

# Isolation, identification and antibiotic resistance profile of bacterial strains isolated from 'charmout', a dried meat sold in Chad

## ABSTRACT

This study aimed to investigate the presence of pathogenic bacteria in *charmout* and assess the antibiotic resistance of the isolated strains. A total of 50 *charmout* samples were collected from vendors across five provinces of Chad and analyzed using appropriate methods for enumeration, isolate characterization and susceptibility testing. The results indicated that none of the samples met the standards based on the three-class plan. Fifteen pathogenic bacterial strains were identified, including *Pseudomonas* spp. (24.8%), *Escherichia coli* (16%), *Bacillus* spp. (12%), *Proteus mirabilis* (9.6%), *Staphylococcus* spp. (7.2%), *Klebsiella pneumoniae* (5.6%), *Citrobacter* spp. (4.8%), *Enterobacter* spp. (4%), *Enterobacter cloacae* (4%), *Pantoea* spp. (4%), *Serratia odorifera* (3.2%), *Hafnia alvei* (2.4%), *Morganella morganii* (0.8%), *Kluyvera* spp. (0.8%) and *Pasteurella testudinis* (0.8%). Antibiotic susceptibility testing revealed that 79.63% (43/54) of strains were susceptible to all tested antibiotics, although some exhibited resistance, particularly to beta-lactam and fluoroquinolone families. This affected 30% (6/20) of *Escherichia coli* strains, 28.57% (2/7) of *Klebsiella pneumoniae*, 16.67% (1/6) of *Citrobacter* spp., 20% (1/5) of *Enterobacter cloacae*, and 100% (1/1) of *Morganella morganii* resistance. These findings highlight hygiene issues in production and storage, as well as the effect of antibiotics use in livestock farming in Chad. Strengthening health regulations and implementing preventive measures is crucial to reducing antibiotic resistance risk and ensuring consumer food safety.

**Key words:** *charmout*, pathogenic bacteria, microbiological quality, antibiotic resistance, Chad.

## 1. INTRODUCTION

Dried meat is one of the most popular meat products and makes up a large proportion of processed meat products [1]. As an excellent source of high-quality protein and many essential nutrients, meat and meat products contribute significantly to human nutrition [2], a role they have fulfilled since ancient times due to their nutritional value [3]. Indeed, dried beef is an important source of digestible and absorbable essential fatty acids, minerals and vitamins, and could therefore be a potential source of nutrients in complementary food formulations [4].

Dried meat products made using different drying and curing methods are very common and well known, with a long history in many countries [5]. However, dried foods, including meat products, are increasingly implicated in outbreaks due to the presence of foodborne pathogens [6]. Raw meat and meat products are likely to harbor a variety of micro-organisms (bacteria, viral pathogens, parasites) during the long chain of slaughter, transport and storage, processing environment, storage environment, equipment, utensils and workers [7]. Bacterial risks are the most important biological hazards in these meat products [8].

Thus, consumption of these products, when contaminated with pathogenic micro-organisms, exposes consumers to health threats and affects global trade [9], as the high prevalence of foodborne pathogens, as along with the number of widely reported cases and outbreaks, have a considerable impact on the lives of individuals, businesses and national economies [10]. The most frequently isolated bacteria contaminating dried meats include *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., *Pseudomonas* spp., *Shigella* spp., *Proteus* spp., *Citrobacter* spp., *Bacillus* spp., *Enterobacter* spp., *Kluyvera* spp., *Serratia* spp., *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp.

Furthermore, these microorganisms could also lead to resistant infections, as antimicrobial resistance (AMR) currently represents one of the most serious threats to global health, causing millions of deaths [11]. These resistances undermine modern medicine, compromise animal production and threaten food

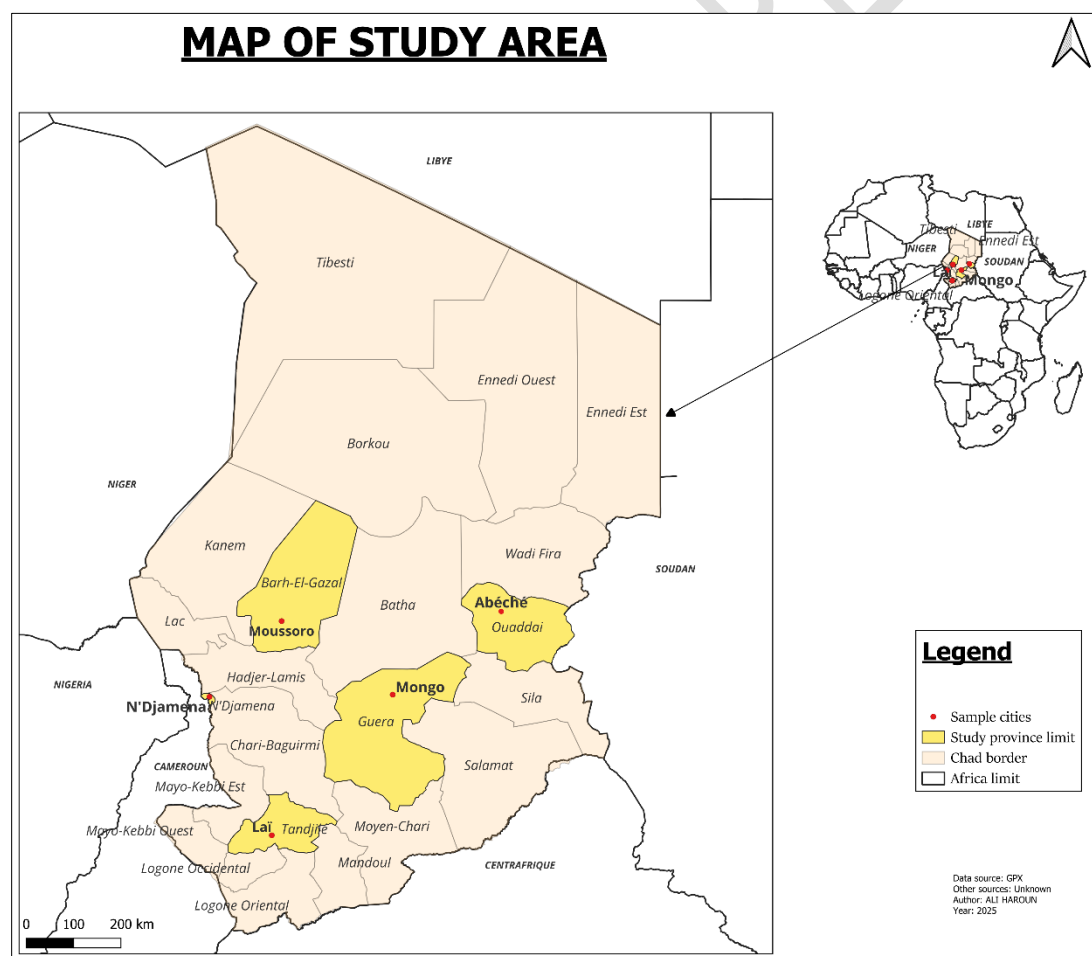
security [12]. The development of antimicrobial resistance among pathogens impacting on human and animal health further reinforces the need for increased surveillance [13].

