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Frequency and Antimicrobial Resistance Patterns of Foodborne Pathogens in Ready-to-Eat Foods: An Evolving Public Health Challenge

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Abstract: Food products that are ready-to-eat have become increasingly popular in recent years due to their efficiency, affordability, and convenience. However, there are concerns about public health because certain products, particularly animal products, may contain antibiotic-resistant bacteria. This study aimed to quickly and accurately identify foodborne pathogens, such as *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), in samples of shawarma and chicken burgers using peptide mass fingerprinting (PMF) technology. Additionally, the prevalence and levels of antibiotic resistance in the pathogens were determined. The study utilized 300 samples obtained from fast food restaurants in Al Qassim, Saudi Arabia. A variety of methods were used to identify foodborne pathogens, including culture on specific media, bacterial counts by numerical dilutions of homogenized samples, and proteome identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The Kirby–Bauer method was applied to detect the susceptibility and resistance of the bacteria to various antibiotics. PCR was utilized to identify antimicrobial resistance genes such as bla_{TEM}, *tet(A)*, bla_{Z}, and *mecA in S. aureus* and *E. coli* isolates. The percentage of *E. coli*, *S. aureus*, *Salmonella*, *Listeria monocytogenes* (*L. monocytogenes*), *Acinetobacter baumannii* (*A. baumannii*), and *Hafnia alevei* (*H. alevei*) was 34%, 31%, 10.67%, 7.33%, 6.67%, and 4%, respectively. Shawarma samples were found to contain the highest levels of pathogens, compared with chicken burger samples. According to the MBT Compass Flex Series Version 1.3 software, all isolates were identified with 100% accuracy. The log score for MBT identification ranged from 2.00 to 2.56. Among *E. coli* isolates, ampicillin, and penicillin had the highest resistance rate (100%), followed by tetracycline (35.29%). A number of antibiotics were reported to be resistant to *S. aureus*, including nalidixic acid (100%), followed by penicillin (96.77%), piperacillin (45.16%), and norfloxacin (32.26%). Some *E. coli* isolates were susceptible to tetracycline (49.02%), nalidixic acid (47.06%), and piperacillin (43.14%), whereas amikacin was the only drug that was effective against 32.72% of *S. aureus* isolates. The proportions of the bla_{TEM} and *tet(A)* genes in *E. coli* isolates were 55.89% and 45.1%, respectively, whereas *S. aureus* strains did not possess either of these genes. However, 21.5% and 47.31% of *blaz* and *mecA* genes were present among various isolates of *S. aureus*, respectively. In contrast, *E. coli* strains did not possess either of these genes. In conclusion, the fast identification and antimicrobial profiles of the foodborne pathogens were useful in identifying which restaurants and fast food outlets may need to improve their food safety practices. Ultimately, our results will be used to devise targeted strategies to control foodborne pathogens.

Keywords: foodborne pathogens; frequency; identification; antibiotic resistance genes; public health

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1. Introduction

Several types of pathogens can cause foodborne illness, including viruses, bacteria, fungi, and parasites [1,2]. The majority of pathogens transmitted through food are bacteria, which cause a wide array of illnesses in both humans and animals [1,3–7]. Among the most common bacteria found in food are *Salmonella*, *Shigella* species, *Listeria monocytogenes*

(*L. monocytogenes*), *Bacillus* species, *Yersinia* species, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Campylobacter* species, and *Vibrio cholera* [8]. Several stages can lead to food contamination, including the environments in which animals are raised and vegetables are cultivated and harvested [9]. Contamination can also occur during the manufacturing process, such as shipment, processing, or handling. Additionally, cross-contamination can occur due to the people who prepare or consume food [10,11].

The Global Burden of Disease Study estimates that 0.1% of people worldwide die as a result of eating contaminated food, with 420,000 deaths occurring annually and over 33 million healthy lives lost [12,13]. The World Health Organization (WHO) estimates that more than half of all foodborne infections lead to diarrhea, resulting in approximately 2 million deaths each year [14]. According to a 2018 study by the World Bank, diseases transmitted through contaminated food are estimated to cost low- and middle-income countries around $95 billion, with treatment for foodborne illnesses alone costing approximately $15 billion annually [15]. In Saudi Arabia, the Food and Drug Authority has developed a coordinated strategy to ensure the safety of food sold by family-owned food trucks and improve the knowledge and practices of food truck vendors. This strategy involves implementing essential legislation. Under this legislation, food vendors would be required to adhere to food safety and cleanliness requirements, ultimately reducing the risk of foodborne illnesses [16].

The use of antimicrobial agents is frequently employed to treat infectious diseases in both humans and animals [17–20]. However, their use has resulted in the development of multidrug-resistant microorganisms, which may be difficult to treat and potentially life-threatening [21,22]. Thus, using antimicrobial agents responsibly and taking measures to prevent their misuse is important. Recently, a serious concern has been raised about the inappropriate and unchecked use of antibiotics in animal medicine [20,23,24]. This has led to the development of multidrug-resistant bacteria, which are transmitted to humans as a result of food consumption. As a result, there is an urgent need for stricter regulations on the use of antibiotics in animal medicine [25]. A major consequence of leftover antibiotics in food products derived from animals is the spread of bacteria that are resistant to antibiotics [26,27].

The existence of multidrug-resistant microorganisms in food products may be responsible for foodborne diseases that are difficult to treat in humans [28,29]. Over time, these bacteria may accumulate and cause infections that are resistant to common antibiotics. The risk of foodborne illness can therefore be reduced by taking preventive measures [18,19,30,31]. As a result, when an antibiotic-resistant strain infects a host, the genes can be passed on to microorganisms inside and outside of the host [32].

