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Recherche des entérobactéries résistantes aux antibiotiques dans la viande de poulet dans la wilaya de M'sila

Présenté par : HAMDINI HANA ; SALAH CHAIMA ; ZIOUCHE NADJET ASSALA

Soutenu le 20/06/2023 devant le jury composé de :

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PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

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Presented by: HAMDINI HANA ; SALAH CHAIMA ; ZIOUCHE NADJET ASSALA

Argued on 20/06/2023 in front of the jury members :

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ملخص

أصبحت العدوى الناتجة عن البكتيريا المعوية التي تنتج بيتا لاكتاماز ممتد الطيف (ESBL) أكثر شيوعًا، وهو مصدر قلق صحي عالمي كبير. وفقًا لتقارير عديدة، يمكن أن تنتقل هذه الجراثيم من الحيوانات إلى الناس عبر البيئة. كان الهدف الرئيسي من هذه الدراسة هو فحص انتشار إنتاج ESBL في الدواجن وصورة مقاومتها في مدينة المسيلة. حيث تم أخذ عينات من ثلاثة أماكن مختلفة في ولاية المسيلة (المسيلة وسط، بوسعادة، سيدي عيسى). بعد الفحص الانتقائي، تم تحديد و تأكيد وجود سلالات مقاومة منتجة للESBL في السلالات المعزولة بطريقة النمط الظاهري. ثم تم استخراج الحمض النووي عن طريق الغليان. ونتيجة لذلك، كانت جميع المناطق الثلاث تحتوي على الإشريكية القولونية المنتجة لـ ESBL. وبالتالي ، احتوت 13 (20.97٪) من العينات على للإشريكية القولونية المنتجة لـ ESBL. كانت جميع السلالات المعزولة مقاومة للجيل الثالث من السيفالوسبورين (cefotaxime) الذي تم اختباره وحساسة للأمينوسيدات وبعض بيتا لاكتامينات (imipenem، cefoxitin). في الختام، تظهر النتائج انتشارًا كبيرًا لـ *Enterobacteriacea* المنتجة لـ ESBL في مدينة المسيلة ، والمزيد من الدراسات ضرورية لتوصيف وتصنيف البكتيريا المقاومة وآلياتها.

الكلمات المفتاحية : مقاومة المضادات الحيوية , بيتا لاكتاماز ذات الطيف الممتد , الإشريكية القولونية , بكتيريا الأمعاء.

Résumé

Les infections provoquées par les entérobactéries qui produisent des Bêta-Lactamases à Spectre Etendu (BLSE) sont de plus en plus fréquentes, ce qui constitue une préoccupation sanitaire mondiale majeure. Selon de nombreux rapports, ces germes peuvent se transmettre des animaux aux humains par l'environnement. L'objectif principal de cette étude était d'examiner la prévalence de la production de BLSE chez les volailles et leur profil de résistance dans la région de M'sila. Des échantillons ont été prélevés à trois endroits différents dans la wilaya de M'sila (M'sila centre, Boussaâda, Sidi-Aïssa). Après criblage sur gélose sélective BLSE, la confirmation de la résistance à la production d'BLSE des souches isolées a été déterminée par la méthode phénotypique. Ensuite, on a procédé à l'extraction de l'ADN par ébullition. Par conséquent, les trois régions abritaient des *E. coli* producteurs d'BLSE. Ainsi, 13 (20,97 %) des échantillons contenaient des *E. coli* produisant des BLSE. Tous les isolats étaient résistants aux céphalosporines de troisième génération (céfotaxime) testés et sensibles aux aminosides et à certaines de bêtalactamines (imipenème, céfoxitine). En conclusion, les résultats montrent une prévalence significative des entérobactéries productrices de BLSE dans la région de M'sila, et des recherches supplémentaires sont nécessaires pour caractériser les gènes de cette bactérie responsables de cette résistance et de leurs mécanismes

Mots clés : Antibiorésistance, Bêta-lactamases à spectre étendu (BLSE), *Escherichia coli*, Entérobactéries.

Abstract

Infections brought on by enterobacteria that produce extended-spectrum beta-lactamases (ESBL) are becoming more common, which is a major global health concern. According to numerous reports, these germs can transmit from animals to humans through the environment. The main objective of this study was to examine the prevalence of ESBL production in poultry meat and their resistance profile in the region of Msila. Samples were taken from three different places in the wilaya of Msila (Msila center, Bousaada, Sidi-Aissa). After screening on selective ESBL agar, confirmation of ESBL production of the isolated strains was determined by the phenotypic method. Then, DNA extraction by boiling was performed. As a result, all the three regions harbored ESBL-producing *E. coli*. Hance, 13 (20.97%) of samples contained ESBL producing *E. coli*. All the isolates were resistant to third generation cephalosporin (cefotaxime) tested and sensitive to Aminosides and some Beta lactamines (imipenem, cefoxitin) and amoxicillin clavulanic acid and Fosfomycin. In conclusion, the results show a significant prevalence of ESBL-producing *Enterobacteriaceae* in the department of Msila. Additional research is required to characterize genes of this bacteria responsible for this resistance and their mechanisms.

Key words: Antibiotic resistance, Extended-spectrum-beta-lactamase (ESBL), *Escherichia coli, Enterobacteriaceae.*

List of Abbreviations

°C	Degree Celsius
ADH	Arginine Di-hydrolase
AK	Amikacine
AMC	Amoxicillin + Clavulanic acid
AMR	Antimicrobial resistance
AMP	Ampicilline
ATB	Antibiotic
ATCC	American Type Culture Collection
BES	Brazilian extended-spectrum β-lactamase
BL	Beta-lactamases
C3G	Third generation cephalosporins
CAZ	Ceftazidime
CIP	Ciprofloxacin
CIT	citrate
CRBt	Biotechnology Research Centre
CRO	Ceftriaxone
CTX	cefotaxime
CTX-M	Cefotaximase-munich
DD	double disk
DHF	tetrahydro folic acid
DHPS	Di-hydropterate synthase
DNA	Deoxyribonucleic acid
ESBL	Extended-spectrum-beta-lactamase
FF	Fosfomycin
FOX	Cefoxitin
GE	Gelatinase
GES	Guyana Extended-Spectrum Beta-Lactamase
GNB	Gram negative bacteria
H	Hour
IBC	Integron Borne Cephalosporinase
IMP	Imipenem
KPC	*Klebsiella pneumoniae* Carbapenemase
MF	McFarland Standards

MH	Muller-Hinton
mm	Millimeters
NB	Nutrient broth
Nm	Nanometer
OD	Optical Density
ODC	Ornithine decarboxylase
OXA	Oxacillin
PCR	Polymerase Chain reaction
PER	Pseudomonas extended resistance
PLP	Proteolipid protein
R	Resistant
RNA	Ribonucleic acid
S	Sensible
SFO	*Serratia fonticola*
SHV	Sulfhydryl Variable
TE	tris EDTA
TEM	Temoniera
TLA-1	Tlahuicas, Indian tribe
URE	Urease
VEB	Vietnamese Extended spectrum Beta-lactamase
VIM	*Verona imipenemase*
VP	Vogues Proskauer
WHO	World Health Organization

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Introduction

Introduction

A vast family of non-spore-forming, Gram-negative bacilli known as *Enterobacteriaceae*. The members of this family are common and can be found in a wide range of biological niches in both terrestrial and aquatic habitats, including the soil, water, plants, and other living things (Jenkins et *al.*, 2017) . Despite the fact that many species of animals, including humans, have natural flora, *Enterobacteriaceae* are frequently linked to both intestinal and extraintestinal illnesses (Donnenberg, 2015). Most of this family members are important pathogens in the setting of public health: *Salmonella, Escherichia, Shigella, and Yersinia*. Together, these pathogens are a significant cause of morbidity and mortality worldwide, and the emergence of multidrug resistance poses important challenges for the control and prevention of *Enterobacteriaceae* infections (Kang et *al.*, 2018).

It is a normal evolutionary process for bacteria to adapt and develop defenses against antibiotic exposure. Antibiotic resistance can arise through a variety of processes, including genetic changes and the horizontal gene transfer of resistance genes.

Beta-lactamases are bacterial enzymes that can inactivate beta-lactam antibiotics. These enzymes degrade the antibiotic molecules' crucial beta-lactam ring structure, rendering them ineffective against bacterial infections. Beta-lactamases are incredibly important in the development of antibiotic resistance because they enable bacteria to grow and survive when beta-lactam drugs are present. Beta-lactamase-producing bacteria make it more difficult to treat infections because they decrease the effectiveness of beta-lactam antibiotics and need the use of complementary and alternative medicines. Novel antibiotics and combination medications are being developed in an effort to combat beta-lactamase-mediated resistance and improve the treatment of bacterial infections. Antibiotic resistance is the ability of bacteria or other germs to withstand the effects of antibiotics, rendering them worthless for treating infections.

Extended-Spectrum Beta-Lactamase (ESBL) is an enzyme produced by certain bacteria that confers resistance to multiple antibiotics, including those commonly used to treat bacterial infections in humans. These ß-lactamases, which are quickly developing, have the same ability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid (Philippon et *al.*, 1989). ESBL-producing bacteria can be present in the gastrointestinal tracts of chickens, either as a result of natural colonization or due to exposure to antibiotics. The use of antibiotics in poultry farming, including for growth promotion and disease prevention, can contribute to the emergence and spread of ESBL-producing bacteria in chicken flocks. These

bacteria can also be transmitted to chickens through contaminated feed, water, or the farm environment.

Contamination of chicken meat by ESBL-producing bacteria is a significant concern in the food industry and public health. During the processing of chicken meat, there is a risk of cross-contamination with ESBL-producing bacteria. Improper handling and inadequate hygiene practices in slaughterhouses and processing plants can lead to the transfer of bacteria from the chicken's intestinal tract to the meat. Equipment and surfaces used during processing can become contaminated, further facilitating the spread of ESBL-producing bacteria.

Enterobacteria producing ESBL are not more pathogenic than others enterobacteria, but the infections they cause are more difficult to treat because of their resistance to many antibiotics. Several studies have been conducted on the Enterobacteria producing ESBL. However, it was in *E. coli* that the ESBL was most often described in different environments (Madec et *al*., 2012). Our work is based on the search for *Escherichia coli* strains producing ESBLs present in chicken meat and studying their resistance profile in the region of Msila.

To do this, our work is divided into two parts. A bibliographic summary that focused on research about *Enterobacteriaceae* and antibiotic resistance, in order to give a general view of emerging phenomena in terms of resistance. In a second place, we have mentioned some generalities about Beta lactamases and ESBL-producing bacteria then we finished it by discussing the presence of ESBL in poultry meat and their situation in global and in Algeria specifically.

In order to develop these aspects, we have taken the following step:

- ✓ Isolation and identification of ESBL-producing *E. coli*.
- ✓ Study of resistance profiles of isolated strains to a few families of antibiotics.
- ✓ phenotypical confirmation of the presence of ESBL.
- ✓ DNA extraction from identified strains.

