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Prevalence, Antimicrobial Resistance and Risk Factors of Zoonotic Foodborne Pathogens Isolated from Camel Meat

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ABSTRACT

Approximately 75% of newly emerging infectious human illnesses are zoonotic (originating mainly from animals). All camel-raising countries, where camel meat is of popular consumption, are susceptible to camel zoonotic illnesses. The goal of the current study was to evaluate the microbiological safety and the associated risk factors of increasing microbial prevalence with the antibiogram status of the examined camel meat at the Elbasatien abattoir, which is the oldest and biggest abattoir in Cairo governorate of Egypt. Three zoonotic foodborne pathogens, including *Salmonella* spp., *Staphylococcus aureus*, and *Escherichia coli*, were successfully isolated with prevalence rates of 8% (8/100), 24% (24/100), and 14% (14/100) in the camel meat samples under examination. The prevalence of the isolated foodborne pathogens in camel meat was found to be higher among the age group of >5 years, among females, among samples collected during warm conditions, and ultimately among camels reared with other animal species in the herd, based on an analysis of the demographic data of the examined slaughtered camels as potential risk factors of acquiring pathogens. Additionally, using antimicrobial susceptibility testing on the three foodborne pathogens isolated revealed that, although the obtained positive *S. aureus* showed resistance against Amoxicillin-clavulanic, Ampicillin, and Ceptaxime, all isolated positive *Salmonella* and *E. coli* were resistant against all ten antibiotic discs used in the study.The discovered antibiogram data shed insight on the risk of foodborne pathogen transmission to humans and the subsequent spread of antibiotic resistance amongst consumers of camel meat.

Keywords: Camel meat, *E. coli*, Foodborne zoonoses, *S. aureus*, and *Salmonella*.

INTRODUCTION

Although meat is a great source of protein for humans, it can easily become contaminated by microorganisms, leading to food spoilage and foodborne illnesses for the consumer (Komba et al., 2012 and Ahmad et al., 2013). Since camel meat is a somewhat emergent non-traditional meat source that is becoming popular in the international meat markets, camel zoonotic illnesses can be found in all camel-rearing countries (Mohammadpour et al., 2020). It is anticipated that camel meat production would rise globally as a substitute for animal-derived meat (Faye, 2020). Egypt roughly containing 120,000 camels, making up 1.1%, 0.9%, and 0.7% of all camels in Africa, the Arabian Peninsula, and the whole world, respectively. Furthermore, according to Ottman et al. (2017), Egyptian camels yield around 0.09, 0.62, 2.3, and 20.8 thousand tons of fibers, meat, hides, and milk, respectively. Camel meat is thought to be healthier than beef from a nutritional perspective since it contains more polyunsaturated fatty acids and has less total fat (Kadim et al., 2006). However, as there is a dearth of information regarding the microbiological safety of camel meat intended for human consumption, every industry that supports the production and consumption of camel meat must determine its safety for public health (Tegegne et al., 2019).

The handling of raw meat in abattoirs and butcheries can lead to bacterial contamination, which is a major issue in most developing countries due to a lack of different technological applications for hygienic meat processing, a lack of trained labor, and low economic status. These establishments are potential sources of bacterial contamination and can harbor meat-borne zoonotic pathogens like *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus (S. aureus)*, which can have an adverse effect on the shelf life of meat, public health, and financial losses (Alemayehu et al., 2003).

The main gram-negative bacterium causing human food-borne gastroenteritis is *E. coli*, which can be directly isolated from camels and their surroundings or even indirectly isolated from meat and meat products contaminated during handling in the abattoir (Farmer et al., 2007). Additionally, the gram-negative Salmonellae are found throughout nature, where humans and animals serving as their main reservoirs. All organisms' digestive tracts are the principal tropism of *Salmonella* species. When a person has one of these infections, they may unnoticeably behave as an ordinary shedder of the organism, typically through faeces. Due to *Salmonella*'s widespread distribution in the environment, rising frequency in the global food chain, virulence, and adaptability, it is easily transmitted and has a significant negative influence on public health, medicine, and the global economy (Molbak et al., 2006).

On the other side, the most significant species of Coagulase Positive *Staphylococcus* is *S. aureus* which is found in everything, including surfaces, the air, dust, and living things like people and animals. Many of them are found in food as a result of contamination from humans, animals, and the environment. Raw meat will naturally include it as a common element of the skin microbiota (Dinges et al., 2000).