In Chad, *charmout*, a dried meat product, is traditionally prepared by drying the meat and is used in the preparation of sauces. The traditional process for preparing this product involves slicing the meat into strips and drying them outdoors under the sun. This drying process is subject to various limitations including lack of control of drying parameters, meteorological uncertainties, labor, limited drying space, and risks of insect infestation, dust contamination, and other foreign matter [14]. The *charmout* thus produced could pose risks to consumers, due to its microbiological quality which has been deemed unsatisfactory by several studies [15, 16, 17]. Moreover, limited scientific research has been conducted on the identification of bacterial contaminants in this product and their resistance to antimicrobials. Therefore, this study aims to investigate the antimicrobial resistance of strains isolated from *charmout* sold in Chad against the most commonly used antimicrobials.

## 2. MATERIALS AND METHODS

### 2.1. Period, study area and sample collection

This study which focuses on the characterization and antibiotic resistance of bacterial strains isolated from *charmout* was conducted from January to October 2024. It was carried out across five provinces of the country, specifically in the towns of Abéché, Mongo, Moussoro, Laï, and N'Djamena (Figure 1). A total of fifty (50) samples were aseptically collected from market vendors, with ten (10) samples collected from each town.



**Figure 1:** Map of the study area

## **2.2. Microbiological analyses**

### **2.2.1. Bacterial enumeration and analysis of results**

A stock solution was prepared by weighing 10 g of crushed *charmout*, which was then introduced into a bottle containing 90 mL of sterile peptone water. Successive dilutions were then carried out in accordance with the AFNOR standard NF V 08 010 (March 1996). To achieve this, 1,000 µL of the stock solution was transferred into test tubes containing 9 mL of sterile physiological water at room temperature. For the enumeration of total aerobic mesophilic flora (TAMF), Plate Count Agar (PCA) was used, following the guidelines of ISO 4833 (2003). The plates were incubated at 30°C for 24 to 72 hours.

Thermotolerant coliforms were enumerated on Methylene Blue Eosin (MBE) lactose agar medium, as described in ISO 4832 (2006). The inoculated plates were incubated at 44°C for 24 to 48 hours.

Yeasts and molds were enumerated in accordance with ISO 21527-1:2008. The plates were incubated at 25°C for 24 to 72 hours.

*Bacillus* spp. were enumerated on Mannitol Egg Yolk Polymyxin (MYP) agar in accordance with the NF EN ISO 7932 standard. The plates were incubated at 30°C for 18 to 48 hours.

Staphylococci were enumerated using Chapman agar, with incubation at 37°C for 24 to 48 hours, in accordance with ISO 6888-1 (1999).

Microbial counts were determined by colony counting, with the assumption that each viable cell forms a distinct colony. The number of colonies on each plate reflects the number of viable microorganisms present in the dilution. As per ISO 7218 (1996), plates with between 15 and 300 colonies are considered suitable for counting.

The results of the microbiological analyses were interpreted using the three-class plan as described in the Burkina Faso standard NBF 01-208 (2017), applicable to *kilichi*.

### **2.2.2. Identification and characterization of isolates**

Strains were isolated and identified using standard microbiological techniques. All isolates obtained by multiple streaking were subjected to Gram staining, the oxidase test and subsequently to the minimum gallery. Several biochemical tests were carried out, including fermentation of glucose, lactose and mannitol, production of hydrogen sulphide, gas and indole, as well as motility, carbon source and urease activity.

For some Enterobacteriaceae strains, the API 20E system (BioMérieux, France) was used for confirmation. Colonies of the identified strains were isolated from an 18–24-hour old culture. They were then inoculated into approximately 4 mL of sterile saline to produce a suspension with an optical density corresponding to 0.5 on the McFarland scale. Using a sterile pipette, the suspension was added to the gallery tubes until they were filled and began to open. For the [CIT], [VP] and [GEL] tests, the tubes were filled with approximately 3 to 4 additional drops of suspension. For ADH, LDC, ODC, H<sub>2</sub>S and URE, 2 to 3 drops of paraffin oil were added to create an anaerobic environment ensuring no bubble formation. Readings obtained using the digital profile and the API database (ApiWeb). Additionally, the automated VITEK system was employed for strain confirmation.

### **2.2.3. Antibigram of isolated bacterial strains**

Antimicrobial susceptibility profiling was performed using the EUCAST disc diffusion method [18], and results were interpreted according to the recommendations of the French Microbiology Society's Antibigram Committee [19]. Fifteen commonly used antibiotics in Chad, representing four families were tested, namely: Betalactam family (Amoxicillin + clavulanic acid, Ampicillin, Ceftriaxone, Cefoxitin, Ticarcillin, Ceftazidime + Clavulanic acid, Aztreonam, Piperacillin, Cefepime, Cefotaxime), Fluoroquinolones (Ciprofloxacin), Carbapenems (Ertapenem) and Aminosides (Gentamicin, Tobramycin, Amikacin).

## **2.3. Data processing**

Data processing was conducted using R software version 3.2.5. was used for. Analysis of variance (ANOVA) was used to compare the means of the parameters studied. QGIS software version 3.38.2 was employed to generate the map of the study area.