Bibliographic Part

1. Enterobacteriaceae

1.1. Definition of Enterobacteriaceae

A diverse family of bacteria with several species is known as Enterobacteria. They are straight Gram negative bacilli at the phenotypic level; mobile by peritrichous flagella, or immobile; not sporulated; optional aerobic; producing acid from glucose; not requiring sodium or stimulation; positive catalase; negative oxidase; typically reducing nitrates to nitrite (not N2); and possessing gamma-proteobacterial 16S RNA r (Cristian et *al.*, 2008).

More specific criteria, such as the fermentation of various sugars, the generation of sulphide, indole, urease, the presence or lack of metabolic0 enzymes (deaminases, decarboxylases), etc., determine the distinctions between the numerous genera and species (Meziani & Hamidechi, 2012).

Their presence in the gut flora of warm-blooded animals and humans is their principal point of affinity. Although they are found in plants and the environment (soil and water), their spread in nature is nonetheless wider. Some enterobacteria participate in the organic matter cycle naturally as a result of their metabolic traits, whereas others can colonize and degrade agricultural goods or lead to occasionally life-threatening diseases in humans and animals (Cristian et *al.*, 2008).

1.1.1. Classification of Enterobacteriacea

Enterobacteriaceae include many different species (Tab **2**), some of which are important pathogens for humans and animals, while others are normal commensals of the gastrointestinal tract.

They are classified according to their biochemical reaction in laboratory tests, which distinguish them from each other. The main classifications are:

Table 1: Hierarchical subdivisions of enterobacteria classification (Boone et *al.*, 2001)**.**

Taxonomic ranks	**Classification**
Domain :	Bacteria
Phylum :	Proteobacteria
Class :	Gammaproteobacteria
Order :	Enterobacteriales
Family :	Enterobacteriaceae

The species most commonly isolated in clinical bacteriology belong to 12 genera: *Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Morganella, Proteus, Providencia, Salmonella, Serratia, Shigella, Yersinia* (Pilet et *al.*, 1975).

Table 2: Classification of the species of Enterobacteria most frequent in Human Clinic (Perriere, 1992).

	Family	**Genus**	**Species**
Group 1	Edwardsielleae	Edwardsiellea	
	Salmonnelleae	Selmonella	*Salmonella typhi* *Salmonella paratyphi* *Salmonella enteritidis*
Group 2	Escherichieae	Escherichieae	*Escherichieae*
		Escherichieae	*Shigella dysenteriae* *Shigella flexneri* *Shigella boydii* *Shigella sonne*
	Levineae	Levinea	
Group 3	Klebsielleae	Klebsiella	*Klebsiella pneumoniae* *Klebsiella oxytoca*
		Enterobacter	*Enterobacter aerogenes* *Enterobacter cloaceae*
		Serratia	*Serratia marcescens*
		Erwinia	
Group 4	Proteae	Proteus	*Proteus mirabilis* *Proteus vulgaris* *Proteus rettgerii*
		Providencia	
Group 5	Yersinia	Yersinia	*Yersinia enterolitica* *Yersinia pseudotuberculosis*

1.2. Definition of *Escherichia coli*

The bacterium *Escherichia coli* (*E. coli*) is a rod-shaped, gram-negative, facultative anaerobic member of the *Enterobacteriaceae* family. Peritrichous flagella on its 2.0-6.0 m long and 0.25-1.0 m in diameter allow it to move freely in a liquid environment.

Warm-blooded animals' lower intestines frequently include *E. coli*, which is well known for its capacity to digest lactose and generate indole. It is a helpful model organism for genetic and molecular biology studies because to its well-characterized single, circular chromosomal genome. While the majority of *E. coli* strains are not harmful to humans, a few of them have been linked to sepsis, gastroenteritis, and urinary tract infections (Blattner et *al.*, 1997).

1.2.1. Cultural characteristics of *E. coli*

E. coli grows on agar media in 24 hours at 37°C, producing circular colonies that are smooth, have regular edges, are 2 to 3 mm in diameter, and are not colored. inside blood agar. *E. coli* colonies are 2-4 mm in diameter and are smooth, dull grey in color. Lactose-positive *E. coli* colonies on MacConkey media are pink to red, flat, dry, and 2-3 mm in diameter. They are frequently encircled by a deeper pink zone of precipitated bile salts. On Macs, *E. coli* Lactose-negative colonies form 2-3 mm-diameter colorless colonies (Haouzi, 2013).

1.2.2. Antibiotic sensitivity

Antibiotics are typically effective against *E. coli*. Beta-lactams, group A (amino penicillin) penicillin, carboxypenicillins, cephalosporins, acyluridopenicillins, carbapenems, and monobactams are a few of them. Additionally active are polypeptides, aminoglycosides, fluoroquinolones, first-generation quinolones, and cotrimoxazole. The creation of penicillinase and cephalosporin, two enzymes that hydrolyze beta-lactams, can change this sensitivity (Ohmura-Hoshino et *al.*, 2003).

2. Antibiotic resistance

2.1. Antibiotics

2.1.1. Definition of Antibiotics

A biologically produced antibacterial agent known as an Antibiotic (ATB) is one that can either kill or limit the growth of other microorganisms, such as microscopic fungus and bacteria (Yala et *al.*, 2001).

Specifically, according to the World Health Organization (WHO), antibiotics are defined as "natural, semi-synthetic or synthetic substances that selectively inhibit or kill microorganisms, especially bacteria, without harming host tissues". ATBs are widely used in the treatment of bacterial infections, but their inappropriate use can lead to the development of resistance in bacteria, which can make infections more difficult to treat (Organization, 2001).

ATBs must be chosen based on their effectiveness on the bacteria to be fought, which can be tested with a susceptibility test (Spellberg et *al.*, 2008).

2.1.2. Types of Antibiotics

Antibiotics can be classified according to several criteria: origin, chemical nature, mechanism of action and spectrum of action (**Appendix 1 :** Tab **16, 17, 18, 19 and 20**).

2.1.3. Risk of Antibiotics use in general

One of the biggest risks to human health acknowledged by scientists worldwide is antimicrobial resistance (AMR). Recent results indicate that resistant organisms have also been found in patients receiving primary care, even though antibiotic resistance has historically mostly been a clinical issue in hospital settings (Llor & Bjerrum, 2014). According to a recent report from the World Health Organization (WHO), the issue of AMR is now present all over the world and is not just a phenomena affecting underdeveloped or impoverished countries (Organization, 2014).

Data indicate a direct link between antibiotic use and resistance. Antibiotic resistance is more prevalent in nations with higher antibiotic usage (Goossens et *al.*, 2005);(Riedel et *al.*, 2007). In addition to increasing resistance, overprescribing antibiotics is linked to additional hazards, including (Llor & Bjerrum, 2014) : antimicrobial resistance rising, increase in more serious illnesses, increase in the duration of the illness, Increase of the risk of complications, a rise in mortality rates, cost of healthcare rising, Increase of the risk of adverse effects, some being life-threatening, Increase of re-attendance due to infectious diseases and more self-limiting infectious diseases are being treated medically.

Patients who take antibiotics run the risk of negative side effects. The majority of these side effects are minor, however some have been identified as being potentially fatal, such as hepatotoxicity brought on by amoxicillin and clavulanate (Chang & Schiano, 2007). Overprescribing antibiotics has been demonstrated to increase patient re-visits since it medicalizes self-limiting diseases (Little et *al.*, 1997); the more visits, the more antibiotics are prescribed and used.

2.2. Resistance

2.2.1. Definition of Antibiotic resistance

Resistance to antibiotics, or antibiotic resistance, is the ability of a microorganism to resist the effects of antibiotics. While some bacteria are naturally resistant to antibiotics, the resistance acquired over time from misuse is of greater concern. It refers to the appearance of resistance to one or more antibiotics in a bacterium that was previously sensitive to it.

Since 1950, pathogenic bacteria that have become resistant to common ATBs have been identified, and this phenomenon has continued to increase. The effectiveness of ATBs has led to the excessive and often inappropriate use of ATBs in human and animal health (Organization, 2001).

2.2.2. Types of Antibiotic resistance

2.2.2.1. Natural resistance

Some bacterial species have natural resistance because of their structure or metabolism. Gram-negative bacteria, for instance, have an exterior barrier that prevents hydrophilic medicines like penicillin from entering the cell. Similar to mycobacteria, which cause leprosy and tuberculosis and have lipid-rich cell walls, they are resistant to a wide range of antibiotics (Davies & Davies, 2010).

Table3 : Natural resistance in Enterobacteria.

Bactérie	AMP/AMX	AMC	TIC/PIP	C1G	FOX	GEN	TCY	COL	NIT
***Klebsiella* spp.**	R		R						
C.koseri	R		R						
C.freundii	R	R		R	R				
E.cloacae	R	R		R	R				
E.aerogenes	R	R		R	R				
S.marcescens	R	R		R				R	
P.mirabilis							R	R	R
P.vulgaris	R			R			R	R	R
M.morganii	R	R		R			R	R	R
P.stuartii	R	R		R		R	R	R	R
Y.enterocolitica	R	R	R	R	R				

R: natural resistance

2.2.2.2. Acquired resistance

However, is a gradual phenomenon brought on by bacteria being exposed to antibiotics. Bacteria can acquire antibiotic resistance by horizontal gene transfer or mutation. Antibiotic targets and transport pathways can both be changed by mutations. Through conjugation, transformation, or transduction, horizontal gene transfer can take place, enabling bacteria to share plasmids or integrals encoding resistance genes.

Since acquired resistance can spread quickly across bacterial populations and reduce antibiotic effectiveness, it is particularly concerning. Antibiotic use should be prudent and reasonable, and infection control procedures should be followed to stop the spread of resistant microorganisms (Davies & Davies, 2010).

2.2.3. Mechanisms of Antibiotic resistance

There are three different types of antibiotic resistance mechanisms (**Fig.** 1) that microbes have developed. The first is the production of antibiotic inactivating enzymes (e.g. b-lactamases). The modification of antibiotic targets preventing the action of the latter is the second, as in the case of fluoroquinolone resistance through the alteration of class II topoisomerases. Finally, the third mechanism is the reduction of intracellular antibiotic concentrations. This can be due to impermeability (such as imipenem resistance caused by alteration or deletion of OprD porin in *Pseudomonas aeruginosa*) and/or active transfer to the outside of the cell through membrane transporters known as efflux pumps (Walsh, 2000).

So, Bacteria can exchange resistance genes with other bacteria, allowing them to develop resistance to several types of antibiotics at the same time. In addition, bacteria can survive antibiotics by altering their own metabolism, activating mechanisms for pumping drugs out of their cells, or forming biofilms that protect them from the effects of antibiotics (Martinez, 2009).