For the aforementioned reasons, the current study was conducted to evaluate the quality and microbiological safety as well as the associated risk factors of increasing prevalence of foodborne pathogens in camel

meat at the Elbasatien abattoir, which is the oldest and main abattoir in Cairo governorate of Egypt in order to declare the hygienic quality of the meat production and distribution in that region.

MATERIAL AND METHODS

1. Ethical consideration:

The research ethics was performed according to the regulations of Institutional Animal Care and Use Committee (IACUC) with oversight of the Faculty of Veterinary Medicine, University of Sadat City (approval No: VUSC-002-1-24).

2. Sample collection and preparation:

The current study was carried out from January 2023 to September 2023, a total of 100 meat camel samples were randomly aseptically collected from slaughtered camels at Elbasatien abattoir in Cairo governorate, Egypt, along with an accompanying history questionnaire (as taken from owners), in accordance with the technique recommended by Salam et al. (2024). The topside cut was chosen because it is inexpensive, very flavorful, and extremely lean, making it a favourite among Egyptian consumers. A sterile sharp knife was used to collect all meat samples aseptically. The samples were then placed into sterile plastic bags with 90 milliliters of sterile buffered peptone water, sealed into an icebox, and sent as soon as possible to the Zoonoses laboratory at Faculty of Veterinary Medicine, University of Sadat City, Egypt. There, they were homogenized using a stomacher (Lab. Blender 400, Seward Lab, London) and incubated for twenty-four hours at 37 °C to facilitate more traditional bacteriological analysis and evaluation.

3. Bacteriological examination:

3.1. Isolation of Salmonella spp.:

As Collee et al. (1996), every sample was individually injected into recently made nutrient broth (NB). Following a 24-hour aerobic incubation period at 37°C, turbid inoculations were chosen based on the presence of bacterial growth. Similarly, colonies that were smooth, round, opaque, and translucent began to develop on the nutrient agar plates, indicating growth. Additionally, the organisms generated pinhead- or lentil-sized, elevated, round or circular smooth, glistening, opaque, and colorless (transparent or translucent) colonies on *Salmonella shigella* agar (SSA) plates.

3.2. Isolation of Staphylococcus aureus (S. aureus):

After being macerated, the meat specimens were aseptically streaked on tryptic soy broth, which contains 10% sodium chloride and 1% sodium pyruvate, and staphylococcal enrichment broth media. They were then individually incubated for eighteen hours at 37°C. To detect *S. aureus* colonies (yellow colonies with yellow zones), one loopful (10 µl) of the enriched cultures was then subcultured on the surface of mannitol salt agar (MSA) media and incubated aerobically at 37°C for 36 hours (Dallal et al., 2015). In addition, to confirm their identity, the developed bacterial colonies underwent additional biochemical testing (Catalase, Oxidase, and Coagulase tests).

3.3. Isolation of Escherichia coli (E. coli) spp.:

A loopful of the incubated nutrient broth was reportedly scattered onto MacConkey agar media (Oxoid) and incubated at 37°C for 24 to 48 hours, according to Quinn et al. (2002). In order to identify *E. coli* further, all suspicious colonies from each plate that had distinctive characteristics were removed, streaked over buffered peptone water (BPW), and incubated at 37°C for 18 to 24 hours. After being incubated for 24 hours at 37°C, one milliliter of the BPW was transferred to Eosin Methylene Blue (EMB) (Oxoid) agar substrate. For additional identification, the purified colonies were

smeared over nutritional broth and cultured for 18 to 24 hours at 37°C.

4. Determination of the antimicrobial susceptibility (AMS) of isolated organisms:

All positive isolates were tested using disk diffusion method on Muller-Hinton agar plates (Oxoid, Ltd, Basingstoke, Hampshire, UK) according to Abdeen et al. (2020) for susceptibility to 10 commonly used antibiotics (Oxoid, UK). Antibiotic discs that were used in the present study for *E. coli* and *Salmonella* (Gram negative bacteria) included, Ciprofloxacin (CIP 10), Tetracycline (TE 30), Doxycycline (DO30), Amoxicillin/Clavulanic Acid (AMC 30), Streptomycin (S10), Nalidixic Acid (NA30), Cefotaxime (CTX30), Imipenem (IPM10), Ceftriaxone (CRO30), and Ampicillin (AM 10).

