

Isolation and identification of pathogenic bacteria from ready-to-eat chicken shawarma in Duhok district

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Abstract

Since many people eat sandwiches as part of the global food culture, examining the microbiological quality and hygienists are crucial steps in determining the risk factors for food-borne illnesses. This study investigated the bacterial contamination in 51 chicken shawarma sandwiches randomly selected from local restaurants in Duhok district. The results showed that *E. coli* was the predominant bacterial species isolated from the sandwiches (59.40%), thus, it has been suggested to represent the highest source of bacterial contamination in the screened samples. followed by *proteus* (15.60%), *Salmonella* (10.90%), *Shigella* (6.30%), *Pseudomonas* (4.70%), and *Klebsiella* (3.10%). The bacterial sensitivity to the antibiotics showed that all tested pathogens were mostly resistant to the results showed that all species have shown the highest rate of resistance was seen in CAZ, CTX, AX, were 100% followed by AM, TE in the same ratio (98.4%) and, CRO, AK, ATM (96.9, 95.3, 93.8%) respectively. The results of this study demonstrated that the bacterial contamination in the tested sandwiches were relatively high and were produced under low hygienic conditions. This was supported by the finding that the increased bacterial count in the sandwiches and the high resistant level to antibiotic have indicated a high-risk factor for acquiring food-borne illnesses.

Keywords: Ready to eat foods, Chicken shawarma bacterial contamination, Antibiotic resistance, Duhok district

Introduction

Shawarma and other ready-to-eat meat products are among the most important food categories that are more vulnerable to microbial contamination because they are prepared, served, and distributed in public or crowded areas that frequently lack the basic hygienic conditions required for any food establishment (Asiegbu *et al.*, 2016). Bacteria are the most common pathogens spread through food, and they can cause a variety of diseases in both people and animals (Elbehiry *et al.*, 2021; Bintsis, 2017). In food, there are several common bacteria that are encountered such as *Salmonella* species, *Shigella* species, *Listeria monocytogens*, *Bacillus* species, *Yersinia* species, *Campylobacter* species, *Clostridium botulinum*, *Clostridium perfringens*, *Echerichia coli*, *Staphylococcus aureus* and *Vibrio cholera* (Cremonesi *et al.*, 2014; Jam *et al.*, 2025). 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 (Kamala & Kumar, 2018;

Cardoso *et al.*, 2021). Every year, one-third of the world's population is afflicted by food-borne illnesses. Both developed and developing nations, including the United States, have spent billions of dollars treating food-borne illnesses that impact over 48 million patients annually (Scallan *et al.*, 2011). Annually, diarrheal illnesses caused by food and water cause the deaths of about 2.2 million people worldwide (WHO, 2006). One of the most important steps for preventing foodborne infections is to ensure that the production line is run logically, high standards for cleanliness are implemented and biocides and sanitizers are used as frequently as possible (Sillankorva *et al.*, 2012).

Widespread antibiotic-resistant infections that can be challenging to cure and even fatal are caused by the widespread use of antibiotics in poultry as growth promoters or therapeutic prophylactics in livestock (Schroeder *et al.*, 2004, Moghavvemi *et al.*, 2025). Food contamination by antibiotic-resistant bacteria may also pose a considerable concern to public health

since antibiotic resistance determinants can be passed on to other pathogenic bacteria, limiting the treatment of serious bacterial infections. Antimicrobial resistance in food-borne illnesses has increased during the previous several decades. (Van *et al.*,2007). Thus, utilizing antimicrobial drugs appropriately and taking precautions to avoid their overuse is critical. Recently, a substantial worry has been raised concerning the inappropriate and uncontrolled use of antibiotics in animal treatment (Rajaei *et al.*,2021; Om & McLaws, 2016). Therefore, this study aimed to isolation and identification of food pathogens. Whether collected samples from fast food outlets in the Duhok district are contaminated with multidrug-resistant. This study offers vital information regarding the possibility of antibiotic-resistant microorganisms in fast food.