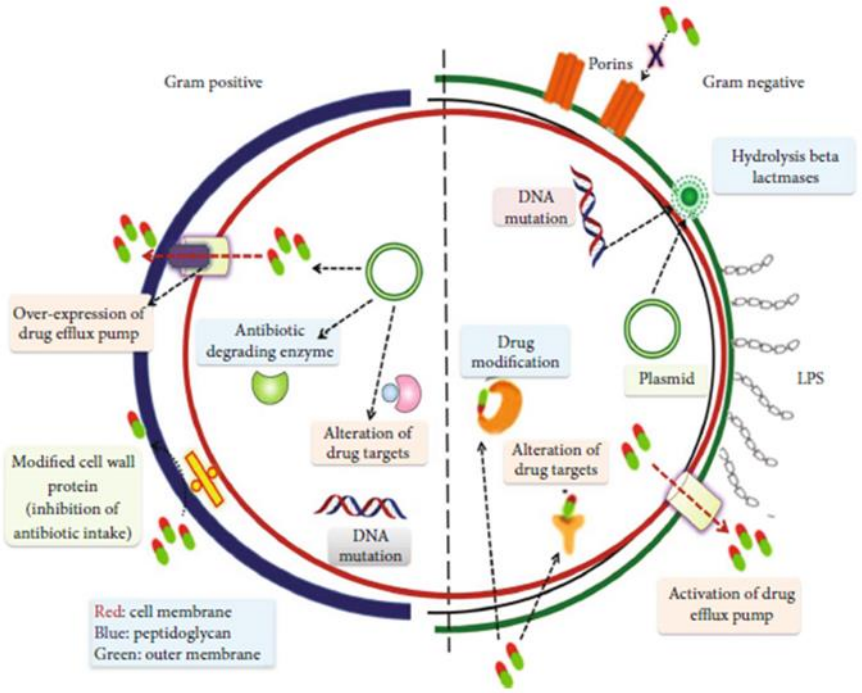

Figure 1: Resistance mechanisms found in Gram-positive and Gram-negative bacteria (Bharadwaj et al., 2022).

2.2.4. Multi-resistant bacteria

The term "multidrug-resistant" refers to microorganisms that are resistant to a variety of antibiotics families, which restricts the therapeutic options in the event of infection. The spread of antibiotic resistance is facilitated by multidrug-resistant bacteria, which can be challenging to treat. This type of antibiotic-resistant bacterial strain was originally rare and exclusively observed in nosocomial infections, but it is now widespread, on the rise, and poses a very serious threat to human health (Walsh, 2000).

Table 4: Profile resistance of Enterobacteriaceae.

B-lactamins group	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Major Enterobacteria Genera Encountered in Hospital Settings	*Escherichia coli* *Proteus mirabilis* *Salmonella* *Shigella*	*Klebsiella* *Citrobacter koseri*	*Enterobacter* *Serratia* *Morganella* *Providencia* *Citrobacter freundii*	*Yersinia*
Aminopenicillins	S	R	R	R

Carboxypenicillins	S	R	R	R
Ureidopenicillins	S	I/R	S	I/R
CIG	S	S	R	R
C3G	S	S	S	S
Carbapenems	S	S	S	S
Mechanism of resistance	Absence of B-lactamase	Low level penicillinase	Low-level cephalosporine	Penicillinase + cephalosporinase

S: Sensitive, **I:** Intermediate, **R:** Resistant

3. Beta lactamases

3.1. Definition of Beta lactamases

Certain bacteria create β-lactamases, which are enzymes that enable them to resist antibiotics from the beta-lactam family, including penicillin, cephalosporins, and carbapenems.

By hydrolyzing the amide bond present in the antibiotic molecule, BL's causes the beta-lactam nucleus to change chemically, resulting in antibiotic resistance. The antibiotic's three-dimensional structure is destroyed during this hydrolysis, rendering it ineffective against bacteria. BL are able to resist a variety of beta-lactam antibiotics due to their varied specificity for various types of these medications (Paterson & Bonomo, 2005).

3.1.1. Classification of Beta lactamases

Amber molecular classification (Ambler, 1980) (Tab **5**) and Bush-Jacoby-Medieros functional classification system (Bush et *al.*, 1995) (Tab **6**),are two ESBL classification methods.

According to their homology of amino acid in the active site, β-lactamases are divided into 4 groups, A to D, in the Amber scheme. Serine-lactamase (class A) and Metallo-lactamase (class B) were the first two classes, with class A containing serine at the active site and class B requiring a bivalent cation, typically Zn2+, to function. Due to the identification of novel serine -lactamases, this class was divided into classes C (Jaurin & Grundström, 1981) and D (Ouellette et *al.*, 1987).

Table 5: Classification of β-lactamases according to Amber molecular scheme (Paterson & Bonomo, 2005) (Stürenburg & Mack, 2003).

Class		**B -lactamases**	Examples
Serine B-lactamases	A	Broad spectrum ß-lactamases	TEM-1, TEM-2 SHV-1
		ESBL TEM-type	TEM-3
		ESBL SHV-type	SHV-5
		ESBL CTX-M-type	CTX-M1, CTX-M9
		Carbapenemases	KPC
	C	AmpC cephamycinases (chromosomal encode)	
		AmpC cephamycinases (plasmid encode)	CMY, DHA
	D	Broad spectrum ß-lactamases	OXA-1, OXA-9
		ESBL OXA –type	OXA-2, OXA-10
		Carbapenemases	OXA-48, OXA-23
Metallo ß-lactamases	B	Metallo B- lactamases	VIM, IMP A

Bush-Jacoby-Medeiros introduced the new enzymatic activity-based classification method for β-lactamases in 1995. They divided ESBLs into 4 categories (1-4) and 6 subcategories (a-f).

Table 6: Classification of β-lactamases according to Bush-Jacoby-Medieros system (Babic et *al.*, 2006).

Group	**Type**	**Exemples**
1	Cephalosporinases	AmpCs, CMY-2
2	clavulanic acid susceptible	
2a	penicillinases	PC-1 from *S.aureus*
2b	Broad-spectrum Penicillinases	TEM-1, SHV-1
2be	ESBLs	SHV-2, TEM-10, CTX-Ms
2br	Inhibitor resistant	TEM, IRT
2c	Carbenicillin hydrolyzing	PSE-1
2d	Oxacillin hydrolyzing	OXA-10, OXA-1
2e	Cephalosporinase inhibited by clavulanate	FEC-1
2f	Carbapenemases	KPC-1, SME-1
3	Metallo-beta-lactamases	IMP-1, VIM-1
4	Miscellaneous	

3.2. Definition of ESBL

The extended-spectrum beta-lactamase (ESBL) family of bacterial enzymes is enormous and extremely diverse. They are brought on either by plasmids (common) or by a mutation of the native genome encoding a beta-lactamase SHV in *Klebsiella spp*. Affected bacteria can hydrolyze a variety of penicillins and cephalosporins via both methods. Most cases of ESBL are caused by genetic changes in naturally occurring beta-lactamase, including TEM-1, TEM-2, and SHV-1. They have a modest level of activity against first-generation cephalosporins and a high level of activity against penicillins (Paterson & Bonomo, 2005).

Third-generation cephalosporins (ceftazidime and cefotaxime) and monobactams (aztreonam) are both impacted by the genetic alterations that cause ESBL, which also broaden the range of these enzymes. Clavulanic acid, tazobactam, and sulbactam, the traditional beta-lactam inhibitors, inhibit ESBL-producing bacteria because they do not hydrolyze cephalomycins (cefoxitin) or carbapenems (imipenem) or hydrolyze them. Fluoroquinolone resistance and the existence of ESBLs are frequently linked (Paterson & Bonomo, 2005).

3.2.1. Classification of ESBL

The majority of ESBLs fall into one of three categories: TEM, SHV, or CTX-M. The other ESBL groups, like VEB, PER, GES, TLA, IBC, SFO-1, BES-1, and BEL-1, have, nevertheless, been reported (Thirapanmethee, 2012).

- **TEM β-lactamases**

The most common subtype in this group is TEM-1. According to reports, more than 90% of ampicillin-resistant *E. coli* are caused by this enzyme. The derivative of TEM-1 with an amino acid substitution is called TEM-2. The first TEM-type -lactamase to exhibit ESBL phenotypic characteristics is TEM-3. The enzyme is able to attach to oxyimino -lactams thanks to this change in amino acid. The enzyme becomes more susceptible to Beta-lactamase inhibitors by opening the active site to bind to the -lactam ring. ESBL phenotypic diversity results from amino acid substitutions at positions 104, 164, 238 and 240. Most ESBLs have mutations in more than one location of an amino acid. 190 different TEM group kinds have been recorded as of May 2011 (Jacoby, 2012).

- **SHV β-lactamases**

The SHV-1 and TEM-1 share 68% of their amino acid sequences. Similarities in structure are also present *K. pneumoniae* frequently contains the SHV-1 -lactamase, which contributes to ampicillin resistance. Similar to TEM-1, these ESBLs also feature amino acid alterations at the active site, which are often found at positions 238 or 238 and 240. At the end of May 2011, 141 different forms of SHV have been reported (Jacoby, 2012). This group is typically found around the world, not just in Europe and the United States. These group's most prevalent enzymes are SHV-5 and SHV-12 (Thirapanmethee, 2012).

- **CTX-M β-lactamases (class A)**

Because it is more resistant to cefotaxime than to other oxyimino -lactams such CAZ, CRO, and cefepime, this group earned its name. The acquisition of plasmid-encoded -lactamase from the chromosome of non-pathogenic bacteria in the genus *Kluyvera*, particularly *K. ascorbata* and *K. georgiana*, is what led to the formation of this enzyme rather than mutation (Bonnet, 2004). Only 40% of this enzyme's homology to TEM and SHV. 120 different types of enzymes have been reported as of the end of May 2011(Jacoby, 2012) and They are divided into five categories by their amino acid sequences: CTX-M1, CTX-M2, M8, M9, and M25. It is the ESBL with the highest frequency in South Africa. The most prevalent CTX-M identified in UK-isolated *E. coli* right now is CTX-M15 (Livermore & Hawkey, 2005).

- **OXA β-lactamases (class D)**

Oxacillin and anti-staphylococcal penicillin can both be hydrolyzed by this enzyme. This group belongs to functional group 2d and molecular class D. It is unaffected by clavulanic acid, but it is resistant to AMP, cephalothin, oxacillin, and cloxacillin. *E. coli*, *K. pneumoniae*, and *Enterobacteriaceae* are typically home to this enzyme. The amino acid homology within this group was extremely low since it was previously categorized using a phenotypic system rather than a genotypic one (Thirapanmethee, 2012).

- **PER**

About 25–27% of the amino acids of PER ESBLs and TEM and SHV are similar. Penicillins and cephalosporins are effectively hydrolyzed by PER-1 -lactamase, although clavulanic acid inhibits it. PER was formerly typically isolated from *P. aeruginosa*, however it is now now found in *Acinetobacter sp* and *Salmonella enterica serovar Typhimurium.* Though

present everywhere, the PER-type ESBL is particularly common in Europe (Thirapanmethee, 2012).