The increase in drug resistance is recognized as a global problem [33]. In the animal husbandry and health sectors, it has been demonstrated that the proper use of antimicrobials is critical. According to these studies, the number of diseases caused by bacteria that are multidrug-resistant and are acquired from numerous sources, including food chains, is on the rise [34–36]. A higher risk of therapeutic complications and unsatisfactory clinical results can result from the difficulty in obtaining therapeutic medications due to antimicrobial resistance [37].

There has been a great deal of concern about the public health implications associated with the existence of extended-spectrum antibiotic resistance genes in bacteria, including extended-spectrum beta-lactamases [38,39]. By expressing these genes, bacteria can become resistant to certain antibiotics, making the treatment of diseases caused by these bacteria difficult [40]. Due to industrialization, people are increasingly interested in using ready-to-eat foods [41]. These products may be prepared under low-hygiene conditions by people handling food [42], making them potential vectors of bacterial foodborne infections [43]. Meat is a great source of essential nutrients like protein, vitamin B, phosphorus, and zinc, which are all necessary for proper growth and development [44]. Furthermore, meat

contains healthy fats that prevent inflammation and raise cholesterol, which is conducive to the growth of bacteria [45].

The majority of foodborne zoonotic diseases are transmitted through food originating from animals, such as meat and its derivatives. Numerous cases of food poisoning and related human deaths have been linked to *Salmonella*, *L. monocytogenes*, *E. coli*, *S. aureus*, and other zoonotic bacteria worldwide [46–48]. Several health issues, such as meningitis, septicemia, and gastroenteritis can be caused by these bacteria, which can be spread from contaminated food, drink, and surfaces to other people. In order to reduce the risk of disease transmission, proper food safety practices must be followed.

Pathogenic bacteria resistant to antibiotics may also spread through meat and its derivatives [49,50]. In recent research [51,52], multidrug-resistant pathogens have been identified in animal products, highlighting the importance of tracking the prevalence of multidrug-resistant pathogens and informing policy makers of the severity of this problem, as well as the effectiveness of preventive measures [30,52]. In the Al Qassim region of Saudi Arabia, antimicrobial resistance levels for ready-to-eat foodborne pathogens have rarely been evaluated, and there is still a lack of knowledge regarding the relationship between antibiotic resistance phenotypes and resistance genes in pathogenic organisms isolated from ready-to-eat meat products.

It is therefore the aim of the current investigation to determine whether fast food samples collected from fast food outlets in the Al Qassim region are contaminated with multidrug-resistant *E. coli*, *Salmonella*, *S. aureus*, and *L. monocytogenes*. We hypothesize that these bacteria will be present in the samples, and that the level of contamination will vary significantly between outlets. A phenotypic and proteomic analysis of these bacteria was also performed, as well as an examination of genes associated with antibiotic resistance, including bla_{TEM} and *tet*(*A*) in the case of *E coli* isolates and *blaz* and *mecA* in the case of *S. aureus* isolates. The findings of this study provide valuable insights into the risk of antibiotic-resistant bacteria in fast food.

2. Materials and Methods

2.1. Sample Collection

A total of 300 samples of chicken burgers and shawarma sandwiches were collected from fast food outlets in the Saudi Arabian region of Al Qassim from January 2023 to June 2023. Immediately after collection, the specimens were transferred to the lab under preservation (4 °C). The investigation was performed in the College of Public Health and Health Informatics at Qassim University's microbiology laboratory for public health.

2.2. Total Bacterial Count

A sterile Pulsifer bag was filled with 25 g of chicken burgers or shawarma samples and 225 mL of sterile peptone water containing 0.1% peptone [53]. To homogenize the contents of the bags, they were placed on the stomacher (Thomas Scientific, Swedesboro, NJ, USA) and homogenized for two minutes. We created numerical dilutions of the homogenized samples with 9 mL of peptone water containing 1% peptone. In this experiment, one ml of each dilution was used as a solution on Count Agar (Merck, Darmstadt, Germany). Observed colonies were used to calculate colony counts after two days of incubation at 37 °C [54].

2.3. Pathogenic Bacterial Isolation and Identification

For the isolation of various foodborne pathogens, the FDA approach was used [55]. This approach involves three stages: pre-enrichment, enrichment, and detection. Pre-enrichment involves concentrating the pathogens from a large sample, enrichment involves growing the pathogens, and detection involves testing for the pathogens. The samples were tested for *L. monocytogenes* using Listeria-enrichment broth with Listeria-Selective Enrichment Supplement, whereas *E. coli* was tested using Lauryl tryptose (LST) broth (Merck Group, Darmstadt, Germany). The enrichment broth and LST broth were incubated

at 37 °C for 48 h. *Salmonella* were cultivated on specific media, such as Hektoen Enteric Agar, Xylose Lysine Deoxycholate Agar (XLD), and Bismuth Sulphite Agar (BSA), all of which were purchased from Oxoid, Basingstoke, UK. *S. aureus* was further isolated using Baird–Parker agar as a selective medium. The samples were observed for any signs of bacterial growth. If bacterial growth was detected, further testing was conducted to determine the type of bacteria.

2.4. MALDI-TOF Spectrometry Based Screening of Foodborne Pathogens

In accordance with the ethanol–formic acid and acetonitrile extraction protocol developed by Bruker Daltonics in Bremen, Germany, MALDI-TOF MS [56] was used to identify different isolates of *E. coli*, *Salmonella*, *S. aureus*, *L. monocytogenes*, and *A. baumannii* from the shawarma and chicken burger samples. The isolates were identified based on their mass spectra, and the spectra were compared to previously defined spectra. The identifications were confirmed by a molecular method using real-time PCR.