2. Materials and Methods

2.1 Sample collection

A total of 51 experimental samples of shawarma sandwiches (more popular) were collected randomly from 51 different local restaurants as served to the consumer at 7 am to 11:59 am from fast food outlets in Duhok district from January 2025 to May 2025. Immediately after collection, in an ice box the specimens were transferred to the lab. The investigation was performed within less than one hour from the collection in the Duhok Technical Institute at Duhok Polytechnical University's microbiology laboratory.

2.2 Sample processing and pathogenic bacterial isolation and identification

The obtained samples were completely blended upon arrival at the laboratory, and 50 g from each sample was added to 90 ml of buffered peptone water (BPW) and incubated aerobically at 37°C for 18-24 hr. followed by 1 ml of BPW incorporated to 9 ml of Rappaport broth and incubated overnight at 42°C. A loopful of broth streaked onto the different media (MacConkey agar, Xylose Lysine Deoxycholate (XLD) agar, Blood agar).

Pure colony sub-cultured on various media to identify it morphologically such as Eosin Methylene Blue (EMB) agar to identify *E. coli* by green metallic

sheen and pink colonies on MacConkey agar.

Cetrimide Agar used for isolation and identification of *Pseudomonas* by the typical yellow-green to blue color which indicates the production of pyocyanin. *Proteus* species exhibit distinctive swarming behavior on blood agar. *Klebsiella* colonies appear large, mucoid, and red with diffusing red pigment on MacConkey agar. In addition, to using XLD agar, *Salmonella Shigella* (SS) agar used. For further identification (IMVIC, Oxidase test, TSI) was conducted to determine the type of bacteria.

2.3 Susceptibility test for antimicrobials.

All confirmed strains of pathogenic bacteria were subjected to antibiotic susceptibility tests using Kirby-Bauer disc diffusion method by spreading the inoculated sterile swab on Mueller-Hinton agar incubated overnight aerobically at 37°C according to (Hudzicki, 2009). Eighteen antibiotic discs were utilized, supplied by (Bioanalyses, Turkey), including Amikacin (AK; 10 mg), Cefotaxime (CAZ; 30 mg), Amoxicillin (AX; 25 mg), Cefotaxime (CTX; 30 mg), Nitrofurantoin (F; 100 mg), Gentamicin (CN; 10 mg), Imipenem (IPM; 10 mg), Tetracycline (TE; 10 mg), Ciprofloxacin (CIP; 10 mg), Amoxicillin/Clavulanic acid (AMC; 30 mg), Aztreonam (ATM; 30 mg), Ceftriaxone (CRO; 10 mg), Ampicillin (AM; 25 mg), Chloramphenicol (C; 10 mg), Cefixime (CFM; 5 mg), Norfloxacin (NOR; 30 mg), Azithromycin (AZM;15 mg) and Meropenem (MEM; 10 mg).

The diameter of the inhibition zone around antibiotic disks was measured according to the Clinical and Laboratory Standards Institute (CLSI, 2000).

3. Result

3.1 Different types of foodborne pathogens and their prevalence

According to the results shown in Figure 1, *E. coli* was found to be the most prevalent bacteria observed in the shawarma sandwich samples (38 isolates, 59.4%). Among the samples tested, 10 *Proteus* isolates (15.6%), 7 *Salmonella* isolates (10.9%), 4 *Shigella* isolates (6.3%), 3 *Pseudomonas* isolates (4.7%), and 2 *Klebsiella* isolates (3.1%).