- **Other types of ESBLs**

Many ESBL varieties, including VEB, GES, and IBC, are not covered in the list above. Compared to TEM, SHV, and CTX-M, the predominance of other groups is minimal. They are usually separated from *P. aeruginosa* and only occur in a small geographic area, such as South East Asia for VEB-1 and VEB-2 or South Africa, France, and Greece for GES-1, GES-2, and IBC-2. BES-1, IBL-1, SFO-1, and TLA-1 are examples of the rare type ESBLs, which are exclusive to the *Enterobacteriaceae* family (Thirapanmethee, 2012).

3.2.2. ESBL-producing bacteria

3.2.2.1. Enterobacteriaceae

The majority of antibiotics used to treat infections brought on by *Enterobacteriaceae* are -lactamines because of their variety, low toxicity, bactericidal activity, and broad spectrum of action. The amount of antibiotic in contact with the target, the antibiotic's affinity for the target, and the formation of the -lactamase that inactivates the antibiotic all play a role in how effective -lactams are. All strains of the species have some level of resistance due to these factors, but some strains have gained resistance as a result of mutations or the acquisition of genetic material like plasmids, transposons, or integrals.

A native phenotype of -lactam resistance, also known as the "sensitive phenotype," is distinguished from acquired phenotypes of resistance, also known as the "resistant phenotypes," for each species. For some compounds, resistance can be expressed at low levels in vitro, but it can also be the cause of treatment failures. Knowing the resistance phenotypes enables one to:

- Compen-sate for the limitations of the in vitro sensitivity tests by using interpretive reading rules.
- Establish a list of -lactams not to be studied but to be used.
- check the consistency between the identification and the resistance phenotype.
- direct the therapeutic choice.

Penicillin in classes G, V, and M are naturally resistant to enterobacteria. The phenotypes of resistance will therefore be defined using the β-lactamines belonging to the other groups of names alone and associated with the inhibitors of β-lactamases: aminopenicillin (amoxicillin),

carboxypenicillin (ticarcillin), ureidopenicillin (piperacillin) First-generation cephalosporins (cephalin), second-generation cephalosporins (cefixime and cephalomycins, cefoxitin), third-generation cephalosporins (cefotaxitin and ceftazidime), fourth-generation cephalosporins (cefoxymine and cefpirome), monobactams (aztreonam) and carbapenems (imipenem) (Bingen, 2008).

3.2.2.2. Non fermentative bacteria

- ***Pseudomonas aeruginosa***

Pseudomonas aeruginosa is a gram-negative bacillus, a major pathogen mainly responsible for infections acquired in the hospital and in general difficult treatment, it is an agent of pneumonia, bacteremia and urinary tract infections. It has the particularity of associating a high level of natural resistance to antibiotics, in particular βlactamines, and different mechanisms of resistance to essentially enzymatic antibiotics that sometimes add up within the same strain. An increasing number of acquired β- lactamases have been described in *P. aeruginosa* in recent years. The first β- lactamases, had a relatively narrow spectrum while the most recent have a hydrolysis spectrum extended to the cephalosporins of 3rd and 4th generation and/or carbapenems. These different β- lactamases belong to classes A, B, C, D, of the Ambler classification based on amino acid sequences (Bingen, 2008).

- ***Acinetobacter***

Bacteria of the genus *Acinetobacter* have long remained unknown due to their low pathogenicity. Since the 1970s and 1980s, these bacteria have developed multiple resistance mechanisms: antibiotics and have taken an important place among the multidrug-resistant bacteria responsible for nosocomial infection and more particularly of hospital epidemics. *Acinetobacter* is responsible for an average of 9 to 10% of hospital-acquired infections in resuscitation. Levels of β- lactam resistance in the international literature range from 5% to 50% for carbapenems and from 50% to 100% for all other β- lactams (Bingen, 2008).

3.2.3. Mechanism of action of ESBL

ESBL modify the chemical structure of the beta-lactam nucleus by hydrolyzing the amide bond found in the antibiotic molecule. This hydrolysis destroys the three-dimensional structure of the antibiotic and makes it inactive against bacteria. ESBL have a variable specificity for different types of beta-lactam antibiotics, allowing them to resist a wide range of these drugs.

ESBL are often encoded by genes that are transferable between different bacterial strains, facilitating their spread and dissemination. The increase in the use of beta-lactam antibiotics in recent decades has led to the emergence and spread of ESBL-producing bacteria, which can cause difficult-to-treat infections and pose a public health challenge. Understanding how ESBL works is essential for the development of new antibiotics and effective therapeutic strategies to combat bacterial infections caused by these resistant organisms (Paterson & Bonomo, 2005).

4. The presence of ESBL in chicken meat

4.1. Definition of meat

According to the World Organization for Animal Health, meat means all edible parts of an animal and the word "animal" in this context means any mammal or bird. This vocabulary includes the pulpit of mammals (sheep, cattle, goats, camels, etc.) and birds (chicken, turkey, guinea fowl, etc.).

Generally, meat is the flesh of animals. It is essentially formed by the striated muscles after their evolution post mortem, which are eaten after cooking (Drieux, 1962). From a nutritional point of view, meat and meat products are classified among the seven food groups because of their energy value, their protein richness, their intake of certain trace elements and vitamins, which are not abundant in other foods , But the quality of the meat depends on the age, sex, and breed of the animal (Fosse, 2003);(El Rammouz, 2005).

Meat is important to the food industry, economies, and cultures around the world. There are nonetheless people who choose to not eat meat (vegetarians) or any animal products (vegans), for reasons such as taste preferences, ethics, environmental concerns, health concerns or religious dietary rules (Lawrie & DA, 2006). There are two types of meat, red and white.

4.2. Definition of white meat " Chicken meat "

All items that are currently marketed in various forms, including carcasses, restructured meats, and cutting and processing products, are collectively referred to as white meat (Bourgeois et *al.,* 1988). Due to its appealing protein intake and low fat content, poultry meat is crucial for human consumption (Mussa et *al.*, 2022). The muscle tissue of chickens, which are domesticated birds raised for their meat and eggs, is known as chicken meat. The table below show the chemical composition of chicken meat (**Tab.** 7).

Table 7: Average chemical composition of chicken meat (g/100g).

Components	**Quantity per gram**
Water	73
Proteins	22
Lipids	4
Carbohydrate	Traces
Minerals	1,4
Energy in Kcal	130

4.3. Consumption of chicken meat

4.3.1. In Global

After pork, chicken is the second most popular meat consumed globally. It was calculated that there were just over 93 million tons of carcass equivalent consumed worldwide in 2008. With 18.6 million tons, the Chinese are the biggest consumers of poultry meat, closely followed by Americans with 16.3 million tons. While Americans choose chicken and turkey, the Chinese primarily eat chicken and duck. But in 2009, there was a tiny uptick. The developed world consumes the most poultry meat, with the United States leading the pack with about 53 kg consumed annually per person in 2009. Products made from poultry are processed at a very high level.

As a result, products that are cut and heavily processed (such as grilled, smoked, or marinated products) have replaced products that were purchased raw and whole. Although there was a minor increase in 2009, the only country where apparent consumption has decreased since 2005 is the United States (Quebec Poultry Industry Monograph, February 2011). Figure 2 depicts the apparent per capita consumption of poultry meat in the major consuming nations from 2004 to 2008(**Fig.** 2).

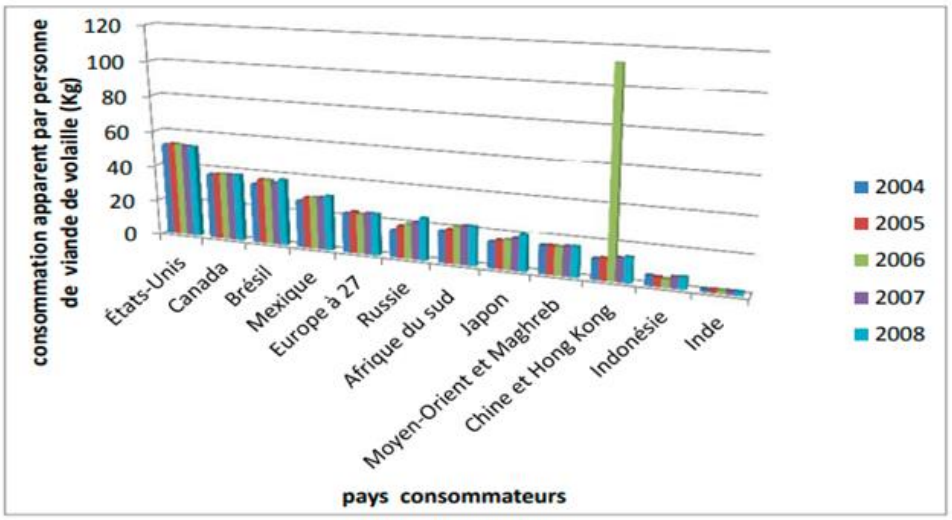

Figure 2: Per person consumption of poultry meat in the main consumer countries, 2004 to 2008.

4.3.2. In Algeria

Poultry meat consumption in Algeria According to official statistics from 2014, which have been repeated for three years, Algeria produces 350,000 tons of red meat and 250,000 tons of white meat annually. A total of 600000 tons per year for a national consumption requirement of about 1 million tons. During this year, the Ministry of Agriculture reported that red meat production has increased by 100,000 from the UGCAA (Union General des Commerçants et Artisans Algerians) which gave these figures on the sidelines of a press conference on the meat market (The evening of Algeria, Sunday, June 8, 2014). According to BOULENOUAR (2014), Algerians still consume below the global meat standard. While the recommendations of «WHO», and «FAO» are 25 kilos of meat per person per year, the Algerian consumes between 18 and 20 kilos only (the evening of Algeria, presse : le soir d'algerie Sunday 8 June 2014).

4.4. Contamination of meat by ESBL

4.4.1. Origin of contamination

ESBL-E infections were initially primarily a hospital-related issue with hospital acquisition or contact-related transmission. This has altered over the past 20 years, and now persons who have never received medical care are also ESBL-E rectal carriers (Livermore et *al.*, 2007) (van den Bunt et *al.*, 2019). By employing a one-health approach that considers people, animals, and the environment as an integrated entity, research efforts have been concentrated on identifying the

transmission pathways, reservoirs, and resistance genes of antimicrobial-resistant microbes (Huizinga et *al.*, 2019).

It has been proposed that contaminated food may be a source of ESBL-E (Overdevest et *al.*, 2011). Exchange of microbes or genetic material between humans and animals has also been theorized, for example, between farmers and their livestock where the epidemiological connection is rather clear (Dierikx et *al.*, 2013). Due to larger spatial-temporal differences, transmission of ESBL-E isolates or plasmids carrying resistance genes from bacteria on retail chicken meat to humans in the general population is more challenging to demonstrate (Huizinga et *al.*, 2019).