2.5. Susceptibility Test for Antimicrobials

Susceptibility testing to antimicrobial agents was conducted according to the Kirby–Bauer method, as recommended by the Clinical Lab Standards Institute (CLSI) [57]. The test was conducted using the recommended concentration of antimicrobial agents and plates, and the zone of inhibition was utilized to detect the vulnerability of the bacteria to the antimicrobial agent. According to CLSI's descriptive categorization and zone diameter breakpoints, inhibitory zone diameters were classified as resistant, moderate, or susceptible. This classification helps to guide the clinician in determining the most appropriate antimicrobial therapy for a patient. The antimicrobial drug classes that are most popularly used in the healthcare of people and animals were chosen for the study. Trypticase Soy Broth (TSB) (Sigma Aldrich, Burlington, MA, USA) was used as the incubation medium for the isolates. The turbidity was measured using a Sensititre™ Nephelometer (Thermo Fisher Scientific, Waltham, MA, USA) and recorded as 0.5 McFarland. The colonies were spread distinctly on Müller–Hinton agar (Sigma Aldrich) and incubated at 37 °C for 24 h with the antibiotic discs positioned on the agar at 3 cm intervals. An analysis of the zones of growth inhibition was conducted in accordance with CLSI [58] criteria.

2.6. Molecular Detection of Antibiotic Resistance Genes for Foodborne Pathogens

2.6.1. Extraction of Nucleic Acid

DNA extraction was accomplished using the DNeasy kits as stated by the producer's directions (QIAGEN, Hilden, Germany) [59]. At room temperature, the DNase treatment (QIAGEN) was carried out for 30 min to break the double-stranded DNA molecules into single strands by degrading the helical structure of the DNA. This process is necessary for the DNA to be further processed and used in molecular biology experiments. The enzyme was then inactivated by adding an equal volume of Stop Solution. The sample was centrifuged, and the supernatant was collected, thus making the sample ready for the next step.

2.6.2. Primers and Standards for Real Time PCR

The Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was employed to identify the antibiotic resistance genes of *S. aureus* and *E. coli*. To recognize the existence of antibiotic resistance genes (bla_{TEM}, *tet*(*A*), *blaz* and *mecA*), the isolates were tested using the designated primers (Table 1). Primers were selected based on the OIE criteria. There was a total of 15 µL of oasigTM or PrecisionPLUSTM 2 qPCR Master Mix used in this procedure, along with 1 µL of primer and probe combination, and 4 µL of RNAse/DNAse-free water, making up the concentration of the reaction mix.

Table 1. Recognition of antimicrobial resistance genes in *E. coli* and *S. aureus* using primer sets.

Gene Target	Oligonucleotide Sequence	Base Pair (bp)
bla_{TEM}	F: ATC AGC AAT AAA CCA GC R: CCC CGA AGA ACG TTT TC	516
tet(A)	F: GGTTCACTCGAACGACGTCA R: CTGTCCGACAAGTTGCATGA	577
blaz	F: TGA CCA CTT TTA TCA GCA ACC R: GCC ATT TCA ACA CCT TCT TTC	700
mecA	F: AAA ATC GAT GGT AAA GGT TGG C R: AGT TCT GCA GTA CCG GAT TTG C	532

A final volume of 20 µL was generated in each well after adding 15 µL of the solution mixture and 5 µL of DNA template to each well. In addition, 5 µL of RNAse/DNase free water was applied for the negative control wells. Five tubes were labeled and used to produce a standard curve dilution series, each receiving 90 µL of template preparation buffer. The positive control template from tube 1 was transferred to tube 2 for proper mixing, with 10 µL from this tube then transferred to tube 3, and so on, until all the tubes had been used. After transferring 5 µL of the standard template into each well, the final volume was 20 µL.

In the following step, amplification was conducted using 7500 Fast real-time PCR equipment. To prevent PCR contamination, AmpErase® Uracil-N-glycosylase (UNG) was heated for 15 min at 37 °C and then for two minutes at 95 °C. UNG will degrade any uracil residues in DNA, preventing contamination of the sample. It can also be used to remove contaminating primers from the reaction and carryover from previous reactions. A total of 40 cycles were performed, each involving ten seconds of denaturation, and 60 s of annealing and extension processes at 60 °C. Sequence detection system software was used to analyze the data.

3. Results

3.1. Total Bacterial Count

According to the study results, shawarma sandwiches (n = 150) and chicken burger samples (n = 150) had mean values for total colony-forming units per gram (CFU/g) of 7.32 ± 0.26 log CFU/g and 5.98 ± 0.43 log CFU/g, respectively. The shawarma sandwiches had a significantly higher CFU/g (p-value = 0.002) than the chicken burgers. There was also no discernible difference between the CFU/g values of the chicken burgers and shawarma sandwiches (p-value = 0.235).

3.2. Different Types of Foodborne Pathogens and Their Prevalence

According to the results shown in Table 2, *E. coli* was found to be the most prevalent harmful microorganism observed in the shawarma sandwich samples (38.67%). Among the samples tested, 32% contained *S. aureus*, 12.67% contained *Salmonella*, 8.67% contained *L. monocytogenes*, 8% contained *A. baumannii*, and 4.67% contained *H. alevei*. The samples of the tested chicken burgers were found to be contaminated with *E. coli*, *S. aureus*, *Salmonella*, *L. monocytogenes*, *A. baumannii*, and *H. alvei* at 29.33%, 30%, 8.67%, 6%, 5.33%, and 3.33% respectively. According to the analysis, the percentage of *E. coli*, *S. aureus*, *Salmonella*, *L. monocytogenes*, *A. baumannii* and *H. alevei* isolated from shawarma and chicken burger fast food was 34%, 31%, 10.67%, 7.33%, 6.67%, and 4%, respectively. The bacteria listed above are all pathogenic, which means that they are capable of causing illnesses.