On the other side, all the positive *S. aureus* (Gram positive bacterium) isolates were tested for their antimicrobial susceptibility using various classes of antimicrobials used in veterinary field; Amoxicillin/Clavulanic Acid (30µg), Clindamycin (2 µg), Doxycycline (30 µg), Ampicillin (10 µg), Erythromycin (15 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Vancomycin (30 µg), sulfamethoxazole-trimethoprim (30 µg), Ciprofloxacin (5µg), Ceptaxime (30 µg) and Imipenem (10 µg). Disk diffusion assays were performed (in triplicate) on Muller Hinton agar. The AMS, based on the induced inhibition zones, were recorded where resistance against two or more antimicrobials of different classes was considered as multidrug resistant (MDR) according to Abdeen et al. (2020).

5. Statistical analysis:

As stated by Byomi et al. (2019a and b), SPSS (Statistical Package for Social Science), version 17 was used to analyze all of the data. Additionally, percentages were computed to convey the contamination frequency.

RESULTS

1. Results of bacterial isolation and identification:

Table 1. The prevalence of *Salmonella* spp., *E. coli*, and *Staphylococcus aureus* in the examined camel meat samples:

Sample	*Salmonella* spp.		*S. aureus*		*E. coli*		Total no.
	Positive		Positive		Positive		
	No.	%	No.	%	No.	%	
Camel meat	8	8	24	24	14	14	**100**

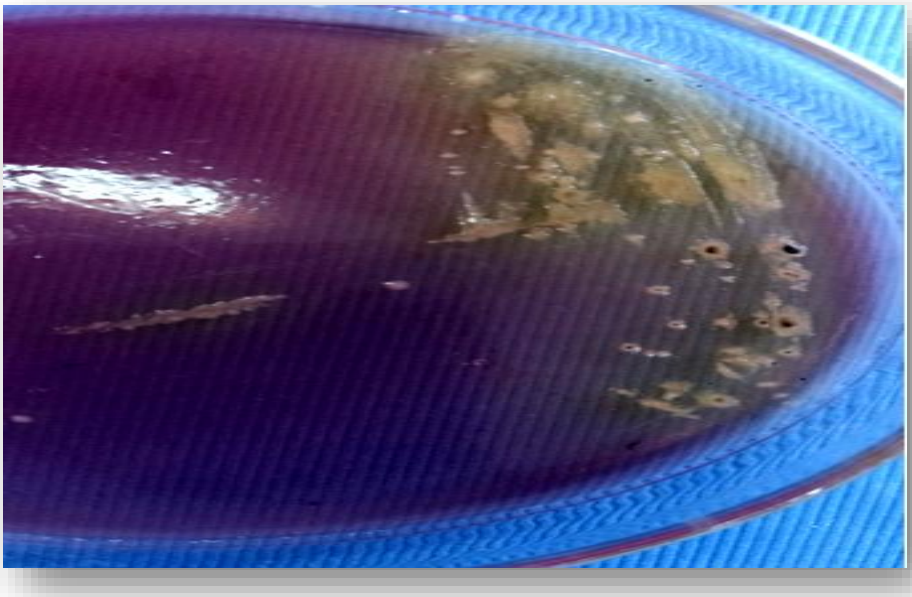

Figure 1. A representative *Salmonella shigella* agar (SSA) plate showing positive *Salmonella* isolates. The organisms were produced pinhead or lentil sized, raised, round or circular smooth, glistening, opaque, colorless (transparent or translucent) colonies.

Figure 2. A representative mannitol salt agar (MSA) plate showing positive *S. aureus* colonies (Yellow colonies with yellow zones).