4.4.2. Impact on human health

Customers' meat consumption exposes them to ESBL-EC Bacteria can be transmitted to people in many different ways. These may include bacteria like ESBL-E that are resistant to antibiotics. Due to ineffective antibiotic therapy during the initial stages of treatment, ESBL infections are linked to longer hospital admissions, greater care expenditures, and higher morbidity and mortality (Kim et *al.*, 2002). The prevalence of community-acquired ESBL infections is rising, despite the fact that they are often linked to earlier health care-related exposures (Zhanel et *al.*, 2006). *E. coli* is the primary cause of several common bacterial diseases, including gastroenteritis, urinary tract infections (UTI), septicemia, and newborn meningitis (Allocati et *al.*, 2013). Different symptoms are brought on by ESBL bacteria depending on where the infection is. Urinary tract, fever, and gastrointestinal tract are common locations and symptoms. diarrhea (which could be bloody), abdominal pain, stomach cramps, gas, and loss of appetite shortness of breath, a skin injury, chills, nausea, and confusion.

4.4.3. Situation in the world

In one study in France, between 2016 and 2019, 1,935 meat samples were tested for the presence of ESBL- Ec, AmpC or carbapenemase. No carbapenem-resistant *E. coli* was isolated from these samples. Of the samples tested, 296 (15.3%) contained at least one suspected *E. coli* strain that produced an ESBL or AmpC enzyme. The highest prevalence's were observed in chicken meat samples (62% in 2016 and 26% in 2018) while those observed in pork and beef samples did not exceed 1.2%, regardless of the year of observation.

4.4.4. Situation in Algeria

Enterobacteria, and particularly *E. coli*, are considered important indicator bacteria that could be used to track antibiotic resistance in different ecosystems, and it is not only a worldwide

threat. *E. coli* avian is recently considered in Algeria as one of the most important causes of economic losses in the poultry sector (Halfaoui et *al.*, 2017). Many studies in Algeria has reported the presence of ESBL-EC in poultry meat specifically and in the food chain in general. Among them, in one study, 20 strains of *E. coli* resistant to third-generation cephalosporins were isolated from 61 cloacal swabs collected from broiler chicken. Of these, 16 were ESBL producers and 4 were plasmid cephalosporinases producers (Belmahdi et *al.*, 2016). This confirms the risk of exposure to ESBL-EC and associated diseases.

Problematic and Objective

Problematic and Objective

Escherichia coli isolates that produce the extended-spectrum ß-lactamase (ESBLs) pose a threat to human health on a global scale and have been found in humans, animals, and the environment. In *Enterobacteriaceae,* ESBLs are currently the most significant resistance mechanisms. Modern extended spectrum cephalosporin and monobactam medications may become less effective as a result. The use of antibiotics to treat sick animals and promote growth in cattle may result in the emergence of highly resistant bacterial strains that can spread to people via the food supply. *E. coli* has been regarded as an excellent indicator of the selection pressure put on the intestinal population of bacteria in food animals by the use of antimicrobials, and may also be used as a representative of the *Enterobacteriaceae* to monitor the emergence and changes in proportion of bacteria possessing ESBLs.

In Algeria, few studies investigated the problem of ESBL producing *Enterobacteriaceae* in poultry meat. Moreover, to our knowledge, no published data exist about this problem in Msila.

Based on the presented background, we conducted a study to isolate and characterize ESBL producing *E. coli* in poultry meat from the 3 different regions (Msila center, Bousaada, Sidi aissa) of Msila, with the following sub-objectives:

- Isolation of 3GC resistant *Enterobacteriaceae*.
- Determination of ESBL prevalence.
- Determination of the antimicrobial resistance pattern of these isolates.

Materials and Methods

Materials and Methods

1. Study conception

The study design is presented in Figure 3.

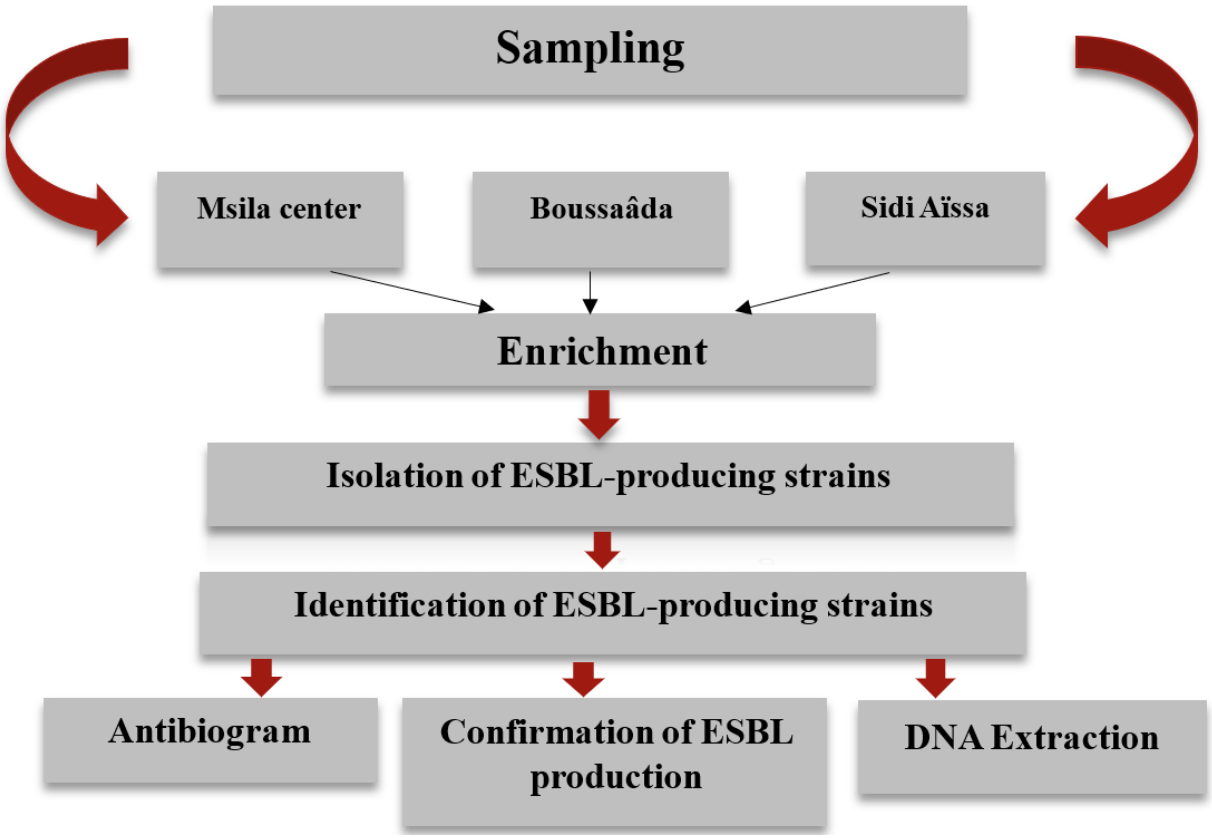

Figure3 : Study conception.

In the three regions, sampling was carried out in the most frequented areas in order to cover as many as possible. Next, ESBL-producing strains were isolated and identified. And then, the antibiotic sensitivity of the isolated strains and the confirmation of the production of ESBL were realized. Finally, the extraction of DNA from ESBL-producing strains.

2. Sampling

2.1. Sampling area

Sampling of chicken meat was carried out from butcheries distributed on 3 localities (Dairas) of the wilaya of Msila (Msila center, Bousaada, Sidi-Aissa).

Figure4 : Map of the sampling areas.

The number of samples taken from each area is represented in the table below.

Table 8: The number of samples taken from each site.

Sampling sites	Samples number	Rate (%)
Msila	43	69,35
Sidi Aissa	12	19,35
Bousaada	7	11,29
Total	62	100

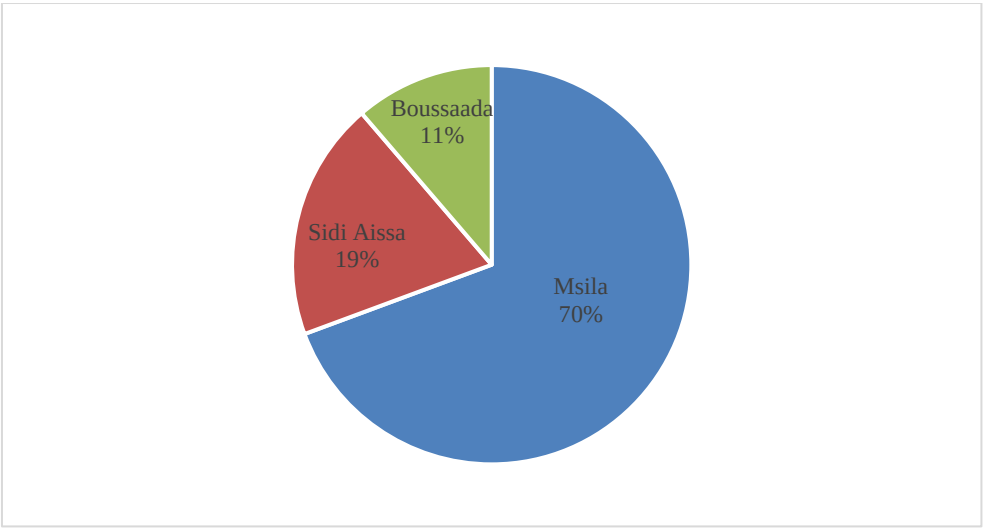

Figure5 : Repartition of samples according to the sampling site.

2.2. Sampling period

A total of 62 samples were taken over the period of February 28 to March 21, 2023.

2.3. Sampling method

Sampling was carried out accordance with the practical procedures to ensure that the samples represent the situation of contamination at the sampling time and place. To obtain sample, a piece of poultry meat was bought from the butcheries. No instructions were given to the butcher and he was not aware that samples will be used for a research purpose. We processed samples to not make any contamination after the butcher gave the samples. Then, meat was transported to the laboratory of microbiology in the university of Msila, within one hour. In the laboratory, it was aseptically cut and put in pre-sterilized jars, then frozen until further analysis.

2.4. Sample type

Samples were taken either from "Wings" or "Escalope" from every butchery.

Table 9: Samples type.

Type of sample	Number of samples	Rate (%)
wings	32	51,61
Escalope	30	48,39
Total	62	100

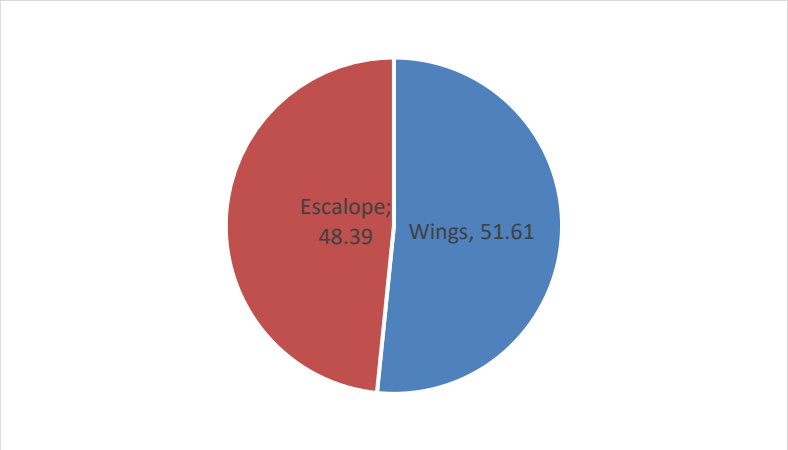

Figure6 : Repartition of samples according to their type.