Table 2. Frequency of various foodborne pathogens in samples taken from shawarma sandwiches (n = 150) and chicken burgers (n = 150).

Foodborne Pathogen	Positive Shawarma Samples		Positive Chicken Burger Samples		Total (n = 300)	
	No.	%	No.	%	No.	%
E. coli	58	38.67	44	29.33	102	34.00
S. aureus	48	32.00	45	30.00	93	31.00
Salmonella	19	12.67	13	8.67	32	10.67
L. monocytogenes	13	8.67	9	6.00	22	7.33
A. baumannii	12	8.00	8	5.33	20	6.67
Hafnia alevei	7	4.67	5	3.33	12	4.00

3.3. Identification of Foodborne Pathogens Using Protein Fingerprinting

The analysis of the isolated bacteria was carried out with the MALDI Biotyper (MBT) equipment, and the produced spectra were compared to those available in the MBT database. The MBT database contains the spectral profiles of recognized bacterial species, and the bacteria were identified on the basis of the results of the analysis. Based on the MBT results, specific details regarding the strains' species and subspecies were provided. According to the MBT Compass software, 100% of the 102 *E. coli* isolates, 93 *S. aureus* isolates, 32 *Salmonella*, 22 *L. monocytogenes*, 20 *A. baumannii*, and 12 *H. alvei* isolates were identified. MBT was able to identify all isolated bacteria in the current investigation, with a log score ranging from 2.00 to 2.56. According to this analysis, the MBT is capable of correctly detecting bacteria. It was observed that several spectrum proteins were distributed throughout the zone's initial bands, ranging from 3000 to 11,000 Daltons (Da), with the strongest peak signals located between 4100 and 9900 Da for the majority of *E. coli* strains (Figure 1). In the analysis of *S. aureus* proteins, the peak signals ranged from 3000 to 18,000 Da, and robust signals were observed between 4300 and 6900 Da. As seen in (Figure 2), all the identifiable strains of *S. aureus* were synchronized with the eight reference strains kept in the MBT's Compass software library.

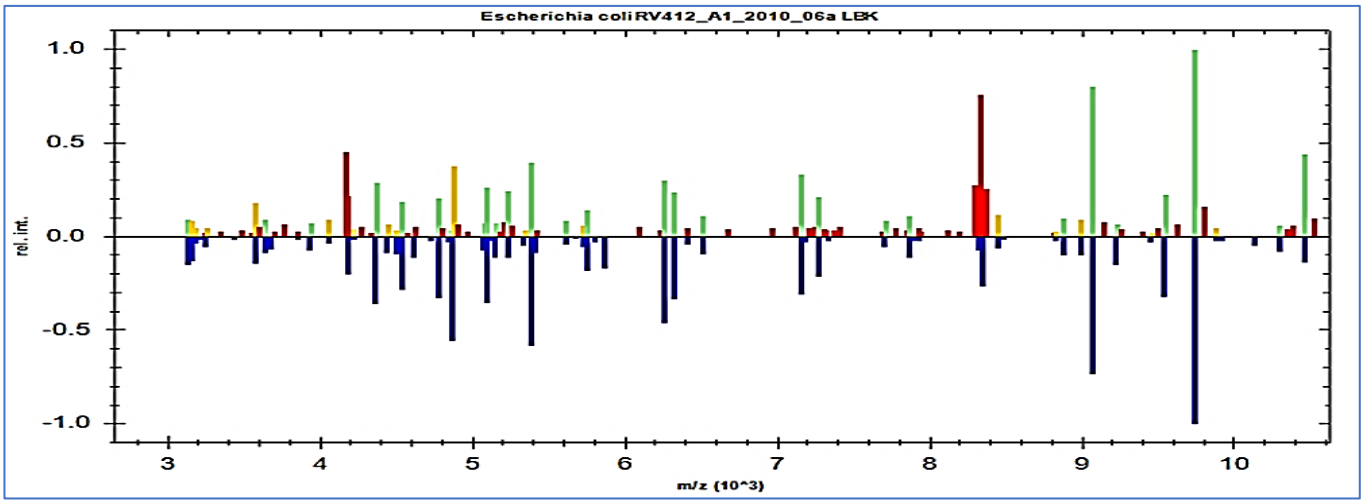

Figure 1. A spectral protein profile analysis of *E. coli* isolated from fast food samples compared to the reference strain RV412_A1_2010_06a LBK maintained in the MBT is shown. The lower parts of the spectra are colored blue, indicating that the stored spectra in the Compass library were used to match the pattern; the upper parts of the spectra are colored green, indicating that the peaks were excellently matched. The upper yellow and red colors of the spectra are characterized by mismatched and intermediate peaks, respectively.