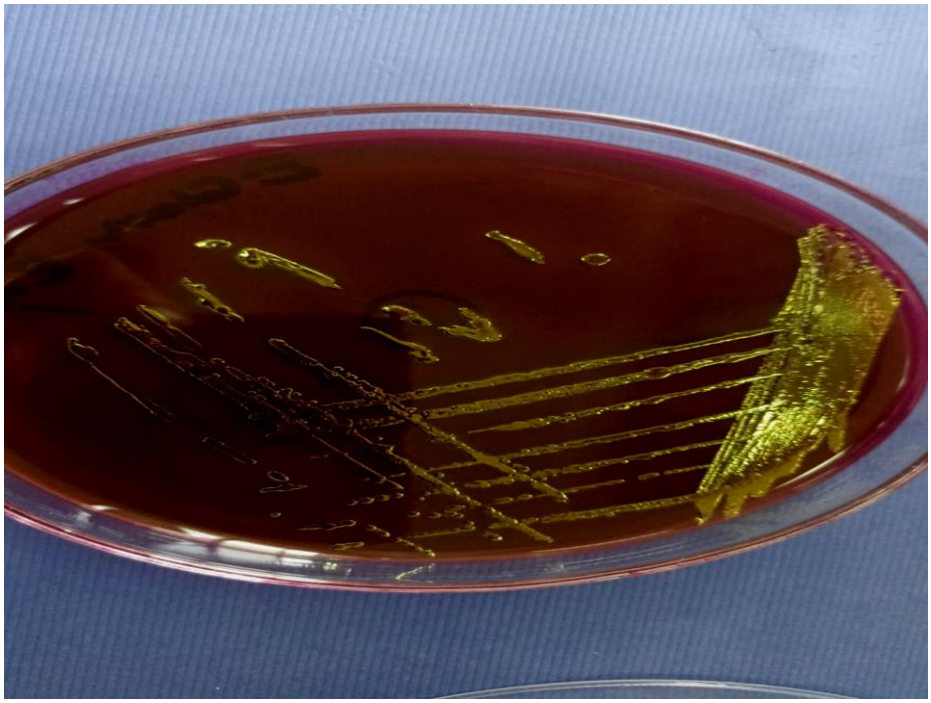

Figure 3. A representative Eosin Methylene Blue agar plates showing positive *Escherichia coli* colonies (metallic sheen).

2. *Prevalence of the isolated bacteria in relation to demographic risk factors:*

Table (2): The prevalence of the obtained isolated bacteria in relation to demographic data of the examined camel meat samples (obtained from owners):

Demographic data	*Salmonella* spp.		P-value	*S. aureus*		P-value	*E. coli*		P-value
	Positive			Positive			Positive		
	No.	%		No.	%		No.	%	
1. Age:									
a. ≤5 years (no. = 60):	3	5	0.33^{NS}	7	11.67	0.001**	5	8.33	
b. >5 years (no. = 40):	5	12.5		17	42.5		9	22.5	0.08^{NS}
Total (no. =100)	**8**	**8**		**24**	**24**		**14**	**14**	
2. Gender:									
a. Males (no. = 55):	2	3.64		9	16.36		6	10.91	
b. Females (no. = 45):	6	13.33	0.16^{NS}	15	33.33	0.08^{NS}	8	17.78	0.49^{NS}
Total (no. =100)	**8**	**8**		**24**	**24**		**14**	**14**	
3. Prevailing climatic conditions during sampling:									
a. Warm climate (no. = 65):	8	12.31	0.08^{NS}	19	29.23	0.16^{NS}	11	16.92	0.39^{NS}
b. Cold climate (no. = 35):	0	0		5	14.29		3	8.57	
Total (no. =100)	**8**	**8**		**24**	**24**		**14**	**14**	

4. Rearing (contact) with other animal species:									
a. Yes (no. = 35):	7	20	0.004**	18	51.43	0.0001**	10	28.57	0.005**
b. No (no. = 65):	1	1.54		6	9.23		4	6.15	
Total (no. =100)	**8**	**8**		**24**	**24**		**14**	**14**	

** Highly significant. ^{NS} non-significant.

3. Results of antimicrobial susceptibility testing of the isolated foodborne pathogens:

Table 3. Antimicrobial susceptibility test of *Salmonella* spp. isolated from camel meat:

Antimicrobial agent	conc. (µg)	R	S
Amoxicillin-clavulanic (AMC)	30	62	38
Doxycycline (DO)	30	87	13
Ampicillin (AM)	10	100	0
Cefotaxime (CRO)	30	62	38
Ciprofloxacin (CIP)	5	75	25
Ceptaxime (CTX)	30	100	0
Imipenem (IPM)	10	87	13
Tetracycline (TE)	10	100	0
Streptomycin (S)	10	100	0
Nalidixic acid (NA)	30	100	0

R: Resistant **S:** Susceptible

Table 4. Antimicrobial susceptibility test of *Staphylococcus aureus* isolated from camel meat:

Antimicrobial agent	conc. (µg)	R	S
Amoxicillin-clavulanic (AMC)	30	100	0
Clindamycin (DA)	2	30	80
Doxycycline (DO)	30	33	67
Ampicillin (AM)	10	75	25
Erythromycin (E)	15	0	100
Cefoxitin (FOX)	30	50	50
Cefotaxime (CRO)	30	33	68
sulfamethoxazole-trimethoprim (SXX)	20	0	100
Ciprofloxacin (CIP)	5	7	93
Ceptaxime (CTX)	30	63	37
Imipenem (IPM)	10	0	100