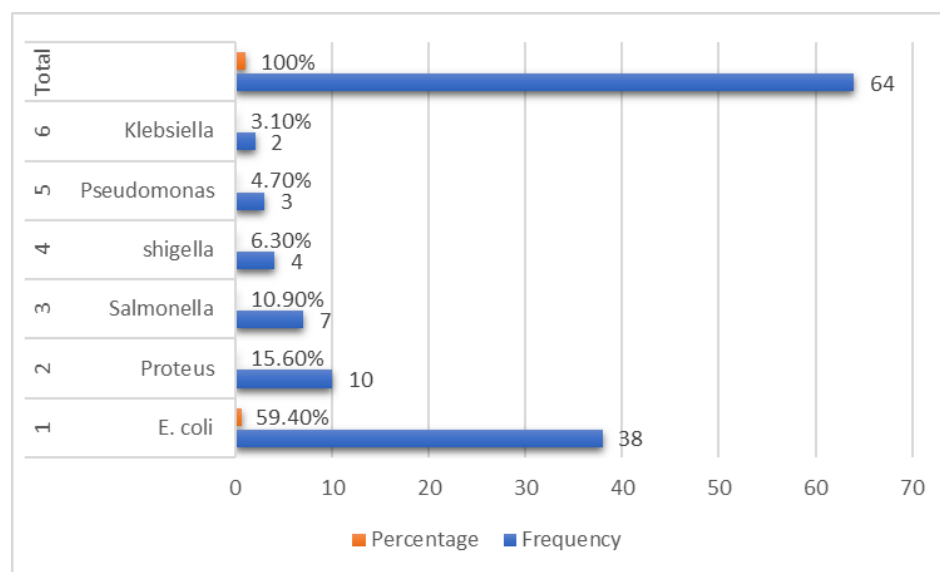


Figure 1: Frequency of various foodborne pathogens isolated from shawarma sandwiches.

Table 2. Illustrates the details of foodborne pathogens distribution pattern among of isolated from shawarma sandwiches. The main point of interest is that 13 of the collected samples had more than one strains of pathogens, 5 out of them had *E. coli*, *Proteus*, and 3 samples had *Salmonella*, *E. coli*. Furthermore, 2 samples possess *Proteus*, *Salmonella* and, the other 2 samples possess *Proteus*, *Pseudomonas*. Moreover, one of the collected samples possess *Shigella* and *E. coli*.

3.2 Antibiotic resistance profiles

The pattern of antibiotic resistance for all bacterial isolates from samples of chicken Shawarma can be seen in Table 1. The antibiotic resistance profiles of the isolated bacteria were varied according to the type of bacteria. Overall, the results showed that all species have shown the highest rate of resistance was seen in CAZ, CTX, AX were %100 followed by AM, TE in the same ratio (98.4%) and, CRO, AK, ATM (96.9, 95.3, 93.8%) respectively. Nevertheless, analyzing each bacterial resistance on its own reveals that *E. coli* was highly resistance to each of CAZ, CTX, AX, and AK was 100% followed by CRO, AM and TE with the equal proportion (97.4%) and, AMC, MEM were (89.5, 81.6%) respectively. In addition, the susceptibility of *E. coli* was 73.7% for IPM and NOR and moderate sensitive to CIP (52.6%). On the other

hand, *proteus* was exhibited complete resistance to CAZ, CTX, AM, AX, TE and 90% for each of CRO, AMC, AK, ATM followed by CN 80% and C, MEM 70% and, AZM, F were 60, 50% respectively. The isolated *proteus*, on the other hand, was moderately sensitive to CIP and F and sensitive (90, 80%) to NOR and IPM, respectively. The isolated *salmonella* was complete resistance to eight of the antibiotics that used in this study which are CAZ, CTX, CRO, AMC, AM, AX, TE, AK followed by CN, C, ATM with the identical ratio 85.7% and, AZM, MEM was 71.4% In contrast, the sensitive rate was (71.4, 57.1%) for each of IPM, CIP respectively. intriguingly, in the current study the isolated *shigella* was exhibited complete resistance to 12 of antibiotics out of 18 which is CFM, CAZ, CTX, CRO, AMC, AM, AX, TE, AZM, AK, F, ATM followed by CN, MEM same Proportional 75%. However, it were sensitive to CIP, NOR in equal fraction 75%. On the other hand, the isolated *pseudomonas* displayed absolute resistance to each of the CAZ, CTX, CRO, AMC, CN, AM, AX, TE, AZM, ATM followed by CIP 66.7%. While the bacteria was sensitive for CFM, IPM, NOR, C, AK, F, MEM in Same scale 66.7%. The current study showed that the isolated *Klebsiella* demonstrated total resistance to CAZ, CTX, CRO, AMC, AM, AX, TE, AK, MEM, ATM. In the same line the isolated *Klebsiella* was entirely sensitive to IPM, CIP, NOR, F followed by mildly sensitive for CFM, C and moderately intermediate for CN, AZM.