### 3. RESULTS AND DISCUSSION

#### 3.1. Assessment of the microbiological quality of samples

Table 1 presents the average results of microbiological analyses of *charmout* samples collected from vendors in urban markets across five provinces of Chad. Analyses included counts of total aerobic mesophilic flora, total and thermotolerant coliforms, yeasts and molds, and *Bacillus* spp.

**Table 1:** Average microorganisms load results by city

Microbiological parameters (germs/g)				
Localities	TAMF	TTC	Y&M	<i>Bacillus</i> spp.
Mongo	$2.32 \times 10^7 \pm 2.53 \times 10^{7ac}$	$2.57 \times 10^3 \pm 3.23 \times 10^{3ac}$	$6.08 \times 10^4 \pm 9.71 \times 10^{4a}$	$7.02 \times 10^5 \pm 4.51 \times 10^{5a}$
N'Djamena	$3.71 \times 10^7 \pm 3.05 \times 10^{7bc}$	$4.42 \times 10^3 \pm 1.69 \times 10^{3c}$	$6.98 \times 10^3 \pm 8.32 \times 10^{3a}$	$6.13 \times 10^5 \pm 6.78 \times 10^{5a}$
Laï	$5.29 \times 10^7 \pm 3.68 \times 10^{7c}$	$4.15 \times 10^3 \pm 1.88 \times 10^{3bc}$	$2.75 \times 10^2 \pm 1.53 \times 10^{2a}$	$4.20 \times 10^5 \pm 2.43 \times 10^{5a}$
Abéché	$1.62 \times 10^6 \pm 2.79 \times 10^{6a}$	$1.10 \times 10^2 \pm 3.14 \times 10^{2a}$	$7.65 \times 10^3 \pm 2.06 \times 10^{4a}$	$1.54 \times 10^5 \pm 1.12 \times 10^{5a}$
Moussoro	$1.20 \times 10^7 \pm 1.63 \times 10^{7ab}$	$1.69 \times 10^3 \pm 1.39 \times 10^{3ab}$	$2.98 \times 10^4 \pm 6.29 \times 10^{4a}$	$7.09 \times 10^5 \pm 8.66 \times 10^{5a}$
Overall average	$2.54 \times 10^7$	$2.59 \times 10^3$	$2.11 \times 10^4$	$5.20 \times 10^5$
P-value	0.000358 ***	3.56e-05 ***	0.0819	0.133
Standard values*	$< 1.10^4$	$< 1.10^1$	$< 1.10^2$	$< 1.10^3$

**TAMF:** Total Aerobic Mesophilic Flora, **TTC:** Thermotolerant Coliforms, **Y&M:** yeasts and molds.

The identical letter a in the same column indicates that there is no statistical difference ( $p = 0.05$ ) between the values of the germs according to the localities.

\*Burkinabe standard NBF 01-208 applicable to *kilishi* (dried meat) (in number of germs per gram of *kilishi*).

Table 1 shows that the average load of total aerobic mesophilic flora in the analyzed *charmout* samples was  $2.54 \times 10^7$  CFU/g. This load is higher than the value reported by **Ali et al. [17]** for *charmout*, which was  $7.38 \times 10^6$  CFU/g. It also exceeds the count found on *kilishi* by **Iyiola et al. [20]**, which ranged from  $5.16 \times 10^3$  to  $36.56 \times 10^3$  CFU/g, as well as the value reported by **Seini et al. [21]**, where microbiological analysis of 'Ja2' type *kilishi* revealed a load of  $> 10^5$  CFU/g, making it unsuitable for consumption. Similarly, the *charmout* load is higher than those reported by **Dahiru and Maigari [22]** who found the highest numbers of aerobic mesophilic bacteria to be  $5.43 \times 10^5$  CFU/g. On the other hand, it is comparable to the value reported by **Mbawala et al. [23]**, who found an average contamination level of  $(2.78 \pm 0.24) \times 10^7$  CFU/g in *kilishi* without pepper. Our results indicate values that significantly exceed the established standards in all towns, with statistically significant differences observed between them, potentially reflecting variations in the conditions of preparation or storage of *charmout*. The higher load of total aerobic mesophilic flora in *charmout* compared to other products could be attributed to the exposure of *charmout* to sunlight by vendors in markets, aimed at preventing rewetting or insect infestations. However, this exposure may also lead to contamination from various environmental sources.

With respect to thermotolerant coliforms which are considered indicators of food hygiene and contamination from human and animal feces [24], the average load found in this study was  $2.59 \times 10^3$

CFU/g. This was lower than the value reported by Ali *et al.* [17] for *charmout* ( $9.34 \times 10^4$  CFU/g) and lower than the counts found in *kilishi* by Olusola *et al.* [25] ( $5.9 \times 10^6$  CFU/g). However, the thermotolerant coliform count in *charmout* was higher than the  $<10$  CFU/g count reported by Seini *et al.* [21] for 'Ja2' type *kilishi*. Similar to total aerobic mesophilic flora (TAMF), the results indicate contamination levels of thermotolerant coliforms that exceed the standard in all towns, particularly in Mongo, N'Djamena, and Laï, where contamination levels are notably high. Abéché also showed values above the standard, though less extreme than the other towns. The observed differences between towns were statistically significant, suggesting variations in preparation, storage, or selling conditions. Local practices such as drying methods, hygiene, and exposure to environmental factors (e.g., temperature, humidity) may significantly influence contamination levels.

The yeasts and molds in *charmout* samples were found at an average concentration of  $2.11 \times 10^4$  CFU/g. This value is lower than the fungal count in *kilishi* found by Olusola *et al.* [25], which reached up to  $5.9 \times 10^6$  CFU/g. However, it was higher than the levels reported on *kilishi* by Igene *et al.* [26] ( $1.03 \times 10^3$  CFU/g) and Iyiola *et al.* [20] (ranging from  $0.35 \times 10^2$  to  $1.00 \times 10^2$  CFU/g). These variations may arise from differences in ingredients used in *kilishi*, such as peanut paste, which could promote microbial contamination, or spices like ginger, which might inhibit fungal growth. Spices are known to stabilize foods against microbial spoilage, and the antifungal activity of spices and spice derivatives has been studied in terms of the number of viable cells, mycelial growth and mycotoxin synthesis [27].