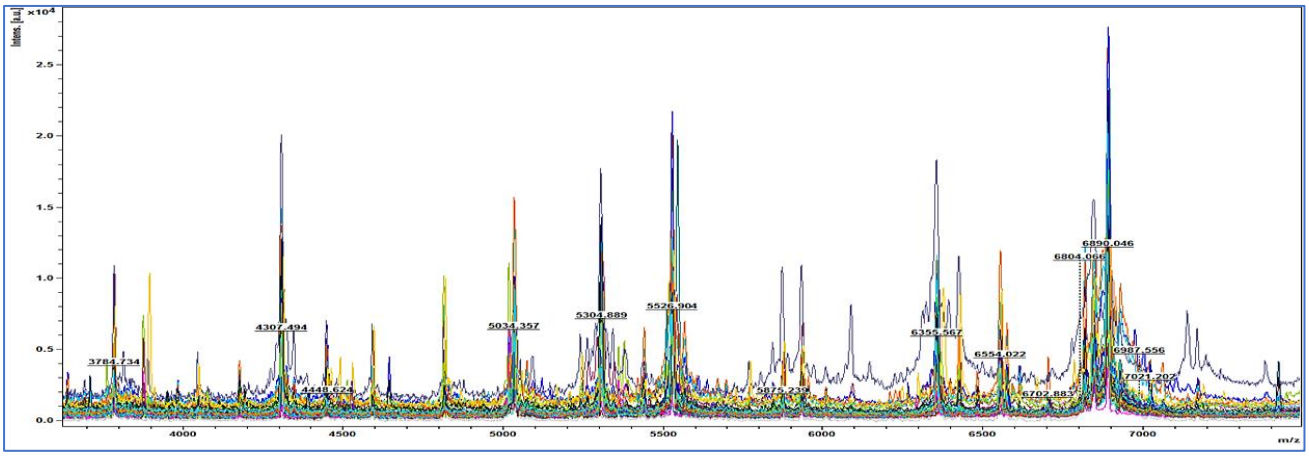

Figure 2. Using Compass Satellite Software of the MBT, 93 *S. aureus* isolates from fast food were compared to 8 *S. aureus* reference strains. Various peak intensities were detected between 3000 m/z to 7000 m/z.

3.4. The Antibiotic Resistance Patterns of E. coli and S. aureus Isolates

The pattern of antibiotic resistance for *E. coli* isolates from samples of Shawarma and chicken burgers can be seen in Table 3 and Figure 3A,B. In terms of antibiotic resistance rates among *E. coli*, β-lactam penicillins (ampicillin and penicillin) had the highest rate (100%), followed by tetracyclines (tetracycline, 35.29%). A number of antibiotics were reported to be resistant to *S. aureus*, including quinolones (nalidixic acid, 100%), followed by β-lactam penicillins (penicillin, 96.77% and piperacillin, 45.16%), and a fluoroquinolone (norfloxacin, 32.26%).

Table 3. The antibiotic resistance of *E. coli* and *S. aureus* isolates recovered from samples of shawarma and chicken burgers.

Antibiotic Class	Conc.	*E. coli* (*n* = 102)						*S. aureus* (*n* = 93)					
		Inhibition Zone Breakpoints (mm)			% of Isolates/Total Isolates			Inhibition Zone Breakpoints (mm)			% of Isolates/Total Isolates		
		S	I	R	R	I	S	S	I	R	R	I	S
Aminoglycoside													
Gentamicin	10 µg	≥27	19–26	≤18	0	98.04	1.96	≥28	19–27	≤17	0	100	0
Amikacin	30 µg	≥27	19–26	≤18	0	68.63	31.37	≥27	20–26	≤19	2.76	64.52	32.72
Quinolones													
Nalidixic acid	30 µg	≥29	22–28	≤21	0	52.94	47.06	≥29	22–28	≤21	100	0	0
Fluoroquinolone													
Norfloxacin	10 µg	≥36	28–35	≤27	0	96.08	3.92	≥29	17–28	≤16	32.26	67.74	0
Ciprofloxacin	5 µg	≥41	30–40	≤29	0	100	0	≥31	22–30	≤21	21.51	73.12	5.38
Tetracyclines													
Tetracycline	30 µg	≥26	18–25	≤17	35.29	15.69	49.02	≥31	24–30	≤23	26.88	64.52	8.60
B-lactam penicillins													
Penicillin	10 IU	≥15	-	≤14	100	0	0	≥15	-	≤14	96.77	0	3.23
Ampicillin	10 µg	≥15	16–22	≤15	100	0	0	≥36	27–35	≤26	100	0	0
Piperacillin	100 µg	≥31	24–30	≤23	0	56.86	43.14	≥31	24–30	≤23	45.16	54.84	0

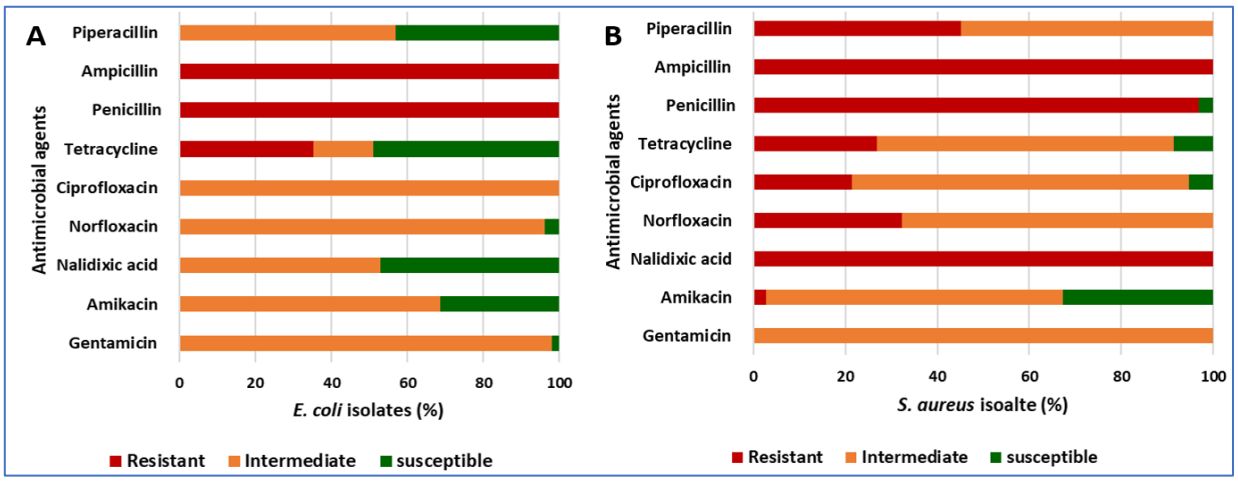

Figure 3. Antibiotic resistance pattern of *E. coli* (**A**); and *S. aureus* (**B**) isolates.