2.5. Sample preparation

In the laboratory, Samples were cut into small pieces (weighing about 5g) using a sterile knife. After that, the samples were put into a sterile jars without forgetting to specify name, number, region and sampling date. Finally, the jars were frozen (-20) until the day of use.

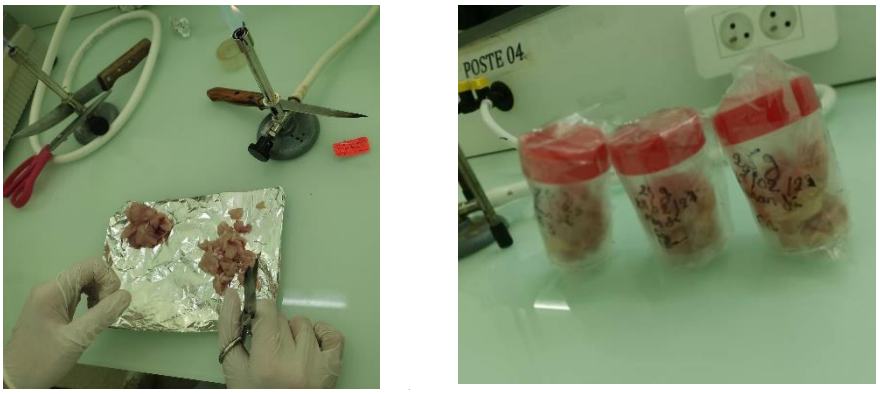

Figure7 : Sampling procedures.

3. Transport of samples to laboratory

All samples, well-sealed and packed to prevent any leakage or contamination, were transported to the «Laboratory of Bacteriology and Virology» at the National Research Centre in Biotechnology (CRBt), in a cooler containing ice packs to keep samples at a temperature of approximately 4°C throughout the trip. When samples arrive in the laboratory, they were either frozen or placed directly in isolation depending on the possibilities (during or after working hours).

3.1. The laboratory

The internship was held in the bacteriology and virology laboratory of the National Biotechnology research center (CRBt) in Constantine and it was created by decision N° 330 of 30 September 2015. This laboratory partly meets the missions and objectives of CRBt's research teams and projects in the field of biotechnology.

The bacteriology and virology laboratory is located on the ground floor of the CRBt, it is of a containment level type 2 according to national and international standards. This type of containment has the advantage of protecting both the manipulator, the sample and the environment.

Figure8 : Bacteriology and virology laboratory in CRBt – Constantine.

4. Isolation of ESBL-producing strains

Isolation of extended-spectrum beta-lactamase-producing bacteria was realized in three steps. First, an enrichment was realized on Nutrient Broth. After that, the screening step was carried out on MacConkey agar supplemented with 1 µg/ml of a 3rd generation cefotaxime as described by Valverde et al. (2004). Finally, confirmation of ESBL aspect was done by the synergy test.

4.1. Enrichment

This step involves seeding a piece of chicken meat directly into a tube containing a sterile nutrient broth (NB) and vortex it. The mixture was then incubated at 37°C for 24 hours. If the medium becomes cloudy, it is considered positive for enrichment.

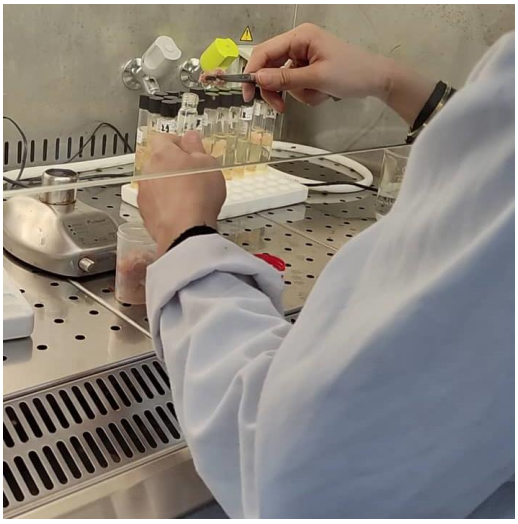


Figure9 : Enrichment in a nutrient broth.

4.2. Screening

4.2.1. Preparation of the cefotaxime supplemented MacConkey medium

First, 500 ml of MacConkey medium was prepared according to the manufacturer's recommendations. Then the antibiotic was added to the medium as follows:

- Preparation of stock solution of 1 mg/ml by diluting 0.01 g (10mg) cefotaxime in 10 ml of sterile distilled water. In our case we diluted 4.7mg in 4,7 ml of sterile distilled water as described in the figure bellow
- Aliquots of 500 µl are made and kept at -20°C,
- Then 500 µl of stock antibiotic solution was added to 500 ml of supercooled MacConkey. medium (45°C), to obtain a final solution of 1 µg/ml.
- The medium was distributed in Petri dishes.
- The medium was kept at +4°C until use.

Figure10 : Weighing the antibiotic powder cefotaxime.

4.2.2. Screening of ESBL-producing bacteria

From the enrichment tube, 50 µl was streaked on antibiotic supplemented medium and incubated at 37°C for 24 hours. Purification of the strains was carried out by repeated cultures until a pure culture was obtained.

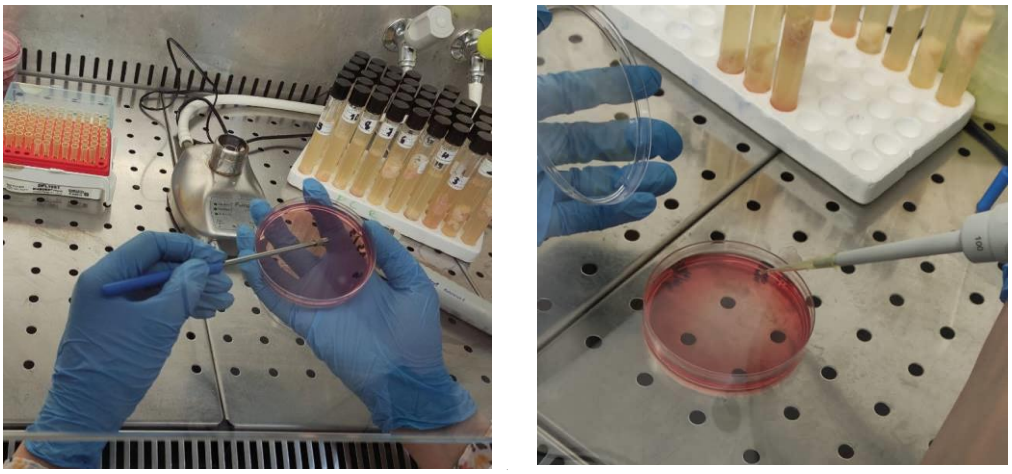

Figure11 : Screening for ESBL-producing bacteria.

4.2.3. Confirmation of ESBL presence

Confirmation of ESBL presence was done by the synergy test. This test was performed and interpreted in accordance with the recommendations of the National Standardization of Antibiotic Susceptibility testing (human and veterinary medicine, 6th version, 2011). Details are presented in the antibiogram section.

- **Technique**

The ESBL test was carried out under standard antibiogram conditions, by applying a 30 mm center-to-center disk of amoxicillin + clavulanic acid (AMC) to a disk of Cefotaxime (CTX) and incubating it for 18 h at 35°C.

- **Interpretation**

Enzyme production can result in the appearance of a synergistic image, or champagne cork, between the AMC and CTX discs.

5. Identification of bacterial strains isolated by Gallery API 20E

Identification of ESBL positive strains was by the API20E system. We followed instructions of the data sheet (GALERIE API 20E ®) supplied by BioMérieux. API 20E galleries

are miniaturized, standardized biochemical test galleries that can be used with complete identification databases (20 characters for *Enterobacteriaceae*).

5.1. Fresh culture preparation

API Gallery identification was performed on fresh 24h culture (exponential growth phase). Colonies were streaked on MH agar medium and incubated at 37°C for 18 to 24 hours.

5.2. Inoculum preparation

A bacterial suspension was prepared from a fresh culture, by dissolving 1 to 2 colonies in 5 ml sterile physiological water. The bacterial suspension was well homogenized and adjusted to an opacity of 0.5 MF. To obtain this, we used a densitometer and adjusted the suspension to an OD of 0.08 to 0.10 read at 625 nm.

5.3. Preparation and inoculation of the API 20E gallery

The first step was to assemble the bottom and lid of an incubation dish and mark the tray with the strain identification code. Water was then poured into the alveoli to create a humid atmosphere.

Once the incubation dishes have been prepared and identified, the cups were inoculated with the bacterial suspension adjusted to 0.5 MF opacity, placing the tip against the wall of the cup so as not to create bubbles during inoculation which could falsify the result.

For the ADH, LDC, ODC, H2S and URE tests, the tube was filled with the suspension and then covered with kerosene oil to create anaerobiosis. For the CIT, VP and GE tests, the tube and cup were filled, while for the remaining tests, filling was limited to the tubules.

Figure12 : Preparation of the API gallery 20E.

5.4. Reading galleries

After 18-24 hours of incubation at 35-37°C the gallery was read with reference to the reading chart provided either directly or after the addition of reagents. Strains are identified using an online database (APIWEB™).

6. Antibiogram

Antibiotic susceptibility is tested using the standard antibiogram method by diffusion on Muller-Hinton agar in accordance with the recommendations for national standardization of antibiotic susceptibility testing (human and veterinary medicine, 6th version, 2011).

6.1. Fresh culture preparation

Antibacterial activity was tested on young bacterial strains in exponential growth phase. Cultures were reactivated by subculturing on Muller-Hinton (MH) medium in petri dishes, then incubated at 37°C for 18 to 24 hours.

6.2. Inoculum preparation

From pure cultures grown on MH medium for 18 to 24 h, a few well-isolated, perfectly identical colonies were transferred using a Pasteur pipette and discharged into 5 ml sterile physiological water, then homogenized and adjusted to 0.5 MF opacity. The opacity was determinaed as an optical density OD of 0.08 to 0.1 on a wave length of 625 of spectrophotometer.