The contamination levels of yeasts and molds in *charmout* were above the acceptable limits in all the towns surveyed, with the highest levels observed in Mongo, N'Djamena, Abéché, and Moussoro. These elevated contamination levels raise concerns about the product's quality in these regions. Laï also showed slightly elevated contamination levels, though closer to the acceptable limits, indicating potential quality concerns even in this town. While these differences were not statistically significant, they highlight variability within each town, which may be linked to inconsistencies production, storage, and hygiene practices.

Regarding *Bacillus* spp. counts, the average contamination level was  $5.20 \times 10^5$  CFU/g. As reported for *kilishi* by Jabaka *et al.* [28], *Bacillus* spp. Presence may arise from environmental contamination during processing, handling, and packaging, as this bacterium is commonly found in soil. Moreover, *Bacillus* spp. can produce toxins, especially in improperly handled or preserved food products [29]. In all the towns surveyed, *Bacillus* spp. contamination levels were well above the standard, posing a potential food safety risk if toxins are produced by these bacteria. These findings highlight the need for improved production and storage practices across all towns.

Based on the Burkinaabè microbiological standards applicable to *kilishi* dried meat, the microbiological analysis shown in Table 2 indicates that the *charmout* samples collected from urban markets in Chad were all unsatisfactory at rates of 98%, 80%, and 92%, respectively, with respect to the standards set for total aerobic mesophilic flora, thermotolerant coliforms, and *Bacillus* spp. Regarding yeasts and molds, 54% of the samples were found to be acceptable. However, when applying the three-class plan, none of the samples complied with the standard.

**Table 2:** Contamination levels and declaration of conformity of samples

Cities	TAMF	TTC	Y&M	<i>Bacillus</i> spp.
Mongo (n=10)	0/10 (00%)	1/10 (10%)	3/10 (30%)	0/10 (00%)
N'Djamena (n=10)	0/10 (00%)	0/10 (00%)	5/10 (50%)	0/10 (00%)
Laï (n=10)	0/10 (00%)	0/10 (00%)	10/10 (100%)	0/10 (00%)
Abéché (n=10)	1/10 (00%)	8/10 (80%)	7/10 (70%)	2/10 (20%)
Moussoro (n=10)	0/10 (10%)	1/10 (10%)	2/10 (20%)	2/10 (20%)
Total compliant (n=50)	1/50 (2%)	10/50 (20%)	27/50 (54%)	4/50 (8%)

### 3.2. Prevalence of bacterial strains isolated from *charmout*

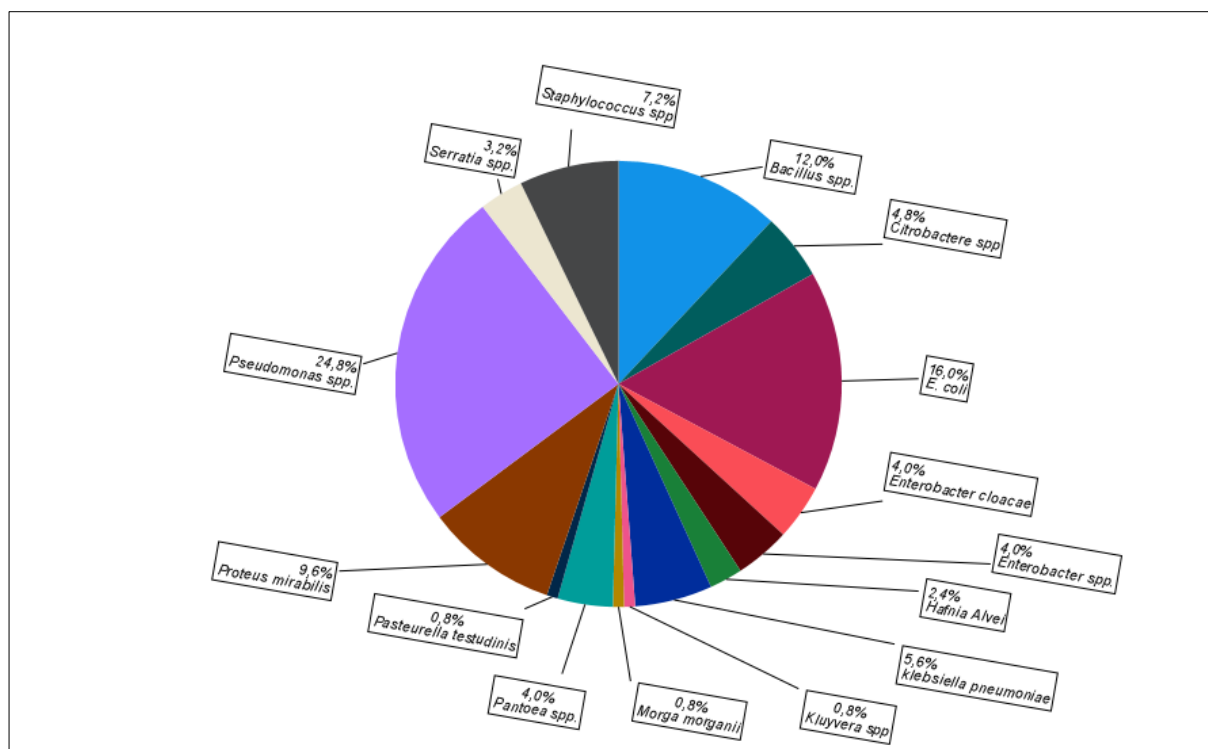
The biochemical characterization of strains isolated from *charmout* samples (Table 3) demonstrated considerable bacterial diversity, with 15 strains identified. These bacteria, commonly found in food environments, may pose health risks if not properly controlled.