R: Resistant **S:** Susceptible

Table 5. Antimicrobial susceptibility test of *E. coli* isolated from camel meat:

Antimicrobial agent	conc. (µg)	R	S
Amoxicillin-clavulanic (AMC)	30	100	0
Doxycycline (DO)	30	100	0
Ampicillin (AM)	10	100	0
Cefotaxime (CRO)	30	100	0
Ciprofloxacin (CIP)	5	100	0

Ceptaxime (CTX)	30	100	0
Imipenem (IPM)	10	57	43
Tetracycline (TE)	10	100	0
Streptomycin (S)	10	100	0
Nalidixic acid (NA)	30	100	0

R: Resistant **S:** Susceptible

DISCUSSION

Microbially-induced foodborne disease is the most significant issue with food safety. The outbreaks were bacterial in nature, and the main causes of the disease incidence were incorrect holding temperatures and inadequate personal hygiene practices among food handlers (Nossair et al., 2016). Certain microorganisms can survive during common food preparation and storage techniques. Nevertheless, authorities in the fields of public health and food safety link an increase in the prevalence of foodborne illness. Food-borne pathogen contamination of carcass surfaces, where a healthy animal may harbor harmful bacteria on its skin, hair, hooves, and intestinal tract, is a serious public health concern (Hussein, 2007). In the developing countries where tainted labor sources were prevalent, and refrigeration was infrequent, foodborne illnesses could result in billions of illnesses and 406 million deaths annually globally (CDC, 2011).

The current study succeeded to isolate three zoonotic foodborne pathogens in the examined camel meat specimens, *Salmonella* spp., *Staphylococcus aureus*, and *E. coli*, the prevalence of them was 8% (8 out of 100), 24% (24 out of 100), and 14% (14 out of 100), respectively, as shown in table (1) and figures (1), (2) and (3).

From the previous investigations concerned camel meat, *Salmonella* was mentioned in agreement with the current obtained results as: 10% in Menofia governorate, Egypt (Edris et al., 2013), and 8% in Behera governorate, Egypt (Nossair et al., 2016). Higher prevalence rates were detected as: 16.2% in Ethiopia (Molla et al., 2003), and 20% in Nigeria (Musa et al., 2017) whereas Sevilla-Navarro et al. (2021) reported a lower prevalence rate of 5.5% in Iraq.

Regarding *S. aureus*, the obtained results were in accordance with Al-Thani and Al-Ali (2012) who reported a prevalence rate of 23.8% in Qatar. A higher prevalence rate of 45% was declared in Sharkia governorate (Gwida et al., 2019) that was however far away from the prevalence rate of 95.6% in Tunisia (Ben Chehida et al., 2021). On the contrary, Tegegne et al. (2019) reported a lower prevalence rate of 15.71% in Ethiopia.

Furthermore, concerning *E. coli* prevalence, the obtained result was in accordance with that of Edris et al. (2013) who reported a prevalence rate of 15% in Menofia governorate, Egypt. A higher prevalence rate of 44.36% was found in Behera governorate (Nossir et al., 2016). On the other hand, a lower prevalence rate of 5.71% was detected in Ethiopia (Tegegne et al., 2019).

Salmonella, *S. aureus*, and *E. coli* spp. were among the zoonotic foodborne pathogens found in camel meat in the current study. These pathogens may persist in undercooked meat products, putting consumers at risk of contracting them from the unhygienic conditions of the abattoir. Furthermore, meat has an abundance of all the nutrients needed by the bacteria in sufficient amounts, which may account for the pathogens' presence on the meat (Ukut et al., 2010). The availability of hot water, detergents, appropriate uniforms, and rules regulating the hygienic practices of meat handlers at all levels in El Basatien abattoir, however, may have contributed to the lower prevalence rates that were obtained when compared to earlier

studies (Elder et al., 2000; Bogere and Baluka, 2014 and Nossair et al., 2016).

