded data included sample type, site, collection time, collector name, and sample number. All supplies were sterilized, and personnel were trained in sampling procedures. Samples were stored in chilled cooler bags during transport. Media sterility was confirmed by overnight incubation, and positive and negative controls were included for each batch of media, reagents, and procedures.

2.4. Detection and isolation of pathogenic microorganisms from food products

Detection followed FDA-recommended protocols [39], including enrichment, selective plating, and identification. MacConkey and EMB agars (Merck, Germany) were used to isolate Gram-negative bacilli (e.g., E. coli, E. cloacae, H. alvei); Baird–Parker and mannitol salt agars (Sigma–Aldrich, USA) for S. aureus; CHROMagar Acinetobacter® (HiMedia, USA) for A. baumannii; XLD agar (Oxoid, UK) for S. enterica; and egg yolk agar (HiMedia, India) for C. perfringens. Growth was monitored, and isolates were confirmed by Microflex LT MALDI-TOF MS (Bruker Daltonics, Germany). The study focused on bacterial species commonly associated with RTE foods in Saudi Arabia, including E. coli, S. aureus, A. baumannii, S. enterica, C. perfringens, E. cloacae, and H. alvei. Listeria and Campylobacter species were not included due to resource limitations and prioritization based on previous regional studies.

2.5. Identification of bacterial isolates by protein fingerprinting based on the bruker library

Subculturing eliminated potential contaminants prior to MALDI-TOF MS analysis, following Bruker Daltonics' extended direct transfer protocol [40]. Single colonies were applied to a target plate, overlaid with 1 µL of 70% formic acid, dried, and covered with 1 µL of α-cyano-4-hydroxycinnamic acid (Sigma–Aldrich, USA). Calibration was performed before each run using the Bruker Bacterial Test Standard and E. coli DH5α. Spectra (2,000–20,000 Da) were acquired using the Microflex LT and analyzed with FlexControl 3.1 (Bruker Daltonics, Germany). Identification scores were interpreted according to Bruker thresholds. To ensure accuracy, standard procedures were followed, including formic acid extraction, subculturing isolates to purity, and routine calibration with E. coli DH5α and the Bacterial Test Standard. No discrepancies were observed between conventional culture-based identifications and MALDI-TOF MS results.

2.6. Susceptibility test for antimicrobials

Antimicrobial susceptibility was assessed by the Kirby–Bauer disk diffusion method per CLSI guidelines [41]. Inhibition zones were measured for 14 antibiotics: ampicillin (10 µg), amoxicillin–clavulanic acid (20 µg), gentamicin (10 µg), cefoxitin (30 µg), cephalothin (30 µg), trimethoprim–sulfamethoxazole (25 µg), nalidixic acid (30 µg), norfloxacin (10 µg), amikacin (30 µg), cefepime (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), tetracycline (30 µg), piperacillin (100 µg), and imipenem (10 µg). Interpretation followed CLSI breakpoints [42]. Isolates were grown in Trypticase Soy Broth (TSB; Sigma–Aldrich, USA), turbidity adjusted to 0.5 McFarland using a Sensititre™ Nephelometer (ThermoFisher, USA). Cultures were plated on Müller-Hinton agar (Sigma–Aldrich, USA), discs spaced 3 cm apart, and incubated at 37°C for 24 hours.

2.7. Statistical analysis

All statistical analyses were conducted using SPSS version 20.0 based on collected study data.

3. Results

3.1 Bacterial counts

The analysis of viable bacterial counts, expressed as log CFU/g, revealed the following mean concentrations across the sampled food types: chicken shawarma sandwiches (n = 20) showed the highest bacterial load at 9.44 ± 0.86, followed by chicken burger sandwiches at 7.64 ± 0.34, shish tawook sandwiches at 6.56 ± 0.98, and falafel sandwiches at 5.43 ± 0.22. Chicken shawarma samples exhibited a significantly higher bacterial count compared to both falafel ($p = 0.001$) and shish tawook ($p = 0.002$) samples.

3.2. Prevalence of foodborne pathogens using culture techniques

Culture-based analysis (Table 1 and Figure 1) of the 80 collected food samples identified *E. coli* as the most prevalent bacterium, which was isolated in 32.5% of the samples. This was followed by *S. aureus* (27.5%), *A. baumannii* (18.75%), *S. enterica* (15%), *C. perfringens* (10%),

Table 1. Prevalence of various foodborne pathogens in 80 ready to eat food samples.

Bacteria	Chicken shawarma positive samples		Shish tawook positive samples		Chicken burger positive samples		Falafel positive samples		Total positive samples	
	No.	%	No.	%	No.	%	No.	%	No.	%
E. coli	10	50.00	5	25.00	7	35.00	4	20.00	26	32.50
S. aureus	9	40.00	4	20.00	6	30.00	3	15.00	22	27.50
A. baumannii	3	15.00	4	20.00	3	15.00	5	25.00	15	18.75
S. enterica	6	30.00	2	10.00	4	20.00	0	0.00	12	15.00
C. perfringens	3	15.00	3	15.00	2	10.00	0	0.00	8	10.00
E. cloacae	2	10.00	1	5.00	1	5.00	1	5.00	5	6.25
H. alvei	1	5.00	3	15.00	0	0.00	0	0.00	4	5.00

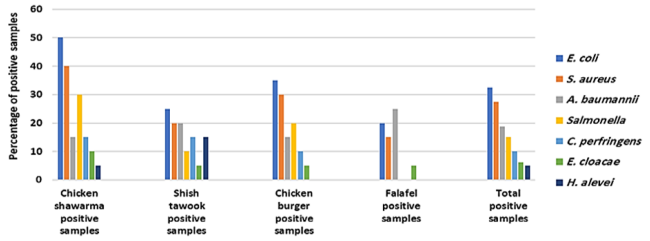

Fig. 1. Percentages of various bacterial species isolated from different types of RTE food samples. The figure shows the distributions of *E. coli*, *S. aureus*, *A. baumannii*, *S. enterica*, *C. perfringens*, *E. cloacae*, and *H. alvei* across chicken shawarma, shish tawook, chicken burger, and falafel sandwiches collected from restaurants in the Al-Qassim region.

Enterobacter cloacae (6.25%), and *Hafnia alvei* (5%). In the chicken shawarma samples, *E. coli* was detected in 50% of the cases, followed by *S. aureus* (40%), *S. enterica* (30%), *A. baumannii* (15%), *C. perfringens* (15%), *E. cloacae* (10%), and *H. alvei* (5%). Shish tawook samples were contaminated with *E. coli* (25%), *S. aureus* (20%), *A. baumannii* (20%), *C. perfringens* (15%), *H. alvei* (15%), *S. enterica* (10%), and *E. cloacae* (5%). In the chicken burger samples, *E. coli* was found in 35% of the isolates, followed by *S. aureus* (30%), *S. enterica* (20%), *A. baumannii* (15%), *C. perfringens* (10%), and *E. cloacae* (5%), while *H. alvei* was not detected. In the peel samples, *A. baumannii* was the most frequently isolated bacterium (25%), followed by *E. coli* (20%), *S. aureus* (15%), and *E. cloacae* (5%). Notably, *S. enterica*, *C. perfringens*, and *H. alvei* were absent in the stool samples. Importantly, all the identified organisms are considered pathogenic, with the exception of *E. cloacae* and *H. alvei*, which are classified as opportunistic pathogens capable of causing infections under specific conditions.