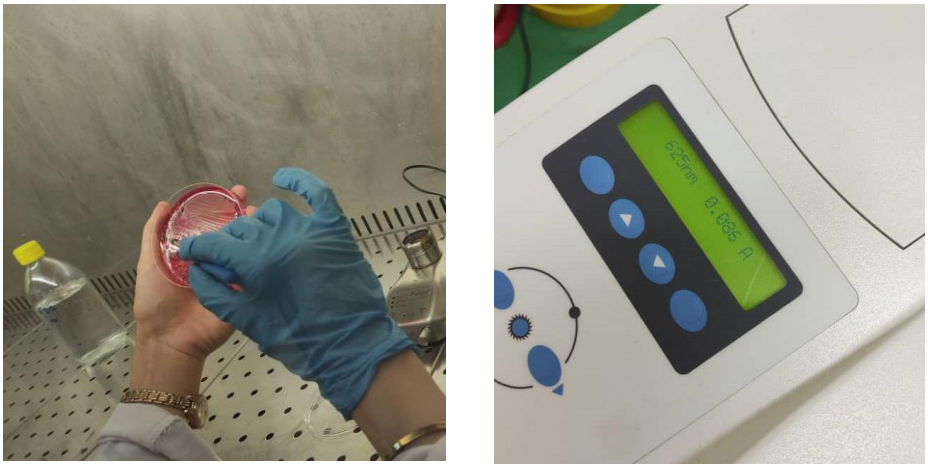

Figure13 : Inoculum preparation.

6.3. Inoculation

For inoculation, a sterile swab soaked in inoculum is squeezed out by pressing firmly (and turning) against the inner wall of the tube, to discharge as much as possible. The swab is then

rubbed over the entire dry agar surface (of petri dishes pre-filled with MH gel culture medium), from top to bottom, in tight ridges. The operation is repeated 2 times, turning the dish 60° each time, without forgetting to turn the swab on itself. At the end of inoculation, the swab is passed over the periphery of the agar.

6.4. Application of antibiotic discs

Each antibiotic disk was pressed with sterile bacteriological forceps, taking care not to move the disks after application.

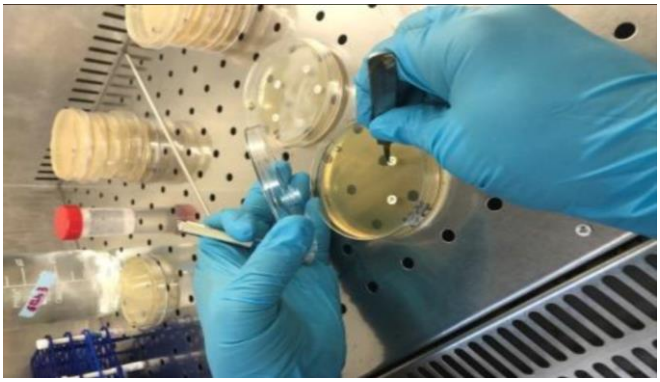

Figure14 : Deposition of antibiotic disks for antibiotic susceptibility.

6.5. Incubation

Inoculated plates were incubated for 18 to 20 hours at 37°C. After incubation, the diameters of the zones of inhibition were read using a ruler. The diameter of the zones of inhibition was interpreted as sensitive, intermediate or resistant, in accordance with the recommendations for national standardization of antibiotic susceptibility testing (human and veterinary medicine, sixth version, 2011) (Appendix 5).

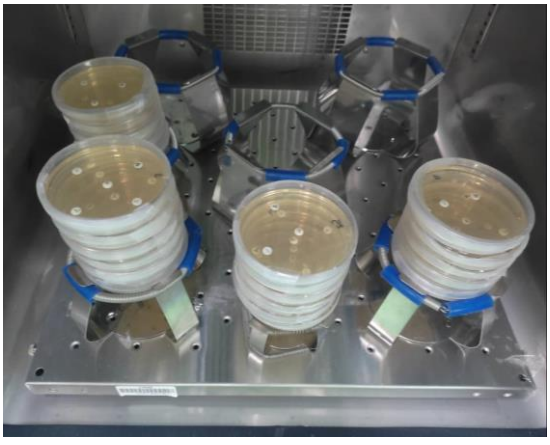

Figure15 : Antibiogram incubation.

7. DNA Extraction

DNA was extracted from all ESBL producing strains.

7.1. Technique

DNA extraction was performed on a young bacterial culture in exponential growth phase. Cultures were reactivated by subculturing on Muller-Hinton (MH) broth. To do so, 1 ml of culture medium was placed in an Eppendorf tube, then one isolated colony was placed in the tube, vortexed and incubated at 37°C for 4 hours. Then a washing step was performed as follow: the Eppendorf tube used for subculturing was centrifuged at 4000xg for 3 minutes and the supernatant was removed. Next, 1 ml of TE buffer pH 8 (wash) was added, and the mixture was centrifuged at 4000xg for 3 minutes and the supernatant discarded.

Then, we started the extraction adding 150 µl of TE buffer the remaining pellet, and it was incubated at 100°C (in a block heater, as shown in the figure bellow) for 10 minutes. After that, it was centrifuged at 12,000xg for 10 minutes.

Finally, 100 µl of the supernatant (containing the dissolved DNA) was taken and distributed to two Eppendorf tubes, one kept at +4°C . For PCR and the other at -20 for longer storage.

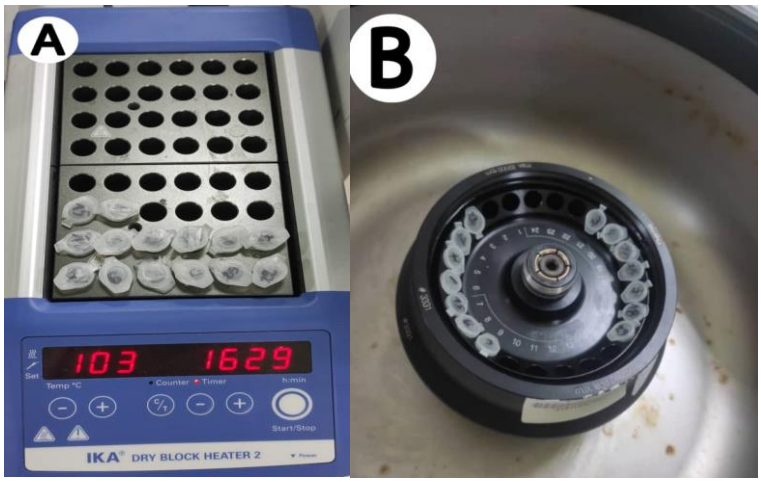

Figure16 : (A) Dry Block Heater (B) Centrifuge , for the DNA extraction.

7.2. Quality and quantity determination

A nanodrop was used to take readings in order to determine the DNA's purity and quantity. Two microliters of each extracted DNA were put into the nanodrop spots (as shown in the figure bellow right). The TE (Tris EDTA) buffer was used as a blank.

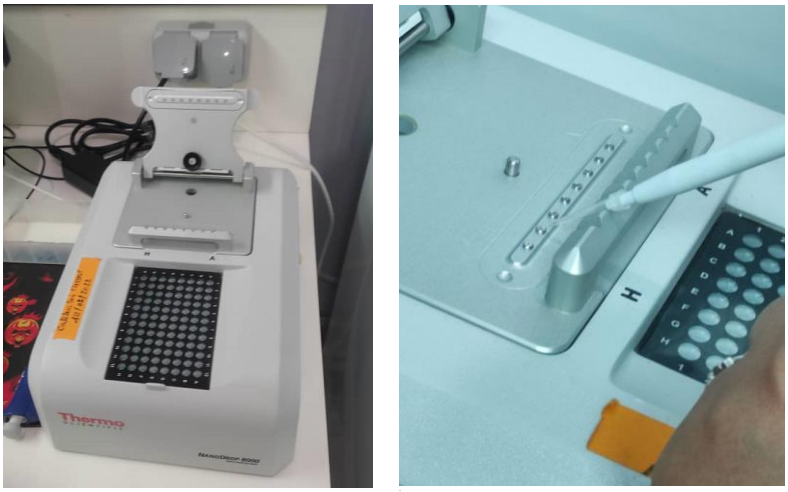

Figure17 : Nanodrop.

Results and Discussion

Results

1. Enrichment

All media got cloudy after enrichment and were considered enriched. This indicates that the samples grew on this medium.

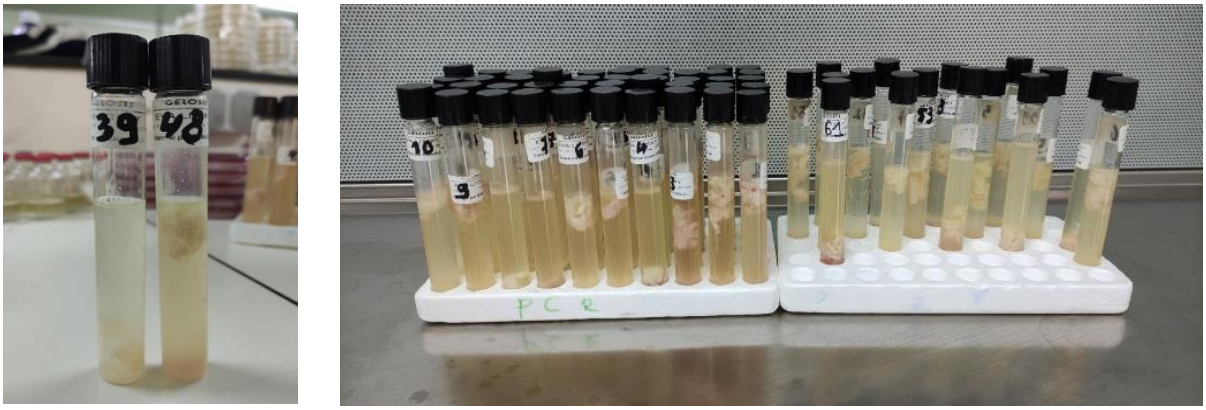

Figure 18 : Tubes after enrichment.

2. Screening

The objective of screening on selective medium (MacConkey) for antibiotic-added (cefotaxime) is the selective growth of antibiotic-resistant enterobacteria. In addition, this medium allows to highlight the fermentation of lactose.

The objective of this work is to isolate *E. coli* strains. The bacteria in this group ferment lactose; the bacterial strains that ferment lactose (transfer of the color of the middle around the colony) are chosen for the next step.

2.1. Reference strain

For this test the reference strain (*E. coli* ATCC 25922) is seeded on a culture medium (MacConkey + cefotaxime) and after incubation under the above conditions, the test revealed that the strain grew only on the antibiotic-free medium.

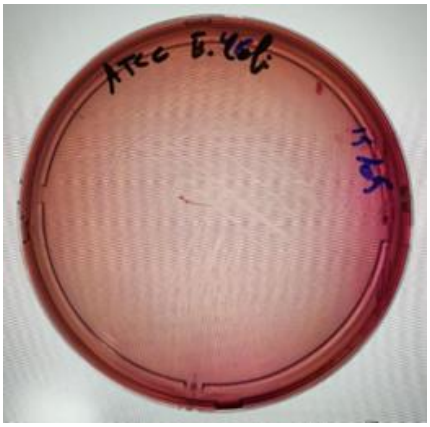

Figure19 : *E. coli* ATCC 25922 in MacConkey medium + cefotaxime.

2.2. Tested samples

Results of the screening are shown in the table and figure bellow. Out of the 62 plated dishes, 18 were negative whereas 44 were positive.

Table 10: Results of general growth.