**Table 3:** Results of biochemical characterization of isolated strains

Strains identified and confirmed with API20E	Num	Shape	Gram	OXY	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	Bacteria identified	Work force		
	1	B	-	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	-	-	+	<i>E. coli</i>	20		
	2	B	-	-	-	-	-	+	-	-	+	+	+	-	-	+	-	-	-	-	-	-	-	-	<i>Morga morganii</i>	1		
	3	B	-	-	+	-	+	±	+	-	-	-	+	±	+	+	+	+	+	+	±	+	+	+	<i>Serratia odorifera</i>	4		
	4	B	-	-	+	-	+	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	<i>Klebsiella pneumoniae</i>	7		
	5	B	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	<i>Enterobacter cloacae</i>	5		
	6	B	-	-	+	-	-	+	+	-	-	-	+	-	-	+	+	-	-	+	+	+	+	+	<i>Kluyvera spp.</i>	1		
	7	B	-	-	+	-	-	-	±	-	-	-	-	-	+	+	+	-	-	-	+	+	+	-	<i>Pantoea spp.</i>	5		
	8	B	-	-	+	-	+	+	±	-	-	-	-	±	-	+	+	-	-	+	-	-	-	+	<i>Hafnia Alvei</i>	3		
	9	B	-	-	-	-	-	-	+	-	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	<i>Proteus mirabilis</i>	12	
Identified strains with minimum gallery		Shapes	Gram	OXY		LAC		GLU		GAZ		H2S		MAN		MOB		CIT		URE		IND						
	10	B	-	-		-		+		+		+		-		+		-		±		-		<i>Citrobactere spp.</i>	6			
	11	B	-	-			+	+		+		-		+		+		+		±		-		<i>Enterobacter spp.</i>	5			
	12	B	-		+		-	+				-		-		+		+		-		-		<i>Pseudomonas spp.</i>	31			
	13	C	+		-		+	+						+		-					+			<i>Staphylococcus spp.*</i>	9			
	14	B	+		-		-	+				-		±		+					±		-		<i>Bacillus spp.*</i>	15		
15	B	-		+								Strain identified only by viteck												<i>Pasteurella testudinis</i>	1			
Total																												125

\*Catalase positive, B: *Bacillus*, C: cocci.

The bacterial strains identified include (Figure 2): *Pseudomonas* spp. (24.8%), *Escherichia coli* (16%), *Bacillus* spp. (12%), *Proteus mirabilis* (9.6%), *Staphylococcus* spp. (7.2%), *Klebsiella pneumoniae* (5.6%), *Citrobacter* spp. (4.8%), *Enterobacter* spp. (4%), *Enterobacter cloacae* (4%), *Pantoea* spp. (4%), *Serratia odorifera* (3.2%), *Hafnia alvei* (2.4%), *Morganella morganii* (0.8%), *Kluyvera* spp. (0.8%) and *Pasteurella testudinis* (0.8%).



**Figure 2:** Prevalence of bacterial strains isolated from *charmout*

More than half of the identified strains correspond to those found in dried meat products, such as *kilishi* [25, 22, 20], *kadid* [30], and *suya* [31, 32]. Common bacterial genera cited in these studies include *Escherichia coli*, *Shigella* spp., *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas* spp., *Citrobacter* spp., *Salmonella* spp., *Kluyvera* spp., *Staphylococcus aureus*, and *Bacillus cereus*, indicating that meat products are highly susceptible to contamination by foodborne pathogens [2]. Many of these pathogens originate from animal reservoirs or contaminate food through fecal contamination, particularly when hygiene practices are inadequate during production, slaughter, or handling [33].

In terms of bacterial prevalence, *Pseudomonas* spp. exhibited the highest prevalence at 24.8%. This reflects a potential risk to meat quality since this bacterium is commonly linked to meat deterioration. The following more abundant bacteria was *Escherichia coli*. Along with *Klebsiella* spp (5.6%) it appears to be the most frequently isolated species in many studies on dried meats. Their presence may reflect post-production contamination [28]. Detection of *Escherichia coli* in *charmout* samples is consistent with findings by Djoulde *et al.* [16] and Ali *et al.* [17], as well as in *kilishi* by Jabaka *et al.* [28], where it occurred in 21.1% of samples. *E. coli* was also found in 27.86% of *carne de sol* samples [34] and in 24.13% of *suya* samples [31]. The presence of this bacteria also suggests inadequate hygiene practices during production or slaughter, as this bacterium is part of the normal intestinal flora but indicates fecal contamination and poses a health risk [13, 28].

Similarly, *Klebsiella* spp. presence may be attributed to poor hygiene, as their detection often signals unhygienic food handling, undercooking, and suboptimal storage, especially when isolated from ready-to-eat foods [35]. These bacteria can persist in contaminated environments, such as slaughterhouses, and are known to cause extra-intestinal infections in humans [36].

Other bacteria, such as *Enterobacter* and *Proteus* spp., were also identified in *charmout* our samples. Their presence, particularly in *kilishi*, has been linked to inadequate processing practices, including poor handling, packaging, and contamination from soil or water [22]. *Proteus* spp., including *Proteus mirabilis*, is a known indicator of unsanitary conditions in food processing, as it is part of the normal flora of the human gastrointestinal tract and can be found in contaminated environments [37, 38].

*Bacillus* spp., identified in this study, have been attributed to pre-production contamination, that is contamination of raw meat. In addition, they are known to be heat-resistant and can produce toxins that remain active even after cooking [28].

The variety of bacterial strains identified points to significant contamination during the *charmout* production process, further underlining the need for enhanced production, storage, and hygiene practices to minimize microbiological risks. These could include improved temperature control, regular cleaning, and continuous microbiological monitoring which are essential to ensure food safety.

The antimicrobial susceptibility profile of strains isolated from *charmout* sold in markets in Chad is presented in Table 4.

**Table 4:** Resistance profile of the strains identified

[illegible]



FOX (30)	S	19 (95%)	0 (00%)	7 (100%)	0 (00%)	1 (100%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
	R	1 (5%)	6 (100%)	0 (00%)	5 (100%)	0 (00%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
TC (75)	S	19 (95%)	6 (100%)	0 (00%)	5 (100%)	0 (00%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	1 (5%)	0 (00%)	7 (100%)	0 (00%)	1 (100%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
ETP (10)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	1 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
CAL (40)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	1 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
ATM (30)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	1 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
PRL (100)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	0 (00%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	1 (100%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
CN (120)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	1 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
TOB (10)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	1 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
FEP (30)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	1 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
AK (30)	S	20 (100%)	6 (100%)	7 (100%)	5 (100%)	1 (100%)	1 (100%)	4 (100%)	5 (100%)	5 (100%)
	R	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)	0 (00%)
CTX	S	18 (90%)	6 (100%)	7 (100%)	4 (80%)	1 (100%)	0 (00%)	4 (100%)	5 (100%)	5 (100%)
	R	2 (10%)	0 (00%)	0 (00%)	1 (20%)	0 (00%)	1 (100%)	0 (00%)	0 (00%)	0 (00%)

AUG : Amoxicilline + acide clavulanique, AMP : Ampicilline, CRO : Ceftriaxone, CIP : Ciprofloxacin, FOX : Céfoxitine, TC : Ticarcilline, ETP : Ertapénème, CAL : Ceftazidime+Acide Clavulanique, ATM : Aztréonam, PRL : Pipéracilline, CN : Gentamicine, TOB : Tobramycine, FEP : Cefepime, AK : Amikacine, CTX : Céfotaxime.