3.3. Mass spectral identification of characterized bacterial cultures

A total of 92 bacterial isolates were analyzed via the Microflex LT MALDI-TOF MS system, with spectra compared against the Bruker Daltonics Compass 2.0 database. The accuracy of identification for each species, as shown in Table 2 and Figure 2, was as follows: E. coli (26/26, 100%), S. aureus (21/22, 95.45%), A. baumannii (15/15, 100%), S. enterica (12/12, 100%), C. perfringens (7/8, 87.5%), E. cloacae (5/5, 100%), and H. alvei (4/4, 100%). Among all the isolates, 48 (52.17%) were confidently identified at the species level, with score values between 2,300 and 3,000. These included 16/26 E. coli (61.54%), 9/22 S. aureus (40.9%), 6/15 A. baumannii (40%), 7/12 S. enterica (58.33%), 4/8 C. perfringens (50%), 3/5 E. cloacae (60%), and 3/4 H. alvei (75%). An additional 42 isolates (45.65%) were identified at the species level with score values between 2,000 and 2,299, comprising 10/26 E. coli (38.46%), 12/22 S. aureus (54.55%), 9/15 A. baumannii (60%), 5/12 S. enterica (41.67%), 3/8 C. perfringens (37.5%), 2/5 E. cloacae (40%), and 1/4 H. alvei (25%). Only two isolates—one each of S. aureus and C. perfringens—yielded probable genus-level identifications (score range 1.7–1.99). Importantly, all the isolates were successfully identified, with no cases of nonidentification reported.

As illustrated in Figure 3, the mass spectral protein profiles of the identified bacterial isolates—including E. coli, S. aureus, A. baumannii, S. enterica, C. perfringens, E. cloacae, and H. alvei—exhibited distinct peak patterns distributed within a mass range of 3,000 to 11,000 Daltons (Da), varying according to species. E. coli isolates showed peaks between 3,200 and 11,200 Da, with prominent signals at ~4,800, 5,400, 6,200, 8,400, 9,000, 9,450, and 9,750 Da. S. aureus isolates displayed major peaks at 3,500, 5,000, 5,500, and 6,250 Da. For A. baumannii, intense signals were recorded at 4,200, 5,700, and 8,500 Da. S. enterica showed characteristic peaks at 3,750, 4,200, 5,200, and 7,800 Da. C. perfringens had strong peaks at 4,250, 5,000, 6,000, 6,300, and 7,150 Da. E. cloacae spectra featured notable peaks at 3,200, 3,500, 5,800, 6,250, and 7,400 Da. Finally, H. alvei exhibited distinctive peaks at 4,250, 4,750, 5,750, 6,200, 7,200, 7,800, and 9,500 Da.

A Microflex LT instrument was utilized to generate gel

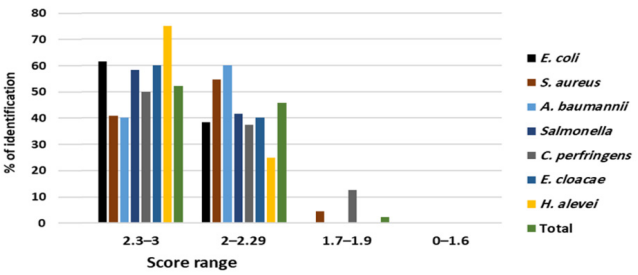

Fig. 2. Score values for 92 bacterial isolates obtained from foodborne samples, identified using the Microflex LT instrument. The majority of isolates were identified at the species level, with score values ranging from 2.0 to 3.0. Only two isolates—1 out of 22 S. aureus (4.5%) and 1 out of 8 C. perfringens (12.5%)—had scores between 1.7 and 1.9, indicating genus-level identification. No isolates yielded score values below 1.7, confirming that all isolates were successfully identified.

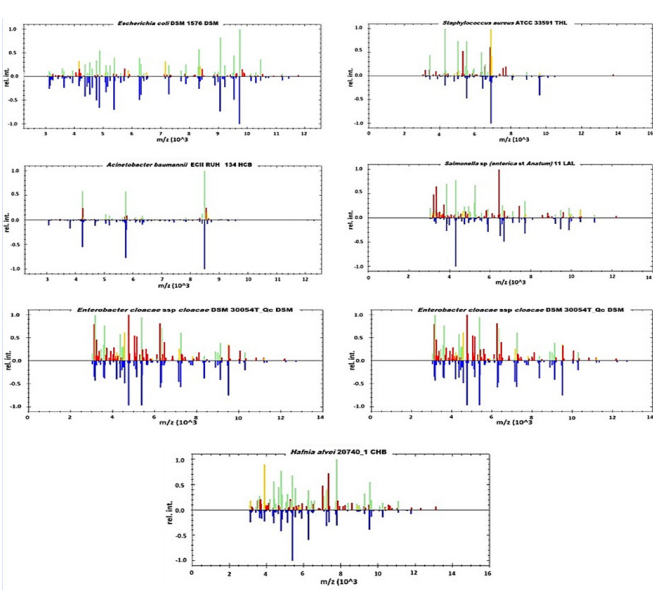

Fig. 3. Mass spectral protein profiles of various bacterial isolates obtained from RTE food samples, including E. coli, S. aureus, A. baumannii, S. enterica, C. perfringens, E. cloacae, and H. alvei. These profiles were compared against reference spectra from the Microflex LT's IVD Compass software. In the spectra, blue lines represent archived reference peaks, green lines indicate matching peaks, red lines denote nonmatching peaks, and yellow lines signify intermediate matches.

Table 2. The score values for 92 bacterial isolates obtained from foodborne samples, as detected by the Microflex LT machine.

Score range	Detection level	Foodborne pathogens															
		E. coli (N=26)		*S. aureus* (N=22)		*A. baumannii* (N=15)		*S. enterica* (N=12)		*C. perfringens* (N=8)		*E. cloacae* (N=5)		*H. alvei* (N=4)		*Total* (N=92)	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
2.3–3	Species	16	61.54	9	40.90	6	40.00	7	58.33	4	50.00	3	60.00	3	75.00	48	52.17
2–2.29	Species	10	38.46	12	54.55	9	60.00	5	41.67	3	37.50	2	40.00	1	25.00	42	45.65
1.7-1.9	Genus	0	0.00	1	4.55	0	0.00	0	0.00	1	12.50	0	0.00	0	0.00	2	2.17
0–1.6	Not detected	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00

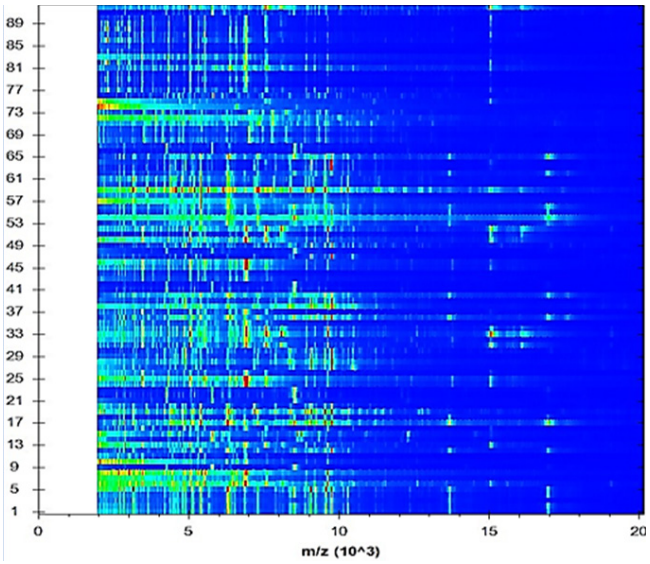

Fig. 4. Gel view representation of protein spectra from multiple foodborne bacterial isolates obtained from RTE food samples collected across various restaurants. Yellow bands indicate areas of spectral protein accumulation at varying intensities. Most prominent peaks are observed within the 2,000 to 10,000 Da range, highlighting the characteristic mass distribution of the detected bacterial proteins.