3.5. The Frequency of Antibiotic Resistance Genes among E. coli and S. aureus Isolates

Using the PCR test results obtained in Table 4 and Figure 4, the incidence levels of resistance genes were determined for isolates of *E. coli* and *S. aureus*. Among the various isolates of *E. coli* isolated from both samples of shawarma and chicken burgers, the proportions of the bla_{TEM} and *tet(A)* genes were 55.89% and 45.1%, respectively. *S. aureus* strains, on the other hand, do not possess either of these genes. There were 21.5% and 47.31% of *blaz* and *mecA* genes present among the various isolates of *S. aureus* isolated from shawarma and chicken burgers, respectively. In contrast, *E. coli* strains did not possess either of these genes.

Table 4. The frequency of antimicrobial resistance genes in *E. coli* and *S. aureus* isolates recovered from shawarma and chicken burgers samples.

Foodborne Pathogen		Antibiotic Resistance Genes							
		bla_{TEM}		*tet(A)*		bla_z		*mecA*	
		No.	%	No.	%	No.	%	No.	%
E. coli	Total (N = 102)	57	55.89	46	45.1	0	0	0	0
	Shawarma (N = 58)	33	56.9	27	46.55	0	0	0	0
	Chicken burger (N = 44)	24	54.55	19	43.18	0	0	0	0
S. aureus	Total (N = 93)	0	0	0	0	20	21.5	44	47.31
	Shawarma (N = 48)	0	0	0	0	9	18.75	23	47.92
	Chicken burger (N = 45)	0	0	0	0	11	22.92	21	43.75

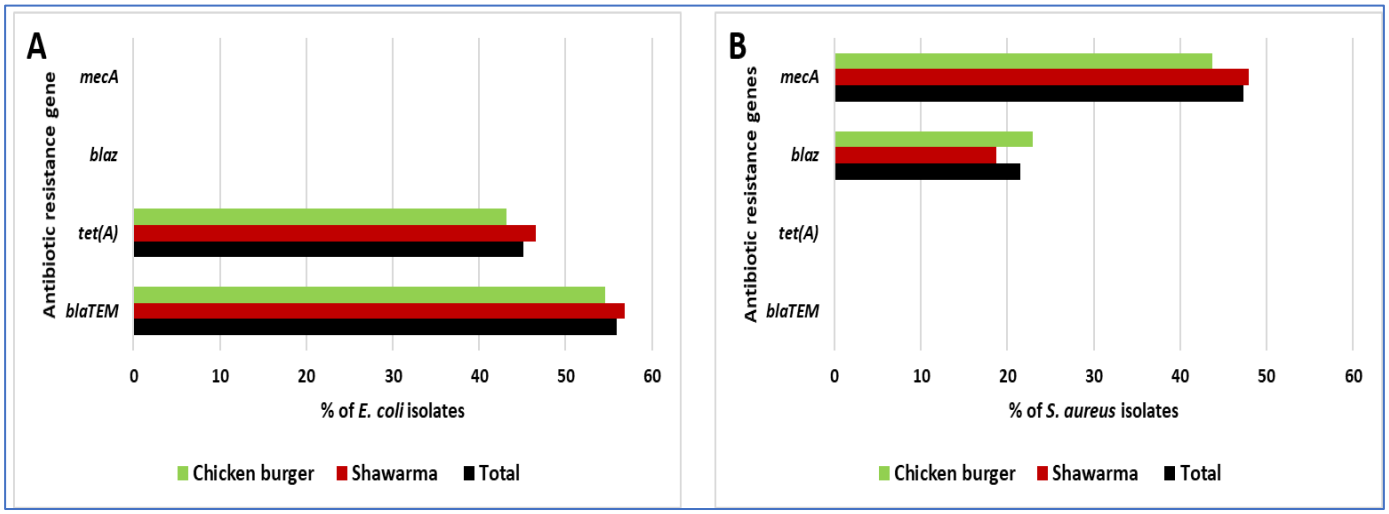

Figure 4. Distribution of antibiotic resistance genes in *E. coli* (**A**); and *S. aureus* (**B**) strains.

4. Discussion

In the current investigation, we examined the possibility of pathogenic microorganism contamination in chicken burgers and shawarma. Pathogenic microorganisms, such as *E. coli*, *Salmonella*, *S. aureus*, and *Listeria*, can be found in chicken meat and can cause foodborne illnesses. We tested samples to determine the presence of bacteria, distribution of resistance genes, and patterns of antimicrobial resistance amongst *E. coli* and *S. aureus* isolates. The results revealed that some of the samples had high levels of pathogenic microorganisms and antibiotic resistance genes. Proper food safety and cleanliness practices should be followed when handling chicken burgers and shawarma.

According to an outbreak-tracking summary for foodborne diseases in the United States between 2009 and 2015, food outlets were reported to be the most common location for outbreaks to have occurred at one site of preparation. In terms of location, these are followed by food establishments (14%), and houses (12%). A lack of resources prevents them from managing food properly, which has likely increased contamination [20,59]. Among the leading causes of food contamination are cross-contamination, contact with dangerous bacteria, viruses, and parasites, as well as improper handling and storage [8,60–62]. The study found that many meals were contaminated with high levels of bacteria, including *S. aureus* and *E. coli*. When meat products are exposed to significant levels of bacterial contamination, their characteristics are likely to change quickly [20,63,64]. The total bacteria count (TBC) is an indicator of hygienic quality and can also be used to assess the quality of food [30,65].