The antibiotic susceptibility testing revealed that 79.63% (43/54) of the bacterial strains were susceptible to antibiotics, while 20.37% (11/54) exhibited acquired resistance. This resistance primarily concerned beta-lactams and fluoroquinolones, both critical classes of antibiotics for treating serious infections.

Beta-lactam resistance is particularly concerning, as these antibiotics are widely used in clinical settings. In particular, 30% of *Escherichia coli* strains were resistant to multiple beta-lactams, including amoxicillin + clavulanic acid, ampicillin, ceftriaxone, cefotaxime, cefoxitin, and ticarcillin. This resistance poses a challenge in treating common infections, such as urinary tract and intra-abdominal infections, where *E. coli* is frequently involved [44, 45]. Similarly, resistance to beta-lactams was noted in 28.57% of *Klebsiella pneumoniae*, 16.67% of *Citrobacter spp.*, 20% of *Enterobacter cloacae*, and 100% of *Morganella morganii* strains. Fluoroquinolone resistance, particularly to ciprofloxacin, was also observed in *E. coli* strains, which may be linked to mutations in bacterial gyrases or topoisomerases, reducing the efficacy of these drugs [46, 47].

Several studies confirm similar resistance patterns in other foodborne isolates. *E. coli* resistance to amoxicillin + clavulanic acid and cefotaxime was reported by Matakone et al. [48] and Tamendjari et al. [49]. Resistance to ciprofloxacin (13.04%) and ceftriaxone (21.74%) was observed by Hossain et al. [50] in raw meat isolates. Additionally, *E. coli* from kadid showed resistance to cefoxitin [30], and *Klebsiella spp.* exhibited significant resistance in various studies, including Mazhari et al. [51].

The antibiogram results (Table 2) showed that while wild-type strains dominated (79.62%), a low-level penicillinase was detected in one *E. coli* strain (1.85%).

Tamendjari et al. [49] suggest that the observed antibiotic resistance in foodborne isolates may stem from the widespread availability and overuse of antibiotics on farms, where they are often administered

at the first sign of illness. This emphasizes the need for public health strategies targeting antibiotic resistance in food systems. Assessing antibiotic-resistant pathogens in food is crucial for formulating effective public health interventions [35]. Given the rising threat of antibiotic resistance, it is essential to enforce antibiotic stewardship, which includes active surveillance, rational antibiotic use, and strict infection prevention measures in both healthcare and agricultural settings [11].

#### 4. CONCLUSION

This study highlights the diversity of bacterial strains isolated from *charmout*, a dried meat sold on markets in Chad, and their antibiotic resistance profile. The results indicate that *charmout* contains high loads of total aerobic mesophilic flora, yeasts and molds, as well as a wide variety of pathogenic bacteria. Although these pathogens are generally susceptible to most of the antibiotics tested, acquired resistance to certain antimicrobials was observed, posing a significant health risk to consumers. The findings suggest that microbial contamination of *charmout* is associated with inadequate hygiene practices during preparation and storage, as well as the use of antibiotics in food-producing animals. This underregulate use of antibiotics encourages the emergence of resistant bacteria, which can be transmitted to humans through the consumption of contaminated products such as *charmout*. The data gathered on the microbiological quality and antibiotic sensitivity profiles of the isolated bacteria provide valuable insights for improving hygiene practices in the production and handling of *charmout*.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

#### REFERENCES

1. Hamdi M, Nasri R., Dridi N, Moussa H, Ashour L, Nasri M. Improvement of the quality and the shelf life of reduced-nitrites turkey meat sausages incorporated with carotenoproteins from blue crabs shells. *Food Control*. 2018; 91: 148-159. doi: 10.1016/j.foodcont.2018.03.048
2. Xiang Q, Liu X, Li J, Ding T, Zhang H, Zhang X, et al. Influences of cold atmospheric plasma on microbial safety, physicochemical and sensorial qualities of meat products. *Journal of Food Science and Technology*. 2018; 55 :846-857. <https://doi.org/10.1007/s13197-017-3020-y>
3. Pal M, Ayele Y, Patel AS, Dulo F. Microbiological and hygienic quality of Meat and Meat Products. *Beverage & Food World*. 2018; 45(5):21-27.
4. Habte K, Azene M, Chanyalew Y, Girma S, Bashea C, Yehualshet A, et al. Nutrient Density and Microbial Safety of Open-Air-Dried Beef Meat and Its Biochemical and Organ Histopathology Effects in Albino Rats: A Promising Ingredient for Complementary Food Formulation. *International Journal of Food Science*. 2023; Article ID 2202312. <https://doi.org/10.1155/2023/2202312>
5. Doruk K, Canan H, Hatice U, Kaya F, Hasan A. Bacteriological enumeration, mycological profile and some physicochemical properties of Samarella (Tsamarella), a sun-dried meat product of Cyprus. *Journal of Food Safety & Food Quality*. 2023; 74(1). DOI: 10.53194/0003-925X-74-4.
6. Kehinde OO, Makinde GE, Agbato O, Adebawale OO, Awoyomi OJ, Fasanmi OG. Evaluation of dynamics and prevalence of microbial flora of soaked dry meats (Kundi and Ponmo) in Nigeria. *Nigerian Journal of Animal Production*. 2021; 48(6) :77-87. <https://doi.org/10.51791/njap.v48i6.3278>
7. Stoica M, Stoean S, Alexe P. Overview of biological hazards associated with the consumption of the meat products. *Journal of Agroalimentary Processes and Technologies*. 2014; 20(2), 192-197
8. Fraqueza MJ, Laranjo M, Elias M, Patarata L. Microbiological hazards associated with salt and nitrite reduction in cured meat products: control strategies based on antimicrobial effect of natural ingredients and protective microbiota. *Current Opinion in Food Science*. 2020; 38:32-39. doi: <https://doi.org/10.1016/j.cofs.2020.10.027>