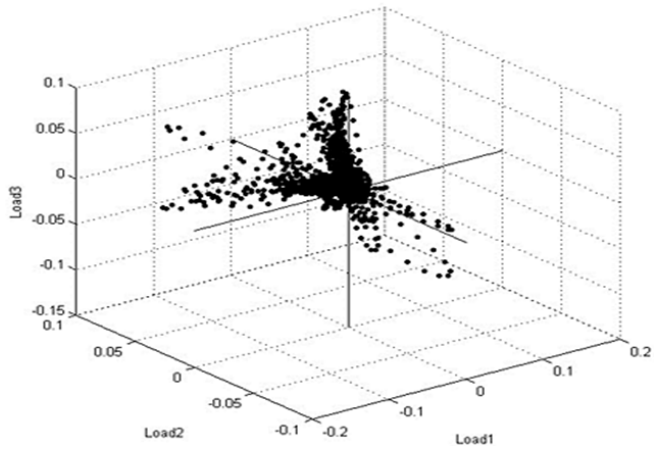

Fig. 5. PCA dimensional plot illustrating the spectral distribution of 92 bacterial strains isolated from RTE food products. Each spot represents a sample's spectral profile, plotted according to the intensity values corresponding to the first three principal components (Loading 1, Loading 2, and Loading 3). The clustering patterns reflect similarities and differences among the bacterial spectra, aiding in species discrimination and classification.

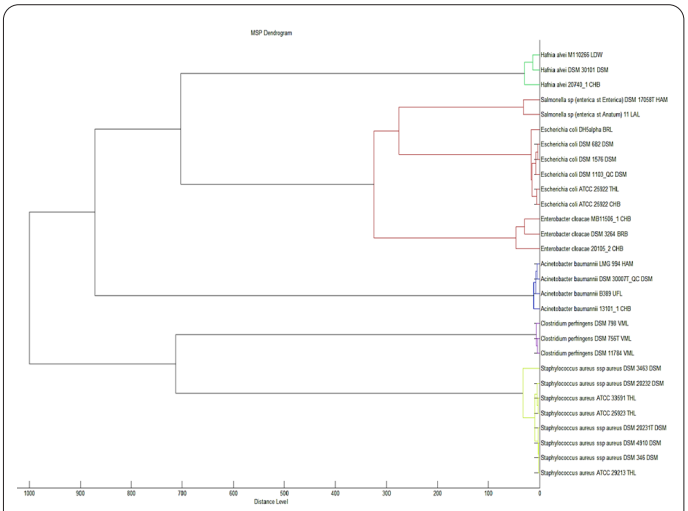

Fig. 6. Minimum Spanning Tree (MSP) dendrogram constructed using Microflex LT Compass Software, showing the clustering of 92 bacterial isolates obtained from RTE food products. The dendrogram illustrates spectral similarity relationships between the test isolates and reference strains from the Bruker database. Isolates formed distinct clusters corresponding to species-level identifications, with a strong correlation observed for 15 reference strains. Notably, closely related strains such as *E. coli*, *S. aureus*, *A. baumannii*, *S. enterica*, *C. perfringens*, *E. cloacae*, and *H. alvei* were clearly differentiated, demonstrating the software's efficacy in taxonomic resolution. Distance levels along the x-axis represent the degree of spectral dissimilarity.

views of 92 well-characterized bacterial isolates obtained from RTE food samples processed in the laboratory. The resulting spectral analysis demonstrated that the majority of peak intensities were distributed within the 2,000--10,000 Da range across all the spectra (Figure 4). Additionally, PCA, a feature of the Compass software integrated with the Microflex LT system, was employed to further analyze the spectral data. PCA serves as a dimensionality reduction technique that enables visualization of the similarities and differences among protein spectra by minimizing dataset variance through algebraic transformation. The 3D PCA plot (Figure 5) displays the clustering of spectral data points, with each dot representing the unique protein profile of an individual bacterial isolate.

To assess the efficacy of Microflex LT Compass Software in distinguishing closely related strains at both the genus and species levels, the spectra of all isolates corresponding to each genus and species were subjected to analysis. This evaluation aimed to determine the software's capability to differentiate between various strains. Subsequently, the spectra were utilized to construct a crosswise minimum spanning tree (MSP) dendrogram based on the aforementioned spectral data. Figure 6 illustrates the process by which the Compass Satellite program generates bacterial spectra for the MSP dendrogram. The MSP dendrogram presented in Figure 6 encompasses 92 isolates of bacterial species, including *E. coli*, *S. aureus*, *A. baumannii*, *S. enterica*, *C. perfringens*, *E. cloacae*, and *H. alvei*. According to the reference strains cataloged in the Bruker library, the constructed dendrogram indicated that 26 evaluated *E. coli* strains were closely associated with 6 reference strains of *E. coli* within the Compass software library. Similarly, 22 *S. aureus* isolates corresponded with 8 reference strains, 15 *A. baumannii* isolates matched with 4 reference strains, 12 *S. enterica* isolates aligned with 2 reference strains, 8 *C. perfringens* isolates correlated with 3 reference strains, 5 *E. cloacae* isolates matched with 3 reference strains, and 4 *H. alvei* isolates were associated with 3 reference strains.

4. Antibiotic susceptibility of foodborne pathogen isolates identified by protein fingerprinting

The antibiotic susceptibility profiles of 26 *Escherichia coli*, 22 *Staphylococcus aureus*, and 15 *Acinetobacter baumannii* isolates recovered from RTE food samples, including chicken shawarma, shish tawook, chicken burgers, and falafel, are summarized in Table 3 and Figure 7. Among the *E. coli* isolates, complete resistance (100%) was observed to ampicillin, amoxicillin-clavulanic acid,

Table 3. The antibiotic resistance profiles of *E. coli*, *S. aureus*, and *A. baumannii* isolates obtained from various RTE food samples.

Antibiotic	Conc. (µg)	*E. coli* (n = 26)				*S. aureus* (n = 22)				*A. baumannii* (n = 15)			
		Susceptible		Resistant		Susceptible		Resistant		Susceptible		Resistant	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	**10**	0	0	26	100	2	9.1	20	90.9	4	26.7	11	73.3
Amoxicillin-clavulanic acid	**20**	0	0	26	100	0	0	22	100	1	6.7	14	93.3
Gentamicin	**10**	17	65.4	9	34.6	15	68.2	7	31.8	11	73.3	4	26.7
Cefoxitin	**30**	0	0	26	100	7	31.8	15	68.2	5	33.3	10	66.7
Cephalothin	**30**	0	0	26	100	0	0	22	100	0	0	15	100
Trimethoprim/ sulfamethoxazole	**25**	18	69.2	6	30.8	20	90.9	2	9.1	12	80	3	20
Nalidixic acid	**30**	14	53.8	12	46.2	0	0	22	100	7	46.7	8	53.3
Norfloxacin	**10**	5	19.2	21	80.8	7	31.8	15	68.2	11	73.3	4	26.7
Amikacin	**30**	19	73.1	7	26.9	2	90.9	20	9.1	5	33.3	10	66.7
Cefepime	**30**	21	80.8	5	19.2	17	77.3	5	22.7	9	60	6	40
Ciprofloxacin	**5**	18	69.2	8	30.8	16	72.7	6	27.3	10	66.7	5	33.3
Chloramphenicol	**30**	15	57.7	11	42.3	9	40.9	13	59.1	6	40	9	60
Tetracycline	**30**	7	26.9	19	73.1	17	77.3	5	22.7	11	73.3	4	26.7
Piperacillin	**100**	14	53.8	12	46.2	11	50	11	50	9	60	6	40
Imipenem	**10**	26	100	0	0	22	100	0	0	14	93.3	1	6.7

cefoxitin, and cephalothin. High resistance rates were also noted for norfloxacin (80.8%) and tetracycline (73.1%). Additionally, moderate resistance was detected for nalidixic acid (46.2%), piperacillin (46.2%), chloramphenicol (42.3%), gentamicin (34.6%), trimethoprim/sulfamethoxazole (30.8%), ciprofloxacin (30.8%), and amikacin (26.9%).