In recent years, there has been an increasing understanding that enteric viruses can cause illnesses in humans. However, it is currently not possible to detect all types of pathogenic viruses in food and water [66]. Enteric bacteria are often used to identify fecal contamination and enteric virus infections due to their high prevalence in the human intestine [66]. The high concentration of enteric viruses in infected individuals' feces is a significant concern for the general public. These viruses have specific characteristics that make them highly resistant to unfavorable conditions and many forms of water treatment. As a result, they have a very low infectious dosage and a high potential for contamination across different environmental sectors [67]. Some scientists have argued [68] that the presence of fecal bacteria indicators in food or water, such as enterococci and fecal coliform bacteria, does not necessarily indicate the presence of enteric viruses. Surprisingly, enteric viruses have been found in aquatic settings at alarmingly high percentages in some cases [69], even when bacterial indicator levels are within guidelines [70].

According to the recommendations made by the European Union, the bacteriological threshold for meat products is 6.7 log CFU/g [71,72]. In this study, the average TBC values

were 7.32 ± 0.26 log CFU/g and 5.98 ± 0.43 log CFU/g in the shawarma and chicken burger samples, respectively. The TBC levels discovered in this analysis are consistent with those found in earlier studies [30,73–76]. Poor processing conditions, including insufficient temperature control or the inadequate cleaning of equipment, can result in raw materials being contaminated with animal products or environmental toxins [77].

In order to determine whether ready-to-eat meals contained *E. coli*, a fecal indicator of food safety, samples of chicken burgers and shawarma were taken. Testing results indicated that the chicken burgers and shawarma samples contained *E. coli*, raising the possibility that eating them could cause foodborne illness [78–81]. Previous studies have shown lower levels of *E. coli* contamination in beef samples from Saudi Arabia (22%) [82], Ethiopia (6%) [83], as well as hamburger samples from Portugal (20%) [43] and Ethiopia (6%) [84]. Food safety standards in these nations, including more testing, stronger laws, and better sanitation in slaughterhouses and meat processing factories, are believed to have contributed to the decline in *E. coli* contamination levels. However, other researchers have reported higher contamination rates, such as 88% in Ghanaian beef samples and 100% in beef products from northwest Spain such as minced meat and hamburgers.

E. coli contamination of animal-derived foods is considered a sign of problems during preparation, storage, transportation, or delivery [85,86]. While temperatures above 55 °C can easily kill *E. coli* in food, there have been significant cases of contamination reported in these foods, indicating a high level of abuse [87]. This includes contamination with fecal matter and cross-contamination with cooked foods. Therefore, *E. coli* is often seen as a sign of contamination in the feces of infants and young children, as well as food poisoning and gastroenteritis in adults [88,89]. Even in low concentrations, *E. coli* contamination of food poses a threat comparable to Salmonella contamination.

During this investigation, strains of *E. coli*, *S. aureus*, *Salmonella*, *A. baumannii*, *L. monocytogenes*, and *H. alvei* were isolated. The MBT Compass software accurately identified all these bacterial isolates at a rate of 100%, with a log value of >2.00. Our research lab has demonstrated that MBT is a highly accurate instrument for identifying microorganisms at the genus and species levels, including fungi, bacteria, and yeasts. The use of mass spectrometry in the field of food safety permits the identification of pathogens quickly and precisely [90–92]. Due to its high sensitivity and the requirement of only one sample, it is easy to use and detects even minute levels of microbes [8]. The results of this study are consistent with several previous studies that used MBT as a validation tool for identifying foodborne pathogens, such as *E. coli*, *Salmonella*, *Staphylococcus*, *Aeromonas*, and *Pseudomonas* species [62,93–96]. The MBT procedure is also fast and economical, making it an attractive choice for the detection of foodborne illnesses. A further advantage of MBT is its cost-effectiveness, which is approximately $0.50 per sample when deployed in the food safety supply chain [97,98]. Consequently, MBT can be used for the detection of foodborne pathogens.

Using MALDI-TOF MS to identify bacteria has some disadvantages, including the requirement for specialized equipment and technical expertise that may not always be available in laboratories. A further limitation of this method is that it frequently fails to distinguish between closely related or poorly defined bacteria. Another possible disadvantage is the price of the MBT. The use of this method might, however, be sufficient in laboratories that are using MBT to detect food products. The use of MBT can reduce sample costs since consumables are so inexpensive and labor costs can be reduced as well [62]. MBT is considered a chemotaxonomic technique because it primarily relies on the similarities and differences between specific biomarkers [98]. The development of the MBT's algorithm for the automated recognition of various isolates utilized a robust and reliable identification approach.

There are several pathogenic bacteria, including *E. coli*, that cause nosocomial infections. Studies have shown that *E. coli* is the major cause of diarrhea [99–102]. Despite the presence of various bacterial species in the human gut, *E. coli* is more resistant to antibiotics than other Enterobacteriaceae [103,104]. Therefore, antibiotic-resistant strains of *E. coli*

present a significant challenge in the global fight against infectious diseases. The genes responsible for antibiotic resistance can be transferred between different species of bacteria. Thus, a species resistant to an antibiotic may be able to transmit its resistance to unrelated species. Consequently, antibiotic resistance can spread throughout the food chain and between individuals [105]. In this investigation, the different isolates of *E. coli* showed the highest level of resistance (100%) to ampicillin and penicillin. They also exhibited 35.29% resistance to tetracycline. Additionally, 55.89% of the isolates had the bla_{TEM} resistance gene, while 45.1% had the *tet(A)* resistance gene. This indicates that the *E. coli* isolates were highly resistant to ampicillin and penicillin, and moderately resistant to tetracycline. The presence of the bla_{TEM} and *tet(A)* genes likely played a significant role in the development of antibiotic resistance in *E. coli*, which could explain the high rates of resistance to ampicillin and penicillin observed in this study.