Table1: The prevalence of all isolated bacteria from chicken shawarma and details of each strain for antibiotic resistance profiles. Keys: antibiotics include; CAZ: Cefotaxime, AX: Amoxicillin, F: Nitrofurantoin, CN: Gentamicin, IPM: Imipenem, AM: Ampicillin, CFM: Cefixime, TE: Tetracycline, AK: Amikacin, CTX: Cefotaxime, CN: Gentamicin, CIP: Ciprofloxacin, AMC: Amoxicillin/Clavulanic acid, ATM: Aztreonam, C: Chloramphenicol, NOR: Norfloxacin, MEM: Meropenem, AZM: Azithromycin.

Bacteria		Antibiotic Sensitivity Test																		Total
		CFM	CAZ	CTX	IPM	CRO	AMC	CN	AM	CIP	NOR	AX	C	TE	AZM	AK	F	MEM	ATM	
<i>E. coli</i>	R	27(71.1)	38(100)	38(100)	5(13.2)	37(97.4)	34(89.5)	21(55.3)	37(97.4)	13(34.2)	8(21.1)	38(100)	30(78.9)	37(97.4)	30(78.9)	38(100)	29(76.3)	31(81.6)	36(94.7)	38
	S	8(21.1)	0	0	28(73.7)	1(2.6)	1(2.6)	10(26.3)	1(2.6)	20(52.6)	28(73.7)	0	7(18.4)	1(2.6)	4(10.5)	0	9(23.7)	7(18.4)	2(5.3)	
	I	3(7.9)	0	0	5(13.2)	0	3(7.9)	7(18.4)	0	5(13.2)	2(5.3)	0	1(2.6)	0	4(10.5)	0	0	0	0	
<i>Proteus</i>	R	8(80)	10(100)	10(100)	1(10)	9(90)	9(90)	8(80)	10(100)	2(20)	1(10)	10(100)	7(70)	10(100)	6(60)	9(90)	5(50)	7(70)	9(90)	10
	S	2(20)	0	0	8(80)	1(10)	0	1(10)	0	5(50)	9(90)	0	3(30)	0	3(30)	1(10)	5(50)	3(30)	1(10)	
	I	0	0	0	1(10)	0	1(10)	1(10)	0	3(30)	0	0	0	0	1(10)	0	0	0	0	
<i>Salmonella</i>	R	4(57.1)	7(100)	7(100)	0	7(100)	7(100)	6(85.7)	7(100)	3(42.9)	3(42.9)	7(100)	6(85.7)	7(100)	5(71.4)	7(100)	4(57.1)	5(71.4)	6(85.7)	7
	S	3(42.9)	0	0	5(71.4)	0	0	1(14.3)	0	4(57.1)	2(28.6)	0	1(14.3)	0	2(28.6)	0	3(42.9)	2(28.6)	1(14.3)	
	I	0	0	0	2(28.6)	0	0	0	0	2(28.6)	0	0	0	0	0	0	0	0	0	
<i>shigella</i>	R	4(100)	4(100)	4(100)	1(25)	4(100)	4(100)	3(75)	4(100)	1(25)	1(25)	4(100)	1(25)	4(100)	4(100)	4(100)	4(100)	3(75)	4(100)	4
	S	0	0	0	1(25)	0	0	0	0	3(75)	3(75)	0	1(25)	0	0	0	0	1(25)	0	
	I	0	0	0	2(50)	0	0	1(25)	0	0	0	0	2(50)	0	0	0	0	0	0	
<i>Pseudomonas</i>	R	1(33.3)	3(100)	3(100)	1(33.3)	3(100)	3(100)	3(100)	3(100)	2(66.7)	1(33.3)	3(100)	1(33.3)	3(100)	3(100)	1(33.3)	1(33.3)	1(33.3)	3(100)	3
	S	2(66.7)	0	0	2(66.7)	0	0	0	0	1(33.3)	2(66.7)	0	2(66.7)	0	0	2(66.7)	2(66.7)	2(66.7)	0	
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Klebsiella</i>	R	1(50)	2(100)	2(100)	0	2(100)	2(100)	1(50)	2(100)	0	0	2(100)	1(50)	2(100)	1(50)	2(100)	0	2(100)	2(100)	2
	S	1(50)	0	0	2(100)	0	0	0	0	2(100)	2(100)	0	1(50)	0	0	0	2(100)	0	0	
	I	0	0	0	0	0	0	1(50)	0	0	0	0	0	0	1(50)	0	0	0	0	
Total	R	45(70.3)	64(100)	64(100)	8(12.5)	62(96.9)	59(92.2)	42(65.6)	63(98.4)	21(32.8)	14(21.9)	64(100)	46(71.9)	63(98.4)	49(76.6)	61(95.3)	43(67.2)	49(76.6)	60(93.8)	64
	S	16(25.0)	0	0	46(71.9)	2(3.1)	1(1.6)	12(18.8)	1(1.6)	35(54.7)	46(71.9)	0	15(23.4)	1(1.6)	9(14.1)	3(4.7)	21(32.8)	15(23.4)	4(6.3)	
	I	3(4.7)	0	0	10(15.6)	0	4(6.3)	10(15.6)	0	8(12.5)	4(6.3)	0	3(4.7)	0	6(9.4)	0	0	0	0	