For *S. aureus* isolates, resistance was also high, with 100% of strains resistant to amoxicillin-clavulanic acid, cephalothin, and nalidixic acid. Resistance to ampicillin was detected in 90.9% of the isolates, while 68.2% of the isolates were resistant to both cefoxitin and norfloxacin, and 59.1% were resistant to chloramphenicol. In the case of *A. baumannii* isolates, cephalosporin resistance reached 100%, followed by amoxicillin-clavulanic acid (93.3%) and ampicillin (73.3%). The resistance rates to other agents included cefoxitin (66.7%), amikacin (66.7%), chloramphenicol (60.0%), nalidixic acid (53.3%), and cefepime (40.0%). Across all the tested species, imipenem demonstrated the highest efficacy, with susceptibility rates of 100% for *E. coli* and *S. aureus* and 93.3% for *A. baumannii*. These findings highlight imipenem as the most effective therapeutic option against multidrug-resistant foodborne pathogens identified through MALDI-TOF MS protein fingerprinting.

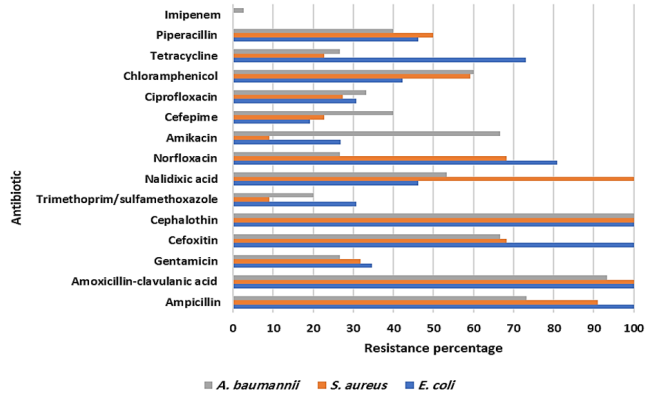

Fig. 7. Comparative antibiotic resistance profiles of E. coli (blue), S. aureus (orange), and A. baumannii (gray) isolates recovered from RTE food samples. The bar graph displays the percentage of resistant isolates to 15 commonly used antibiotics. Notably, all three species exhibited high resistance rates to various antibiotics, with imipenem showing the lowest resistance levels across all tested isolates, indicating its continued efficacy.

4. Discussion

The global market for RTE foods has grown significantly due to their convenience and perceived nutritional value [43]. Despite their popularity, concerns persist among local vendors regarding hygiene and microbial safety [44]. Controlling microbial contamination during processing, transit, and serving is crucial [45]. Improper handling and the introduction of hand microflora during the preparation of RTE items such as sandwiches can introduce foodborne pathogens, raising the risk of food intoxication and poisoning—a threat substantiated by numerous global outbreaks [46].

This study evaluated the microbiological safety, antibiotic resistance patterns, and potential public health threats associated with RTE meals—including chicken shawarma, shish tawook, chicken burgers, and falafel sandwiches—in Al-Qassim, Saudi Arabia. Notably, high prevalence rates of harmful bacteria were observed, including E. coli, S. aureus, A. baumannii, and S. enterica, all of which are associated with foodborne illnesses. The most frequently isolated organism was E. coli (32.5%), followed by S. aureus (27.5%), A. baumannii (18.75%), S. enterica (15%), C. perfringens (10%), E. cloacae (6.25%), and H. alvei (5%).

The microbial loads exceeded regulatory thresholds, thereby posing serious public health concerns. This aligns with previous studies reporting similar bacterial profiles in food samples, including B. cereus, C. perfringens, S. aureus, and Salmonella species [5]. Parallel findings have revealed the presence of S. enterica, E. coli, Klebsiella, Shigella, Enterobacter, S. aureus, B. cereus, and Pseudo-

monas species in a variety of food products [47]. In a comprehensive review and meta-analysis, Mengistu et al. [48] reported that RTE foods in low-income countries frequently exceed acceptable contamination levels, with notable occurrences of S. aureus (30.24%), E. coli (23.8%), and Shigella (34.4%) [49]. This implies that one in every four samples may be contaminated, underscoring a substantial food safety risk. The consumption of contaminated foods elevates the likelihood of foodborne illnesses, which may result in complications beyond acute gastrointestinal distress [50].

A 2021 study [51] investigating 24 RTE sandwiches found that 50% of the 54 bacterial isolates were pathogenic, including Listeria, Staphylococcus, Enterococcus, Yersinia, Aeromonas, and Acinetobacter. The antimicrobial resistance exhibited by these pathogens complicates disease management. Addressing foodborne illnesses demands a multifaceted approach that includes eradicating pathogens at the source, preventing transmission, and safeguarding vulnerable populations [52].

Educating food handlers and consumers is an essential component of a comprehensive food safety strategy. Public awareness campaigns and targeted training programs can significantly reduce foodborne illness incidence and curb the dissemination of antimicrobial-resistant pathogens [12, 13]. Studies have demonstrated that informed food handlers are more likely to implement proper hygiene practices, including handwashing, safe food storage, and temperature control, thereby minimizing contamination risks [13, 14]. Similarly, consumer education on the dangers of antibiotic misuse and the importance of safe food handling is critical to preventing foodborne infections and limiting the spread of antimicrobial resistance through the food chain [52]. Integrating these educational initiatives with existing food safety policies and antimicrobial stewardship programs would enhance their effectiveness and contribute to public health protection.

In Saudi Arabia, there is an urgent need to implement specific countermeasures to prevent food poisoning outbreaks [53]. Notably, food handlers may contribute to the transmission of pathogens such as S. typhi, S. aureus, and E. coli [8], with improper food handling practices further exacerbating pathogen spread.

Globally, pathogenic E. coli strains are a major cause of foodborne illnesses, commonly introduced through contaminated food and water [54]. Contamination typically occurs during slaughter or meat processing, often involving fecal matter from infected animals or humans [55]. S. aureus, known for producing heat-stable enterotoxins, also poses serious food safety risks. These toxins act as superantigens, triggering massive T-cell activation and requiring higher temperatures for inactivation compared to the destruction of the bacteria themselves.

Salmonella species are a leading cause of foodborne illness in the United States, responsible for over one million infections annually, with approximately 19,000 hospitalizations and 380 deaths [56]. These organisms are commonly found in the intestines of cattle and wild animals and are typically transmitted via contaminated foods such as eggs, meats, fruits, and vegetables. C. perfringens ranks as the second most common cause of foodborne illness in the U.S., with an estimated one million cases annually [57]. Key contamination points include restaurants (43%), catering facilities (19%), private homes (16%), and prisons (11%) [57]. Proper storage, cooking, and hygiene practices are critical in minimizing outbreaks [57]. A. baumannii has also been identified in fruits, vegetables, and meat, raising concerns about its dissemination through food and healthcare environments. Medical facility kitchens have been identified as potential sources of antimicrobial-resistant A. baumannii, warranting disinfection of RTE items before consumption [58]. Prompt and accurate detection of foodborne pathogens is, therefore, essential to reducing disease burden.