A previous study by Hemeg [82] indicated that *E. coli* isolates recovered from meat samples were completely resistant to penicillin and amoxicillin–clavulanic acid. Nearly all amoxicillin–clavulanic-acid-resistant strains of *E. coli* contained the bla_{TEM} gene. Alegra et al. [106], reported that over 80% of *E. coli* isolates from food samples carried the bla_{TEM} gene. Similarly, Ramadan et al. [107] found that *E. coli* strains from Egypt exhibited the highest resistance to ampicillin. These isolates also showed resistance to gentamicin, tetracycline, and sulfamethoxazole/trimethoprim. These findings emphasize the importance of closely monitoring antibiotic-resistant bacteria in retail ground beef.

The prevalence rate of *S. aureus* isolated from shawarma and chicken burger samples was 30%, as shown by our findings. Similar results have been reported in other studies on *S. aureus* contamination of meat. Studies conducted in Poland [108] and Tehran [109] found that 20% and 25% of hamburger samples, respectively, were contaminated with *S. aureus*. Arafa et al. [110] conducted an investigation in Egypt and found that 10% of beef burger samples and 30% of minced beef meat samples tested positive for *S. aureus* contamination. Several publications have noted that *S. aureus* isolates are highly resistant to penicillin, ampicillin, nalidixic acid, and tetracycline [111–113], which aligns with the findings of our antimicrobial susceptibility test. Another study by Çetinkaya and Elal Mus [114], found that *S. aureus* isolates recovered from various meals, including uncooked meatballs, were resistant to penicillin (62.9%) and ampicillin (59.3%). A study conducted in the Iranian region of Isfahan using raw retail meat samples found that *S. aureus* isolates showed resistance to multiple antibiotics, including tetracycline (79%), penicillin (73%), and doxycycline (41.7%) [115].

In the current study, it was found that 21.5% of the *S. aureus* isolates possessed the bla_Z gene, while 47.31% had the *mecA* gene. Previous studies conducted by Shahraz et al. [109] and Chajcka-Wierzchowska et al. [108] on hamburger samples found that all methicillin-resistant *S. aureus* isolates had the *mecA* gene. Another study by Arafa et al. [110] discovered that 85.7% of *S. aureus* isolates in minced beef and burger samples had both the *mecA* and bla_Z genes. Baghbaderani et al. [115] also found that 58.33% of isolates from raw retail meat samples carried the bla_Z gene. These two genes play a crucial role in the development of antibiotic resistance in *S. aureus* found in fast food, as well as their ability to survive in food and potentially cause foodborne illnesses.

During the food manufacturing process, antibiotic resistance genes can be transferred via a variety of channels, which raises concerns about food safety [116]. Genetic material can be exchanged and communicated between bacteria throughout the food supply chain. Determining the extent of antimicrobial resistance transmission in food chains is challenging, and little attention has been given to the role of food in the transfer of antibiotic resistance genes, as reported by Andreoletti et al. [117]. Conversely, the presence of antibiotic-resistant bacteria in food can have a harmful impact on human health. The food chain provides numerous opportunities for contamination to be transferred among animals, food handlers, and the surrounding environment [116].

There are various factors contributing to the increased spread of genes associated with antibiotic resistance from their natural environments to pristine and clinical set-

tings [118,119]. These factors include bacterial characteristics, such as selective stress from antimicrobial agents, as well as societal and technological changes that aid in the transmission of resistant bacteria [120]. Several studies have shown that antibiotic-resistant bacteria can develop in the environment [121,122], and horizontal gene transfer can also take place [123]. A community of bacteria may acquire resistance genes due to selective pressure [124]. Antimicrobial agents are widely distributed in the environment, which puts stress on microorganisms and leads to horizontal gene transfer and the exchange of resistance genes as a response to microbial adaptability [121]. In a healthcare environment, for instance, different genera of microorganisms interact with mobile genetic components, allowing for the exchange of genes that can result in the emergence of complex resistance to a wide range of antibiotics [118,119].

There has been an increase in consumer awareness of food safety in recent years, as well as a need for professionals in the food service industry who possess expert knowledge, the right attitude, and practical skills to preserve the quality of food and reduce the risk of foodborne illness [125,126]. Employees in the food sector work in areas such as food preparation, food service, and retail. These workers can contribute to the spread of food disorders either symptomatically or asymptomatically [127]. Food personnel play a critical role in society. In a survey conducted in the Southwest USA to assess food safety knowledge among catering staff, it was found that 71.5% of employees were aware of food hygiene and safety [128]. In Saudi Arabia, numerous organizations are dedicated to enhancing and expanding the understanding and practice of food safety through educational programs, training, seminars, and activities held in public venues such as malls, schools, and homes [129].

5. Conclusions

According to the findings of this study, meat products that are frequently consumed, such as shawarma and chicken burgers, may contain harmful foodborne pathogens like *E. coli* and *S. aureus*. Certain diseases are frequently resistant to the most commonly prescribed antibiotics. Further, improperly prepared ready-to-eat foods could pass these infections on to other foods, rendering them unsafe for human consumption. In order to guarantee the manufacture of secure ready-to-eat beef products, it is crucial that sanitary procedures are followed, appropriate oversight is in place, and rules are followed. To gain a comprehensive understanding of antibiotic resistance in foodborne diseases, future studies should investigate the possibility of spreading drug-resistant organisms in other ready-to-eat foods. Another suggestion for further research is to conduct serotyping investigations on the isolates in order to identify the precise subtypes of antibiotic-resistant bacteria that are frequently found in ready-to-eat meat products.

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