Concerning the prevalence of the obtained isolated bacteria in relation to history of demographic data of the examined camel meat samples that was declared in table (2), it was declared that the infection with pathogenic foodborne zoonoses such as: *Salmonella*, *S. aureus* and *E. coli* spp. become increased with increased camel age, among females, during warm climate, and among camels reared (in contact) with other animal species in the herd. The obtained findings concerning age were in accordance with that concluded by Aqib et al. (2017) who reported the highest prevalence rate of microbes in camel meat was among age group of (6-9 years, i.e., > 5 years) with the least prevalence rate was among age group <5 years. On the contrary, Al-Gburi (2016) found the highest prevalence rate of camel pathogens were among age group <5 years. According to the current findings, camels with older ages had higher prevalence rates because they had greater lifelong exposure with infection sources.

Regarding gender of camels, Devrajani et al. (2010) recorded that the prevalence of bacterial species was higher among females than males whereas Al-Gburi (2016) reported males with higher prevalence rates than females. The distribution of zoonotic foodborne pathogens is almost equal between male and female camel families, according to the non-significant correlations found with the prevalence of various diseases and camel gender.

Furthermore, the obtained results concerning climatic conditions were in agreement with that of Arabi et al. (2014) who represented summer season was the highest season for the prevalence of microbial load in camel meat in comparison with prevalence rates reported in Winter and Autumn seasons. In addition to the fact that the camels under examination had a history of feeding in close quarters in enclosed pens, the study area's hot, humid summer weather may have had an impact on bacterial populations. Nevertheless, additional research on the epidemiology of camel foodborne pathogens in this region is required.

Furthermore, the prior research undervalued the strong correlation between the incidence of foodborne infections and the raising of camels alongside other pastoral animal species. Khalafalla (2017) concluded that the modifications in animal husbandry associated with an increase in camel contacts with other animal species may result in the establishment of diseases and the cross-species transmission of various infections, raising the prevalence of diseases in camels compared to those raised alone.

Concerning the results of antimicrobial susceptibility testing of the isolated foodborne pathogens that were declared in tables (3, 4, and 5), the isolated *Salmonella* and *E. coli* spp. showed resistance to all the used antibiotic discs (Amoxicillin-clavulanic, Doxycycline, Ampicillin, Cefotaxime, Ciprofloxacin, Ceptaxime, Imipenem, Tetracycline, Streptomycin, and Nalidixic acid) whereas the Gram positive *S. aureus* showed resistance against (Amoxicillin-clavulanic, Ampicillin, and Ceptaxime).

From the previously published data upon the antibiotic resistance of pathogens isolated from camel meat, Musa et al. (2017) represented *Salmonella* spp. in Nigeria were susceptible to the antibiotics since the pathogen showed resistance percentages of 25% against Ampicillin, 12.5% against Streptomycin, and 0% against both Ciprofloxacin and Tetracycline. In Ethiopia, Hunduma et al. (2023) declared *E. coli* isolates were resistant against Streptomycin (73%), Ampicillin (100%), and Tetracycline (64%) while they showed susceptibility for Nalidixic acid and Ciprofloxacin. As well, they showed that *Salmonella* isolates were

only resistant against Ampicillin (100%) while were susceptible for the other used antibiotics.

On the other side, *S. aureus* isolates were resistant against Ampicillin (100%), Amoxicillin-clavulanic (70%) while showed susceptibility to Cefotaxime in Pakistan (Aqib et al., 2017) whereas in Kenya, *S. aureus* showed resistance only against Ampicillin (66.7%) (Mwangi et al., 2022).

Reports on the global spread of multidrug-resistant phenotypes among pathogens have increased (Ponce et al., 2008). The use of antimicrobial drugs in veterinary care, animal husbandry, agricultural, aquaculture, and human medicinal practices is thought to be the cause of resistance development (Zhao et al., 2003). According to Okonko et al. (2009), these common behaviours played a significant role in the development of antibiotic-resistant bacteria, which can then spread to people through the food chain. The scenario regarding the risk of foodborne pathogen transmission to humans and the risk of antibiotic resistance spreading among camel meat consumers is explained by the obtained antibiogram results of foodborne zoonoses.

CONCLUSION

The current study shed the light on the presence of pathogenic foodborne bacteria in the examined camel meat samples that did not meet the Egyptian Standards, reflecting the unhygienic conditions during camel slaughtering, handling, and transportation. The routine application of the HACCP system is advised in order to obtain safe and wholesome camel meat, as indicated by the study's recorded results, including proper cooking of meat and proper washing of hands, especially before eating and after using the lavatory or changing diapers, is two ways that consumers can prevent enteric pathogen infections. Furthermore, raw meat and ready-to-eat food should be kept apart to prevent the spread of dangerous bacteria in kitchens.

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