Mass spectrometry has emerged as a rapid, precise, and cost-effective tool for detecting bacteria, particularly those difficult to identify via traditional phenotypic methods [59]. Its application in food safety is well-documented, particularly with MALDI-TOF MS, which allows for reliable genus- and species-level microbial identification [60]. This method offers multiple advantages: high sensitivity, rapid turnaround (approximately 2 hours for 96 samples), and low cost (approximately $0.50 per sample) [19, 61]. In the current study, 92 bacterial isolates were obtained from 80 RTE samples in Al-Qassim. All isolates were successfully identified with a MALDI-TOF MS score of ≥2.00, achieving 100% identification accuracy.

Comparable results were reported by Jadhav et al., who used MALDI-TOF MS to identify L. monocytogenes, S. enterica, and E. coli O157:H7 in red meat samples [62]. Similarly, a 2022 study identified various foodborne bacteria—including S. aureus, E. coli, H. alvei, Pseudomonas spp., S. enterica, and Aeromonas spp.—in beef and mutton using MALDI-TOF MS with full accuracy [4]. In Turkey, MALDI-TOF MS was effectively used to identify S. aureus in dairy products with over 99% sensitivity and rapid turnaround [63].

A study in Saudi Arabia (Ha'il slaughterhouse) employed MALDI-TOF MS and PCA to differentiate E. coli strains across beef carcasses and their sources, confirming its utility for both identification and epidemiological tracking [64, 65]. In China, MALDI-TOF MS-based PCA was used to analyze 30 strains of six major pathogens, demonstrating its robustness and repeatability without the need for protein extraction [24].

Despite its strengths, MALDI-TOF MS has limitations. High equipment costs can be prohibitive, although lower consumable and labor costs make it economically feasible over time [19, 66]. Additionally, closely related species may be difficult to distinguish, and misidentifications may occur if the reference database lacks certain strain entries [67].

Monitoring antimicrobial resistance among foodborne pathogens is imperative, as such resistance complicates treatment and increases public health risks. This study found E. coli isolates to be highly resistant to multiple antibiotics: 100% resistance to ampicillin, amoxicillin-clavulanic acid, cefoxitin, and cephalothin; 80.8% to norfloxacin; and 73.1% to tetracycline. Resistance was also detected for nalidixic acid (46.2%), piperacillin (46.2%), chloramphenicol (42.3%), gentamicin (34.6%), trimethoprim/sulfamethoxazole (30.8%), ciprofloxacin (30.8%), and amikacin (26.9%). These findings are consistent with previous studies in Saudi Arabia that documented resistance among E. coli strains from imported frozen shrimp [68]. and poultry in Mumbai [35], where resistance was attributed to antimicrobial use in food production [69].

S. aureus isolates also exhibited high resistance rates,

particularly to amoxicillin-clavulanic acid (100%) and nalidixic acid (100%), along with significant resistance to ampicillin (90.9%) and cefoxitin (68.2%). Prior studies in the Al-Qassim region reported similar resistance patterns in S. aureus from fast food establishments, and findings from Cameroon and China have likewise highlighted rising resistance trends in S. aureus from RTE foods [70-73].

Currently, there is a limited number of studies examining the presence of Acinetobacter in food products, particularly in RTE items. Hamilton-Miller and Shah [74] identified two Acinetobacter species in salad vegetables and carrots, highlighting the potential for contamination in fresh produce. In the present study, A. baumannii isolates exhibited a high level of antimicrobial resistance, with 100% resistance to cephalothin, 93.3% to amoxicillin-clavulanic acid, and 73.3% to ampicillin. Additionally, resistance was observed in 66.7% of isolates to both cefoxitin and amikacin, 60% to chloramphenicol, 53.3% to nalidixic acid, and 40% to cefepime.

A previous investigation in Saudi Arabia identified 55 A. baumannii strains from 220 samples of various animal-derived food products [75]. These isolates demonstrated substantial resistance to several antibiotics, including amoxicillin-clavulanic acid (89.10%), gentamicin (74.55%), tetracycline (72.73%), ampicillin (65.45%), and tobramycin (52.73%), underscoring the widespread prevalence of multidrug-resistant A. baumannii in raw meat samples. Recent studies have also documented resistance in Acinetobacter species to nitrofurantoin, cotrimoxazole, and erythromycin [76].

A. baumannii is currently recognized by the World Health Organization (WHO) as a high-priority pathogen on its global list of multidrug-resistant organisms [77]. The potential transmission of Acinetobacter from healthcare environments to environmental sources or agricultural settings underscores the critical need for enhanced surveillance. This global designation by the WHO further highlights the urgency of conducting comprehensive monitoring studies and implementing robust containment strategies.

To mitigate the spread of foodborne pathogens, various control measures have been introduced during the handling and processing of food products. Antimicrobial resistance among foodborne pathogens varies regionally and is influenced by factors such as antibiotic usage, agricultural practices, healthcare infrastructure, environmental conditions, and sociocultural norms. Addressing this issue effectively requires region-specific interventions, which may include strengthened antibiotic stewardship, enhanced food safety protocols, expanded surveillance systems, and targeted public education campaigns.

Within the food industry, ensuring food safety and preventing foodborne illnesses remain paramount objectives. The spread of foodborne diseases is driven by several factors, including shifts in manufacturing practices, globalization, climate change, and the emergence of antibiotic resistance [52]. Food safety must be maintained throughout the entire supply chain, as pathogens can be introduced at any stage [78]. Prevention serves as the cornerstone of effective food safety management. Maintaining rigorous hygiene practices, ensuring thorough sanitation, and employing effective pest control measures are essential to minimize contamination risks during food production.

Implementing Good Manufacturing Practices for food industry personnel—including proper food storage, handling, shipping, and processing protocols—is critical. Moreover, integrating the Hazard Analysis Critical Control Point system at all stages of food processing is vital for ensuring the safety and integrity of food products [79]. Consumer education on safe food handling practices also plays a crucial role in reducing contamination risks. Collectively, these measures help establish high standards for food safety and are essential for protecting public health.

5. limitations

This study has several limitations that should be acknowledged. First, the research was geographically restricted to the Al-Qassim region, which may limit the generalizability of the findings to other regions within Saudi Arabia or beyond. Furthermore, while the sample of 80 ready-to-eat (RTE) food items across four food types provided valuable baseline data, a larger sample size encompassing additional food categories and sampling sites would enhance the robustness and applicability of future studies. Second, although the study included a variety of RTE food items, the limited three-month sampling period may not have captured potential seasonal fluctuations in contamination and antimicrobial resistance patterns, underscoring the importance of longitudinal studies that cover different seasons.

Third, although bacterial identification was effectively performed using MALDI-TOF MS, the study did not include molecular techniques to characterize specific resistance genes or virulence factors. Future investigations should incorporate molecular approaches, such as polymerase chain reaction (PCR) and whole genome sequencing, to provide deeper insights into the genetic basis of antimicrobial resistance, its transmission dynamics, and the potential for horizontal gene transfer among foodborne pathogens. In addition, we did not specifically assess extended-spectrum beta-lactamase (ESBL) or carbapenemase production in Gram-negative isolates, which limits our ability to fully characterize key resistance mechanisms; future studies should include such analyses.

The investigation was also limited to bacterial contaminants, excluding other important foodborne agents such as viruses, parasites, and microbial toxins. Finally, food handling practices at the point of sale were not directly assessed, which could have provided further insight into potential sources of contamination. The study also did not evaluate food handlers' or consumers' knowledge and practices regarding food safety and antibiotic use. Future research should consider incorporating educational assessments and targeted awareness initiatives to support interventions, promote safe food handling, and help curb the spread of antimicrobial-resistant pathogens.