9. Falowo AB. Production, nutritional quality and microbial safety of selected Nigerian dried meat products: a review. *Black Sea Journal of Agriculture*. 2023; 6(6):726-733. doi: 10.47115/bsagriculture.1298591
10. Chen JH, Ren Y, Seow J, Liu T, Bang WS, Yuk HG. Intervention Technologies for Ensuring Microbiological Safety of Meat: Current and Future Trends. *Comprehensive Reviews in Food Science and Food Safety*. 2012; 11(2):119-132. doi: 10.1111/j.1541-4337.2011.00177.x
11. WHO (World Health Organization), FAO (Food and Agricultural Organization) and OIE (World Organisation for Animal Health). Antimicrobial resistance and the United Nations Sustainable Development Cooperation Framework: Guidance for United Nations country teams. (2021).
12. FAO (Food and Agricultural Organization). FAO action plan on antimicrobial resistance 2021-2025. 2021; Rome. <https://doi.org/10.4060/cb5545en>
13. Jaja IF, Oguttu J, Jaja C-J, Green E. Prevalence and distribution of antimicrobial resistance determinants of *Escherichia coli* isolates obtained from meat in South Africa. *Plos One*. 2020; 15 (5): e0216914. <https://doi.org/10.1371/journal>
14. Amadou I, Diadie HO, Soumana OS, Balla A. Contribution of an Adapted Hygrometer to Measure Moisture Content of Dried Meat (*Kilishi*). *Journal of Agrobiotechnology*. 2020; 11(1): 1-6
15. Kimassoum D, Bawe N, Sado S, Ngandolo B, Fatou C, Nji M, *et al.* Evaluation of microbial adverse effects on fresh and processed bovine meat in N'Djamena (Chad) and Yaoundé (Cameroun). *African Journal of Microbiology Research*. 2017; 11 (16):637-643.
16. Djoulde DR, Demai BS, NDIH AC, Bayoi J, Bakari D. Processing and quality Of « Charmout » A Dry And Spicy Meat From The Lake Chad Basin. *International Journal of Engineering Science Invention*. 2021; 10(04):17-27. DOI- 10.35629/6734
17. Ali H. H., Tapsoba F., Makhoulouf H., Hama C., Tankoano A., Hassan M. A., Zongo C., Tidjani A. and Savadogo A. (2023). Production and Microbial Quality of “charmout”, a Dried Meat Produced in Chad. *American Journal of Food Science and Technology* 11(1):1-7. doi: 10.12691/ajfst-11-1-1.
18. EUCAST. Determination of antibiotic susceptibility EUCAST diffusion method. Version 4.0, June 2014.
19. CA-SFM / EUCAST. Recommendations 2024. V.1.0 Juin
20. Iyiola VO, Ejimadu JC, Aladi NO, Okoli IC, Okeudo NJ. Sensory characteristics and microbial profile of *kilishi* from different locations in Nigeria. *Nigerian Journal of Animal Production*. 2024; 790–794. <https://www.njap.org.ng/index.php/njap/article/view/4870>
21. Seini SH, Nafiou A, Maazou BA, Sadou H, Ibrahim A, Alma MM, *et al.* Influence of Manufacturing Methods on the Microbiological and Nutritional Characteristics of *Kilichi*, Dry Meat of Niger. *International Journal of Current Microbiology and Applied Sciences*. 2018; 7(12): 231-241
22. Dahiru A. T., and Maigari A. K. (2019). Bacteriological Quality Assessment of *Kilishi* Produced in Kunchi Local Government Area, Kano State, Nigeria. *UMYU Journal of Microbiology Research* 4(1):12-18
23. Mbawala A, Daoudou B, Ngassoum B. Microbiological quality of *kilishi* (dried meat product) produced in the city of N'Gaoundéré (Cameroon). *Tropicultura*. 2010; 28 (3). 153-160
24. Cuq J-L. Microbiologie Alimentaire : Contrôle microbiologique des aliments. Manuel technique, Polytech Département STIA. Université Montpellier. 2007; 2. 119 p.
25. Olusola OO, Abunwune RN, Adeshola AT. Quality evaluation of *kilishi*, an intermediate moisture meat product sold in Zaria metropolis, Nigeria. *Nigerian Journal of Animal Science*. 2017; 19(2), 271-279.
26. Igene JO, Uwadia OE, Ebabhamiegbeho PA, Evivie SE. Shelf-life stability studies of university of Benin (UNIBEN) proff's *kilishi* product. *Asian Journal of Science and Technology*. 2016; 07(01): 2268-2274
27. Souza EL, Stamford, TL, Lima ED, Trajano VN, Barbosa JM. Antimicrobial effectiveness of spices: an approach for use in food conservation systems. *Brazilian Archives of Biology and Technology*. 2005; 48 (4):549-558.
28. Jabaka RD, Ododife Q, Daniel AD, Nuhu UD, Doro EJ, Jibo R, *et al.* Assessment of Bacteria and Parasite Contamination of Dried Sliced Beef (*Kilishi*) Sold within Birnin Kebbi Metropolis, Kebbi State, Northern Nigeria. *South Asian Journal of Research in Microbiology*. 2020; 8(3): 58-66
29. Logan NA. *Bacillus* and relatives in foodborne illness. *Journal of applied microbiology*. 2012; 112(3): 417-429. <https://doi.org/10.1111/j.1365-2672.2011.05204.x>