Table2: Representation of foodborne pathogens distribution pattern among of isolated from shawarma sandwiches.

Bacteria	No. of Strains
<i>Proteus</i> + <i>Salmonella</i>	2
<i>E. coli</i> + <i>Proteus</i>	5
<i>proteus</i> + <i>pseudomonas</i>	2
<i>shigella</i> + <i>E. coli</i>	1
<i>salmonella</i> + <i>E. coli</i>	3
Total	13

Discussion

Since chicken shawarma is more widely available and less costly than beef shawarma, we chose it in the current investigation. Additionally, the majority of the unsold shawarma from the previous night is held over until the next morning. Therefore, we wanted to gather samples early in the morning. In order to identify the pathogenic bacteria that are present and their patterns of antimicrobial resistance amongst all isolated microorganism. The results revealed that some of the samples had high levels of pathogenic microorganisms and antibiotic resistance. Food contamination has become a serious public health hazard around the world, resulting in high morbidity and mortality rates. Infectious diarrhea is reported globally as 3-5 billion cases yearly, with roughly 1.8 million fatalities, primarily in young children (Thanigaivel and Anandhan 2015). The results are concerning, as the majority of tested samples were positive for harmful microorganisms. Food contamination is generally caused by cross-contamination, exposure to hazardous bacteria, viruses, and parasites, as well as inappropriate handling and storage techniques (Elbehiry *et al.*, 2023; Yemmireddy and Hung, 2017; Augustin *et al.*, 2020). In general, bacteria are the most prevalent cause of foodborne illness, and some Bacteria can be found on foods when purchased. Meat contamination during slaughter is a major source of bacterial contamination for consumers (Hassan, 2006 and Mohamed, 2010). The total bacteria count is an indicator of sanitary quality and can also be used to evaluate the quality of food (González-Gutiérrez *et al.*, 2020; Møretrø and Langsrud, 2017). Food contamination with pathogens occurs at any stage of food processing, preparation, storage, handling, distribution, and retail marketing (Ibrahim *et al.*, 2014).

This study found a high threshold of *E. coli* contamination (59.40%). *E. coli*, a member of the Enterobacteriaceae family, is primarily found in the digestive tracts of both humans and animals their existence in food indicates unsanitary conditions and fecal contamination (Bumyut *et al.*, 2021; Parvez *et al.*, 2017). These findings were in agreement with the recent results obtained by several studies in Iraq, Duhok governorate (Taha *et al.*, 2023), Saudi Arabian region of Al Qassim (Abalkhail, 2023), and in

Northern Jordan (Nimri *et al.*, 2014). These finding were disagreement with study conducted in Benghazi city, Libya and highlights the notion that *Klebsiella pneumoniae* was the predominant bacterial species isolated from the sandwiches.