This study revealed a concerning prevalence of pathogenic and potentially multidrug-resistant bacteria in RTE foods collected from the Al-Qassim region of Saudi Arabia. The most frequently isolated microorganisms were *E. coli*, *S. aureus*, *A. baumannii*, and *Salmonella* spp., along with *Klebsiella* spp., *Pseudomonas* spp., *E. cloacae*, *C. perfringens*, and *H. alvei*. Several isolates, including *E. coli*, exhibited resistance to multiple antibiotics, and notable resistance was detected in *S. aureus* and *A. baumannii*. These findings underscore significant public health risks and highlight the urgent need for stricter hy-

giene practices, routine microbial monitoring, and targeted interventions to control the spread of foodborne pathogens and antimicrobial resistance through RTE foods. The use of MALDI-TOF MS in this study proved to be a rapid, accurate, and powerful tool for the identification of bacterial isolates, supporting its value in food safety surveillance and outbreak response. Future surveillance should expand to include larger sample sizes, a broader range of food categories, more food outlets, and longer monitoring periods across different seasons to ensure comprehensive risk assessments and guide effective interventions. Moreover, integrating molecular tools, such as PCR and whole genome sequencing, into future studies will provide critical insights into the genetic determinants of antimicrobial resistance and their transmission dynamics across the food chain. Public health initiatives should also prioritize educational programs for food handlers and consumers to promote safe food practices and raise awareness about the consequences of inappropriate antibiotic use. Finally, our findings support the urgent need for policy measures that enforce stricter food hygiene regulations and promote the rational use of antibiotics in food animal production to mitigate the risk of antimicrobial resistance spread through the food supply. Local authorities should enforce stricter food hygiene regulations for RTE outlets, strengthen microbial monitoring programs (including the use of MALDI-TOF MS), implement food safety and AMR education campaigns, and promote rational antibiotic use in food animal production as essential steps to mitigate the spread of multidrug-resistant pathogens.

Conflict of interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed consent
The authors declare that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request

Authors' contributions
Abdulrahman Almujaidel: Research design and supervision; Abdulrahman Almujaidel, Adil Abalkhail, and Ayman Elbehiry: Perform all laboratory procedures.

Acknowledgments
The authors gratefully acknowledge the financial support of Qassim University, represented by the Deanship of Graduate Studies and Scientific Research, for this research (20040-bhsc-2024-3-4-w) during the academic year 1444 H/2023 AD.

References
1. Alharbi SA, Abdel-Ghaffar MH, Kadher NR (2019) Isolation and identification of pathogenic bacteria from ready-to-eat fast foods in Al-Quwayiyah, Kingdom of Saudi Arabia. Afr J food Agric Nutr Dev 19 (3): 14739-14751. doi: 10.4314/AJFAND.V19I3.17840
2. Ramakrishnan B, Maddela NR, Venkateswarlu K, Megharaj M (2021) Organic farming: Does it contribute to contaminant-free produce and ensure food safety? Sci Total Environ 769: 145079. doi: 10.1016/j.scitotenv.2021.145079
3. Kabiraz MP, Majumdar PR, Mahmud MC, Bhowmik S, Ali A (2023) Conventional and advanced detection techniques of foodborne pathogens: A comprehensive review. Heliyon 9 (4): e15482. doi: 10.1016/j.heliyon.2023.e15482
4. Alzaben F, Fat'hi S, Elbehiry A, Alsugair M, Marzouk E, Abalkhail A, Almuzaini AM, Rawway M, Ibrahem M, Sindi W (2022) Laboratory diagnostic methods and antibiotic resistance patterns of Staphylococcus aureus and Escherichia coli strains: An Evolving Human Health Challenge. Diagnostics 12 (11): 2645. doi: 10.3390/diagnostics12112645
5. Bintsis T (2017) Foodborne pathogens. AIMS Microbiol 3 (3): 529. doi: 10.3934/microbiol.2017.3.529
6. Hoffmann S, Devleesschauwer B, Aspinall W, Cooke R, Corrigan T, Havelaar A, Angulo F, Gibb H, Kirk M, Lake R (2017) Attribution of global foodborne disease to specific foods: Findings from a World Health Organization structured expert elicitation. PloS one 12 (9): e0183641. doi: 10.1371/journal.pone.0183641
7. Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ, Praet N, Bellinger DC, De Silva NR, Gargouri N (2015) World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. PLoS Med 12 (12): e1001923. doi: 10.1371/journal.pmed.1001923
8. Al-Mazrous Y (2004) Food poisoning in Saudi Arabia. Saudi Med J 25: 11-14.
9. Al-Goblan AS, Jahan S (2010) Surveillance for foodborne illness outbreaks in Qassim, Saudi Arabia, 2006. Foodborne Pathog Dis 7 (12): 1559-1562. doi: 10.1089/fpd.2010.0638
10. Lebelo K, Malebo N, Mochane MJ, Masinde M (2021) Chemical contamination pathways and the food safety implications along the various stages of food production: a review. Int J Environ Res Public Health 18 (11): 5795. doi: 10.3390/ijerph18115795
11. Cardoso MJ, Ferreira V, Truninger M, Maia R, Teixeira P (2021) Cross-contamination events of Campylobacter spp. in domestic kitchens associated with consumer handling practices of raw poultry. Int J Food Microbiol 338: 108984. doi: 10.1016/j.ijfoodmicro.2020.108984
12. Altekruse SF, Street DA, Fein SB, Levy AS (1996) Consumer knowledge of foodborne microbial hazards and food-handling practices. J Food Prot 59 (3): 287-294. doi: 10.4315/0362-028x-59.3.287
13. Her ES, Almanza BA, Ma J, Ge L, Liu Y, Lando A, Wu F, Verrill L (2020) Microbial awareness and risk perceptions are key to thermometer ownership and use. Food Control 115: 107268. doi: 10.1016/j.foodcont.2020.107268
14. Abdelhafez AM (2013) Knowledge, attitudes, and practices of food service staff about food hygiene in hospitals in Makkah area, Saudi Arabia. Life Sci J 10: 1097-8135.
15. Mohamed K, Bakri M, AL-Fadil S, AL-Amin F, AL-Taib S, Mohand N, Magdi H (2017) Food Hygiene in Past Ten Years in Saudi Arabia. EC Microbiol 7: 04-13.
16. Wei X, Zhao X (2021) Advances in typing and identification of foodborne pathogens. Curr Opin Food Sci 37: 52-57. doi: 10.1016/j.cofs.2020.09.002
17. Ripolles-Avila C, Martínez-Garcia M, Capellas M, Yuste J, Fung DY, Rodríguez-Jerez JJ (2020) From hazard analysis to risk control using rapid methods in microbiology: A practical