30. Benyagoub E, Mammeri A. Physicochemical, biochemical and microbiological quality of dried and salted camel meat (*kadid*) from the southwestern regions of Algeria. *Fresenius Environmental Bulletin*. 2023; 32(12):3370-3386.
31. Wata I, Musa H, Abdullahi K, Abdullahi I, Hafsat SB, Adamu AM. Isolation and Identification of Bacteria from Hawked *Suya* Meat Sold within Katsina Metropolis. *Sahel Journal of Life Sciences*. 2024; 2(1):179-184. DOI: <https://doi.org/10.33003/sajols-2024-0201-022>.
32. Osunde D, Isoah G, Obasuyi C, Akpogheli P, Oshoma C. Bacteriological Analysis of Ready to Eat *Suya* Meat Sold In Adolor and Oluku Area of Benin City, Nigeria. *Journal of Applied Sciences and Environmental Management*. 2024; 28 (5):1477-1483.
33. Dubois-Brissonnet F, Guillier L. Les maladies microbiennes d'origine alimentaire. *Cahiers de nutrition et de di  t  tique*. 2020 ; 55 :30-38.
34. Cordeiro IR, Vaz JN, Galv  o RC, Palma JM, Neto CM. Resist  ncia antimicrobiana em cepas de *Escherichia coli* isoladas de carne de sol comercializada no munic  pio de te  filo otoni, minas gerais. *dSPACE.doctum.edu.br*. 2022.
35. Theocharidi NA, Balta I, Houhoula D, Tsantes AG, Lalliotis GP, Polydera AC, et al. High Prevalence of *Klebsiella pneumoniae* in Greek Meat Products: Detection of Virulence and Antimicrobial Resistance Genes by Molecular Techniques. *Foods*. 2022; 11(708):1-9. <https://doi.org/10.3390/foods11050708>
36. Davis GS, Price LB. Recent Research Examining Links Among *Klebsiella pneumoniae* from Food, Food Animals, and Human Extraintestinal Infections. *Current environmental health reports*. 2016; 3:128–135. <https://doi.org/10.1007/s40572-016-0089-9>
37. Gwida M, Hotzel H, Geue L, Tomaso H. Occurrence of *Enterobacteriaceae* in Raw Meat and in Human Samples from Egyptian Retail Sellers. *International Scholarly Research Notices*. 2014; Article ID 565671. <http://dx.doi.org/10.1155/2014/565671>
38. Yeh H-Y, Line JE, Hinton AJr. Molecular Analysis, Biochemical Characterization, Antimicrobial Activity, and Immunological Analysis of *Proteus mirabilis* Isolated from Broilers. *Journal of Food Science*. 2018; 0(0). doi: 10.1111/1750-3841.14056.
39. Tang Y-j, Yuan L, Chen C-w, Tang A-q, Zhou W-y, Yang Y-q. Isolation and characterization of the new isolated bacteriophage YZU-L1 against *Citrobacter freundii* from a package-swelling of meat product. *Microbial Pathogenesis*. 2023; 179 : 106098. <https://doi.org/10.1016/j.micpath.2023.106098>
40. Awad N, Al-saadi MJ. Isolation and molecular diagnosis of *Citrobacter freundii* in raw meat (beef, mutton and fish) in AL-Rusafa district of Baghdad city. *International Journal of Health Sciences*. 2022; 6(S8):1482–1491. <https://doi.org/10.53730/ijhs.v6nS8.10026>
41. Penha JC, Franco RM, Duarte MC, Leandro KC. Evaluation of the microbiological and physical-chemical quality of salted bovine meat marketed in establishments and free fairs in the north zone of Rio de Janeiro. *Health Surveillance in Debate: Society, Science & Technology*. 2018 ; 6(4):65-70.
42. Bintsis T. Foodborne pathogens. *AIMS microbiology*. 2017; 3(3):529-563. doi: 10.3934/microbiol.2017.3.529
43. Regenthal P, Hansen JS, Andre I, Lindkvist-Petersson K. Thermal stability and structural changes in bacterial toxins responsible for food poisoning. *PloS one*. 2017; 12(2), e0172445. doi: 10.1371/journal.pone.0172445
44. Nicolle LE. Resistant pathogens in urinary tract infections. *Journal of the American geriatrics society*. 2002; 50(7): 230-235. <https://doi.org/10.1046/j.1532-5415.50.7s.3.x>.
45. Thirion DJ, Williamson D. Les infections urinaires : une approche clinique. *Pharmactuel*. 2003; 36(5).
46. Halawa EM, Fadel M, Al-Rabia MW, Behairy A, Nouh NA, Abdo M, et al. Antibiotic action and resistance: updated review of mechanisms, spread, influencing factors, and alternative approaches for combating resistance. *Frontiers in Pharmacology*. 2024; 14: 1305294. doi: 10.3389/fphar.2023.1305294.
47. Webber MA, Buckner MM, Redgrave LS, Ifill G, Mitchenall LA, Webb C, et al. Quinolone-resistant gyrase mutants demonstrate decreased susceptibility to triclosan. *Journal of Antimicrobial Chemotherapy*. 2017; 72(10) : 2755-2763. doi:10.1093/jac/dkx201.
48. Matakone M, Founou RC, Founou LL, Dimani BD, Koudoum PL, Fonkoua MC, et al. Multi-drug resistant (MDR) and extended-spectrum  $\beta$ -lactamase (ESBL) producing *Escherichia coli* isolated from slaughtered pigs and slaughterhouse workers in Yaound  , Cameroon. *One Health*. 2024; 19 : 100885.
49. Tamendjari S, Saidani K, Chaib L, Aggad H, Bouzebda Z, Bouzebda F. Hygienic quality of food from animal origin and antibiotic resistance of *Escherichia coli* in a border region of Algeria.

*Revista Científica*. 2024; FCV-LUZ / Vol. XXXIV, rcfcv-e34389. <https://doi.org/10.52973/rcfcv-e34389>

50. Hossain S, Jony AH, Saha N, Islam B, Sobur KA, Mowdood S, *et al.* Antibiotic Resistance Genes Detection in *Escherichia coli* Isolated from Raw Meat in Rajshahi Division of Bangladesh. *American Journal of Microbiological Research*. 2024; 12(4):79-84. doi: 10.12691/ajmr-12-4-1.
51. Mazhari BZ, Alanazi F, Abosalif K, Tagwa S, Hussein SE, Agsar D. Microbial Profile and Antibiotic Susceptibility Pattern of Frozen Food in India. *Journal of Pure & Applied Microbiology*. 2023; 18(1):1-12. doi: 10.22207/JPAM.18.1.08.

UNDER PEER REVIEW