As for the other bacterial species that were isolated in the current study, *Proteus* spp. (15.60%), *Salmonella* spp. (10.90%), *Shigella* spp. (6.30%), *Pseudomonas* spp. (4.70%), and *Klebsiella* spp. (3.10%). The majority of these bacteria cause meat deterioration or food poisoning either directly through the secretion of bacterial toxins or indirectly through the creation of biotoxins such biogenic amines (Kung *et al.*, 2010). This is possibly a consequence of the majority of poultry meats were imported into Iraq from various sources. Meat from chickens is stored in freezers, but there is no constant power supply in local markets that use fuel-powered generating sets, so these meats may be exposed to growth-contaminated pathogens that may have originated from contaminated chicken carcasses by their gut containing *Salmonella* and *E. coli*, which were considered to be major foodborne pathogens. Either, their existence could be attributed to the fact that shawarma skewers are often constructed with thick skewers, and the heat from the grilling stage cannot penetrate to the inside, leaving it undercooked and raw product inside the Shawarma skewer core without eliminating the pathogens. Furthermore, 13 of the collected samples had more than one species of microorganism. This could be due to cross-contamination during processing and handling procedures, including storing, cutting, weighing, and packaging are the most critical points during which shawarma can be easily contaminated by various types of bacteria (Zhang *et al.*, 2016; Beshiru *et al.*, 2022; Asiegbu *et al.*, 2016).

Regarding antibiotic resistance characteristics, all isolated microorganism had an increased resistance rate and were all found to be multi-drug resistant (MDR). This concerning resistance could be linked to the simple availability and haphazard application of antibacterial drugs in the veterinary sector, as well as the need for the prophylactic or therapeutic use of subtherapeutic levels in animals consumed by humans (Manyi-Loh *et al.*, 2018; Al-Bahry *et al.*, 2014). For instance, tetracycline is widely administered to animal feed at relatively low doses

for therapeutic and prophylactic purposes, as well as to increase growth, which may select resistant bacterial strains (Michalova & Schlegelova 2004). This was in line with current study in general all isolated microorganism showed 98.4% were resistance to tetracycline. The presence of MDR in local RTE foods is a public health concern since these foods can act as possible vectors for pathogenic bacteria bearing the MDR characteristic, allowing these resistant bacteria or genes to travel to people via food and establish themselves in the gut flora (Guo *et al.*, 2019; Koch *et al.*, 2017).

Antibiotic-resistant microorganisms in food can endanger human health. The food chain creates various opportunities for contamination to spread across animals, food handlers, and the surrounding environment (Wu-Wu *et al.*, 2023). Antimicrobial drugs are widely dispersed in the environment, putting stress on bacteria and causing horizontal gene transfer and the sharing of resistance genes as a reaction to microbial adaptability (Djordjevic *et al.*, 2013). This may be due to the most of the shawarma sold in the early morning is the one that has not been sold were leftover cooked shawarma that was not sold at night, preserved and offered for sale the early morning next day. Either, during the food manufacturing process, antibiotic resistance genes can be conveyed via a variety of pathways, raising concerns about food safety. (Wu-Wu *et al.*, 2023). To ensure the cooking quality by providing an adequate temperature and a correct holding period, and to decrease the microbiological load of RTE foods, reasonable personal hygiene and equipment measures must be followed (Taha *et al.*, 2023; Odeyemi *et al.*, 2020).

Conclusion

In this study, shawarma samples that analyzed contain different pathogenic microorganism as *E. coli*, *Proteus*, *Salmonella*, *Shigella*, *Pseudomonas* and *Klebsiella*. They also state an alarming increase in multidrug resistance in the isolated bacteria. The veterinary healthcare sector would be better if they monitored the guidelines for the use of antibiotics and the careful use of it. Furthermore, it is essential for the health authorities to raise awareness, train, and educate food handlers and keepers. These requirements will guarantee that food safety and hygiene standards are met by retailers, protecting

those who rely on RTE for convenience.

Declaration of conflict of interest

The authors declare that they have no conflict of interest.

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