approach for the food industry. Compr Rev Food Sci Food Saf 19 (4): 1877-1907. 10.1111/1541-4337.12592
18. Wenning M, Breitenwieser F, Konrad R, Huber I, Busch U, Scherer S (2014) Identification and differentiation of food-related bacteria: a comparison of FTIR spectroscopy and MALDI-TOF mass spectrometry. J Microbiol methods 103: 44-52. doi: 10.1016/j.mimet.2014.05.011
19. Elbehiry A, Marzouk E, Hamada M, Al-Dubaib M, Alyamani E, Moussa IM, AlRowaidhan A, Hemeg HA (2017) Application of MALDI-TOF MS fingerprinting as a quick tool for identification and clustering of foodborne pathogens isolated from food products. New Microbiol 40 (4): 269-278.
20. Mangal M, Bansal S, Sharma SK, Gupta RK (2016) Molecular detection of foodborne pathogens: A rapid and accurate answer to food safety. Crit Rev Food Sci Nutr 56 (9): 1568-1584. doi: 10.1080/10408398.2013.782483
21. Akkina RC, Payala V, Maganti SS (2022) Tools for rapid detection and control of foodborne microbial pathogens. In: Foodborne Pathogens-Recent Advances in Control and Detection. IntechOpen. doi: 10.5772/intechopen.103938
22. Crotta M, Prakashbabu BC, Holt H, Swift B, Pedada VC, Shaik TB, Kaur P, Bedi JS, Tumati SR, Guitian J (2022) Microbiological risk ranking of foodborne pathogens and food products in scarce-data settings. Food Control 141: 109152. doi: 10.1016/j.foodcont.2022.109152
23. Ramatla T, Ngoma L, Mwanza M (2021) The utility of MALDI-TOF-mass spectrometry, analytical profile index (API) and conventional-PCR for the detection of foodborne pathogens from meat. J Food Nutr Res 9 (8): 442-448. doi: 10.12691/jfnr-9-8-7
24. Yan W, Qian J, Ge Y, Ye K, Zhou C, Zhang H (2020) Principal component analysis of MALDI-TOF MS of whole-cell foodborne pathogenic bacteria. Anal biochem 592: 113582. doi: 10.1016/j.ab.2020.113582
25. Böhme K, Antelo SC, Fernández-No I, Quintela-Baluja M, Barros-Velázquez J, Cañas B, Calo-Mata P (2016) Detection of foodborne pathogens using MALDI-TOF mass spectrometry. In: Antimicrobial Food Packaging. Elsevier, pp 203-214. doi:10.1016/B978-0-12-800723-5.00015-2
26. Cheng K, Chui H, Domish L, Hernandez D, Wang G (2016) Recent development of mass spectrometry and proteomics applications in identification and typing of bacteria. Proteomics Clin Appl 10 (4): 346-357. doi: 10.1002/prca.201500086
27. Álvarez M, Andrade MJ, Núñez F, Rodríguez M, Delgado J (2023) Proteomics as a new-generation tool for studying molds related to food safety and quality. Int J Mol Sci 24 (5): 4709. doi: 10.3390/ijms24054709
28. Ahmed SK, Hussein S, Qurbani K, Ibrahim RH, Fareeq A, Mahmood KA, Mohamed MG (2024) Antimicrobial resistance: impacts, challenges, and future prospects. J Med Surg Public Health 2: 100081. doi:10.1016/j.glmedi.2024.100081
29. Salam MA, Al-Amin MY, Salam MT, Pawar JS, Akhter N, Rabaan AA, Alqumber MA (2023) Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. Healthcare 11 (13): 1946. doi: 10.3390/healthcare11131946
30. Arsène MMJ, Davares AKL, Viktorovna PI, Andreevna SL, Sarra S, Khelifi I, Sergueïevna DM (2022) The public health issue of antibiotic residues in food and feed: Causes, consequences, and potential solutions. Vet World 15 (3): 662. doi: 10.14202/vetworld.2022.662-671
31. Addis M (2015) A review on antibiotic resistant and implication on food chain. J. Food Sci 42: 9-11.
32. Bastam MM, Jalili M, Pakzad I, Maleki A, Ghafourian S (2021) Pathogenic bacteria in cheese, raw and pasteurized milk. Vet Med and Sci 7 (6): 2445-2449. doi: 10.1002/vms3.604
33. González-Gutiérrez M, García-Fernández C, Alonso-Calleja C, Capita R (2020) Microbial load and antibiotic resistance in raw beef preparations from northwest Spain. Food Sci Nutr 8 (2): 777-785. doi: 10.1002/fsn3.1319
34. Crits-Christoph A, Hallowell HA, Koutouvalis K, Suez J (2022) Good microbes, bad genes? The dissemination of antimicrobial resistance in the human microbiome. Gut Microbes 14 (1): 2055944. doi: 10.1080/19490976.2022.2055944
35. Giri S, Kudva V, Shetty K, Shetty V (2021) Prevalence and characterization of extended-spectrum β-lactamase-producing antibiotic-resistant Escherichia coli and Klebsiella pneumoniae in ready-to-eat street foods. Antibiotics 10 (7): 850. doi: 10.3390/antibiotics10070850
36. Koch N, Islam NF, Sonowal S, Prasad R, Sarma H (2021) Environmental antibiotics and resistance genes as emerging contaminants: methods of detection and bioremediation. Curr Res Microb Sci 2: 100027. doi: 10.1016/j.crmicr.2021.100027
37. Hitchins AD, Jinneman K, Chen Y (2017) BAM Chapter 10: Detection of Listeria monocytogenes in foods and environmental samples, and enumeration of Listeria monocytogenes in foods. Bacteriological Analytical Manual [Internet]: 99-103.
38. Maharjan S, Rayamajhee B, Chhetri VS, Sherchan SP, Panta OP, Karki TB (2019) Microbial quality of poultry meat in an ISO 22000: 2005 certified poultry processing plant of Kathmandu valley. Int J Food Contam 6: 1-9. doi:10.1186/s40550-019-0078-5
39. Feng P, Weagant SD, Grant MA, Burkhardt W, Shellfish M, Water B (2002) BAM: Enumeration of Escherichia coli and the Coliform Bacteria. Bacteriological analytical manual 13 (9): 1-13.
40. Sala-Comorera L, Vilaró C, Galofré B, Blanch AR, García-Aljaro C (2016) Use of matrix-assisted laser desorption/ionization–time of flight (MALDI–TOF) mass spectrometry for bacterial monitoring in routine analysis at a drinking water treatment plant. Int J Hyg Environ Health 219 (7): 577-584. doi: 10.1016/j.ijheh.2016.01.001
41. Bauer A (1996) Antibiotic susceptibility testing by a standardized single disc method. Am J of Clinc Path 45: 149-158.
42. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN (2021) Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. J Clin Microbiol 59 (12): 10.1128/jcm. 00213-00221. doi: 10.1128/JCM.00213-21
43. Fang TJ, Wei Q-K, Liao C-W, Hung M-J, Wang T-H (2003) Microbiological quality of 18 C ready-to-eat food products sold in Taiwan. Int J Food Microbiol 80 (3): 241-250. doi: 10.1016/s0168-1605(02)00172-1
44. Stephan R, Althaus D, Kiefer S, Lehner A, Hatz C, Schmutz C, Jost M, Gerber N, Baumgartner A, Hächler H (2015) Foodborne transmission of Listeria monocytogenes via ready-to-eat salad: A nationwide outbreak in Switzerland, 2013–2014. Food control 57: 14-17. doi: 10.5167/uzh-122093
45. Christison C, Lindsay D, Von Holy A (2008) Microbiological survey of ready-to-eat foods and associated preparation surfaces in retail delicatessens, Johannesburg, South Africa. Food Control 19 (7): 727-733. doi: 10.1016/j.foodcont.2007.07.004
46. Nguz K (2007) Assessing food safety system in sub-Saharan countries: An overview of key issues. Food Control 18 (2): 131-134. doi:10.1016/j.foodcont.2005.09.003
47. Gizaw Z (2019) Public health risks related to food safety issues in the food market: a systematic literature review. Environ Health Prev Med 24: 1-21. doi: 10.1186/s12199-019-0825-5
48. Mengistu DA, Belami DD, Tefera AA, Alemeshet Asefa Y (2022) Bacteriological quality and public health risk of ready-to-eat foods in developing countries: systematic review and meta analysis. Microbiol insights 15: 11786361221113916. doi:

10.1177/11786361221113916
49. Mohamed M-YI, Habib I, Khalifa HO (2024) Salmonella in the food chain within the Gulf Cooperation Council countries. AIMS Microbiol 10 (3): 468. doi: 10.3934/microbiol.2024023
50. Hoffmann S, Scallan Walter E (2020) Acute complications and sequelae from foodborne infections: Informing priorities for cost of foodborne illness estimates. Foodborne pathog Dis 17 (3): 172-177. doi: 10.1089/fpd.2019.2664
51. Camellini S, Iseppi R, Condò C, Messi P (2021) Ready-to-Eat sandwiches as source of pathogens endowed with antibiotic resistance and other virulence factors. Appl Sci 11 (16): 7177. doi: 10.3390/app11167177
52. Hassan A, Khan MKI, Fordos S, Hasan A, Khalid S, Naeem MZ, Usman A (2023) Emerging Foodborne Pathogens: Challenges and Strategies for Ensuring Food Safety. Biol Life Sci Forum 31 (1): 32. doi: 10.3390/ECM2023-16596
53. El Sheikha A (2015) Food safety issues in Saudi Arabia. Nutr Food Technol 1 (1): 1-4. doi:10.16966/nftoa.103
54. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB (2013) Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev 26 (4): 822-880. doi: 10.1128/CMR.00022-13
55. García A, Fox JG, Besser TE (2010) Zoonotic enterohemorrhagic Escherichia coli: a one health perspective. ILAR J 51 (3): 221-232. doi: 10.1093/ilar.51.3.221
56. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, Jones JL, Griffin PM (2011) Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17 (1): 7. doi: 10.3201/eid1701.p11101
57. Grass JE, Gould LH, Mahon BE (2013) Epidemiology of foodborne disease outbreaks caused by Clostridium perfringens, United States, 1998–2010. Foodborne Pathog Dis 10 (2): 131-136. doi: 10.1089/fpd.2012.1316
58. Campos A, Lopes MS, Carvalheira A, Barbosa J, Teixeira P (2019) Survival of clinical and food Acinetobacter spp. isolates exposed to different stress conditions. Food Microbiol 77: 202-207. doi: 10.1016/j.fm.2018.09.009
59. Pereira EM, de Mattos CS, Dos Santos OC, Ferreira DC, de Oliveira TLR, Laport MS, de Oliveira Ferreira E, Dos Santos KRN (2019) Staphylococcus hominis subspecies can be identified by SDS–PAGE or MALDI-TOF MS profiles. Sci Rep 9 (1): 11736. doi: 10.1038/s41598-019-48248-4
60. Zou Y, Tang W, Li B (2022) Mass spectrometry imaging and its potential in food microbiology. Int J Food Microbiol 371: 109675. doi: 10.1016/j.ijfoodmicro.2022.109675
61. de Koster CG, Brul S (2016) MALDI-TOF MS identification and tracking of food spoilers and food-borne pathogens. Curr Opin Food Sci 10: 76-84. doi: 10.1016/j.cofs.2016.11.004
62. Jadhav SR, Shah RM, Karpe AV, Morrison PD, Kouremenos K, Beale DJ, Palombo EA (2018) Detection of foodborne pathogens using proteomics and metabolomics-based approaches. Front Microbiol 9: 3132. doi: 10.3389/fmicb.2018.03132
63. Taban BM, Numanoglu Cevik Y (2021) The efficiency of MALDI-TOF MS method in detecting Staphylococcus aureus isolated from raw milk and artisanal dairy foods. CyTA-J Food 19 (1): 739-750. doi:10.1080/19476337.2021.1977392
64. Elabbasy MT, Hussein MA, Algahtani FD, Abd El-Rahman GI, Morshdy AE, Elkafrawy IA, Adeboye AA (2021) MALDI-TOF MS based typing for rapid screening of multiple antibiotic resistance E. coli and virulent non-O157 shiga toxin-producing E. coli isolated from the slaughterhouse settings and beef carcasses. Foods 10 (4): 820. doi: 10.3390/foods10040820
65. Çevik YN, Mursaloğlu PK (2023) Contribution of MALDI-TOF-MS-based principal component analysis for distinguishing foodborne pathogens. J Food Saf 43:: e13053. doi:10.1111/jfs.13053
66. Haider A, Ringer M, Kotroczó Z, Mohácsi-Farkas C, Kocsis T (2023) The current level of MALDI-TOF MS applications in the detection of microorganisms: a short review of benefits and limitations. Microbiol Research 14 (1): 80-90. doi: 10.3390/microbiolres14010008
67. Rychert J (2019) Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. J Infect Dis Epidemiol 2 (4): 1-5. doi: 10.29245/2689-9981/2019/4.1142
68. Alhabib I, Elhadi N (2024) Antimicrobial resistance pattern of Escherichia coli isolated from imported frozen shrimp in Saudi Arabia. PeerJ 12: e18689. doi: 10.7717/peerj.18689
69. Xu C, Kong L, Liao Y, Tian Y, Wu Q, Liu H, Wang X (2022) Mini-Review: Antibiotic-resistant Escherichia coli from farm animal-associated sources. Antibiotics 11 (11): 1535. doi: 10.3390/antibiotics11111535
70. Esemu SN, Njoh ST, Ndip LM, Keneh NK, Kfusi JA, Njukeng AP (2023) Ready-to-Eat Foods: A Potential Vehicle for the Spread of Coagulase-Positive Staphylococci and Antimicrobial-Resistant Staphylococcus aureus in Buea Municipality, South West Cameroon. Can J Infect Dis Med Microbiol 2023 (1): 9735319. doi: 10.1155/2023/9735319
71. Wang W, Li H, Wang C, Li F, Dong Y, Xiao JJ (2023) Antimicrobial resistance, virulence, and genetic characterization of methicillin-resistant Staphylococcus aureus recovered from ready-to-eat (RTE) food in China: a new challenge for food safety. Zoonoses 3 (1): 964. doi: 10.15212/ZOONOSES-2023-0025
72. Lin Q, Sun H, Yao K, Cai J, Ren Y, Chi Y (2019) The prevalence, antibiotic resistance and biofilm formation of Staphylococcus aureus in bulk ready-to-eat foods. Biomolecules 9 (10): 524. doi: 10.3390/biom9100524
73. Qin Y, Wen F, Zheng Y, Zhao R, Hu Q, Zhang R (2017) Antimicrobial resistance and molecular characteristics of methicillin-resistant Staphylococcus aureus isolates from child patients of high-risk wards in Shenzhen, China. Jpn J Infect Dis 70 (5): 479-484. doi: 10.7883/yoken.JJID.2016.328
74. Hamilton-Miller J, Shah S (2001) Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables. Int J Antimicrob Agents 18 (1): 81-83. doi: 10.1016/s0924-8579(01)00353-3
75. Elbehiry A, Marzouk E, Moussa IM, Dawoud TM, Mubarak AS, Al-Sarar D, Alsubki RA, Alhaji JH, Hamada M, Abalkhail A (2021) Acinetobacter baumannii as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic of biofilm formation and phenotypic pattern of antimicrobial resistance. Saudi J Biol Sci 28 (1): 1158-1166. doi: 10.1016/j.sjbs.2020.11.052
76. Alrehaili J, Almarri FK, Kumar S, Mustafa S, Alshehri H, Haque S, Azzi A, Anwer R (2023) Molecular Characterization of Microbial Quality of Ready-to-eat Salads using Multilocus Sequence Typing. J Pure Appl Microbiol 17 (2): 838-848. doi: 10.22207/JPAM.17.2.10
77. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y (2018) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18 (3): 318-327. doi: 10.1016/S1473-3099(17)30753-3
78. Aworh OC (2021) Food safety issues in fresh produce supply chain with particular reference to sub-Saharan Africa. Food Control 123: 107737. doi: 10.1016/j.foodcont.2020.107737
79. Shamloo E, Hosseini H, Moghadam ZA, Larsen MH, Haslberger A, Alebouyeh M (2019) Importance of Listeria monocytogenes in food safety: a review of its prevalence, detection, and antibiotic resistance. Iran J Vet Res 20 (4): 241.