Samples	Number	Rate (%)
Positive	44	70,97
Negative	18	29,03
Total	62	100

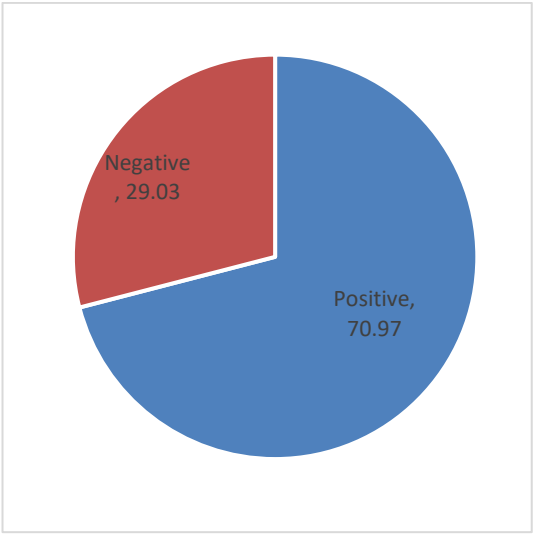

Figure20 : Repartition of results of general growth on selective medium.

2.3. Repartition on region of sampling

Strains that resist to cefotaxime were isolated from the three regions as shown in the table and figure bellow.

- For region 1: MSILA center, 36 samples (36/43) contained lactose + resistant strains.
- Region 2: SIDI AISSA, 5 samples (5/12) contained lactose + resistant strains
- Region 3:BOUSAADA, three samples (3/7) contained lactose + resistant strains.

Table 11: Positive samples according to different regions.

Region	**Total samples**	**Positive samples**	**Rate (%)**
Msila	43	36	83,72
Sidi Aissa	12	5	41,67
Bousaada	7	3	42,86

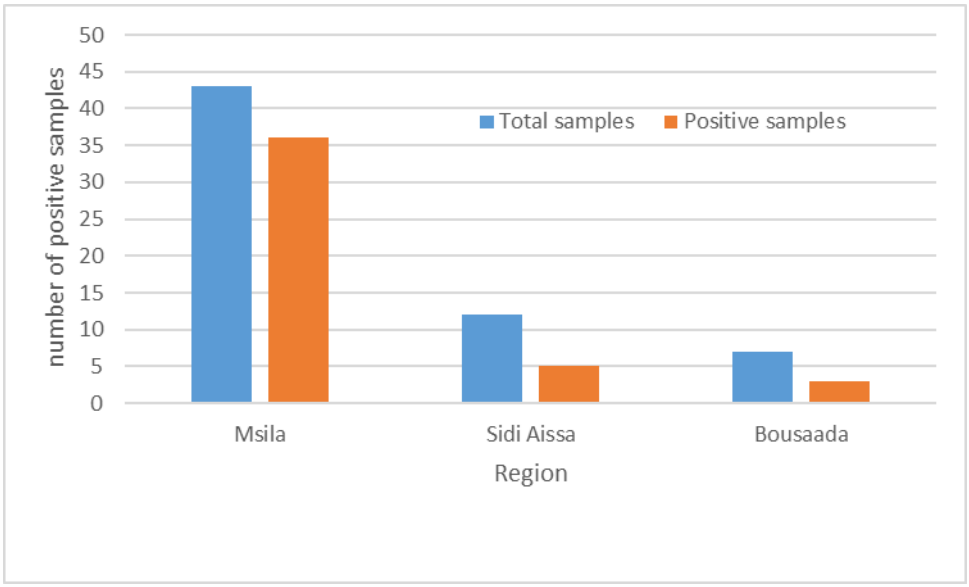

Figure21 : Repartition of positive samples according to different regions.

3. Lactose positive presumptive *E. coli*

Out of the 44 samples having positive culture, 13 contained lactose positive colonies. These colonies had a pink to dark pink, dry and donut-shaped, surrounded by a dark pink area of precipitated bile salts. This aspect is characteristic of *E. coli* in this medium.

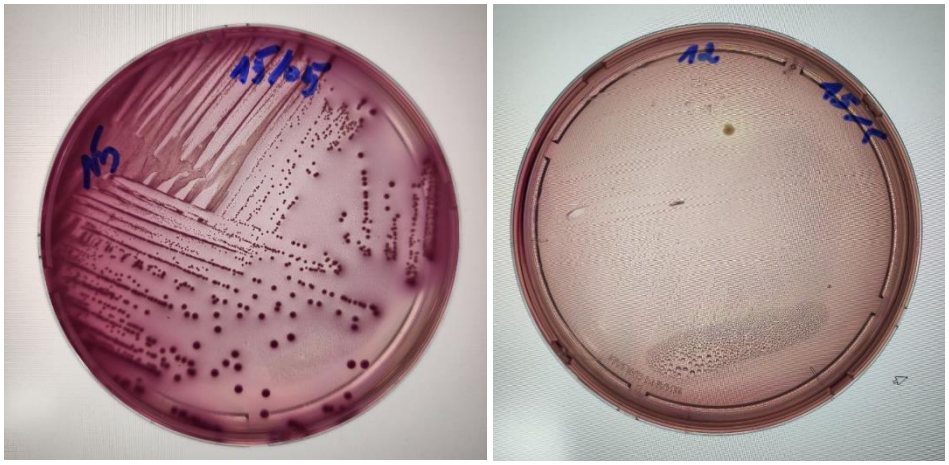

Figure22 : Screening for resistant strains to cefotaxime.

3.1. Repartition of Lactose positive colonies containing samples

Table 12: Summary of strains isolated on selective media of antibiotic-spiked Enterobacteria.

Region	Total samples	Positive samples	Rate (%)
Msila	43	6	13.95
Sidi Aissa	7	3	42,86
Bousaada	12	4	33.33

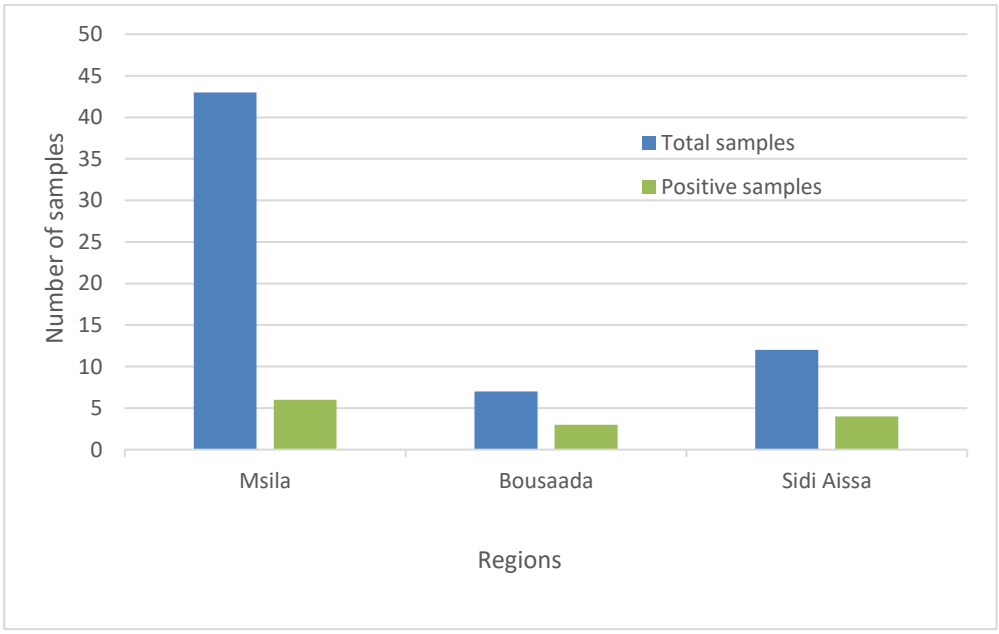

Figure23 : Repartition of isolated strains on selective media of antibiotic-spiked enterobacteria according to different regions.

4. Identification by API 20E system

The 13 lactose positive strains were further identified by API 20 system.

4.1. Quality control

In order to verify that the storage and transport conditions do not have an impact on the performance of the API E20 system, a quality control test of the screw system was carried out with reference strain *E. coli* ATCC 25922. The latter revealed a good identification of the strain tested on the web API software (100%). The aspect of the biochemical reactions of the above-mentioned strain is presented in the figure 24.

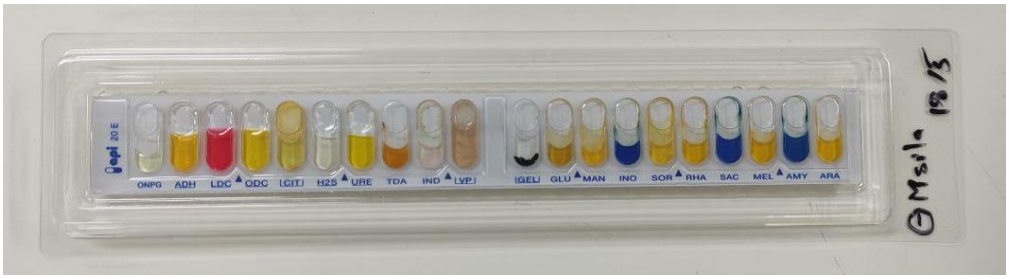

Figure24 : Biochemical profile of reference strain *E. coli* ATCC 25922 on gallery API 20E.

4.2. Identification results

All the 13 strains were identified as *Escherichia coli* with different percentages. It ranged from acceptable to excellent identification.

The API 20E Gallery identification result is presented in Table 13. All strains were *E. coli* with good identification.

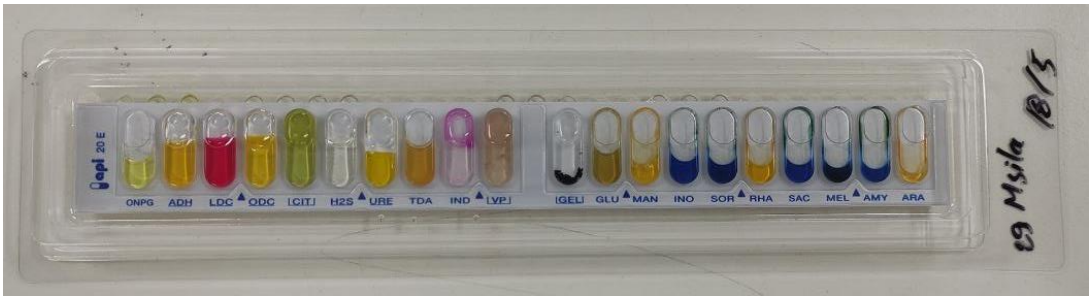

Figure25 : Biochemical profile of strain n°29 on gallery API 20E.

Table 13: Results of biochemical identification of API-isolated strains.

Rate of identification	Number	Rate (%)
100%	7	53,85
99%	1	7,69
96%	1	7,69
90%	1	7,69
84%	1	7,69
74%	1	7,69
45%	1	7,69
total	13	100,00

5. Antimicrobial susceptibility testing

5.1. Quality control

The antibiogram results for *E. coli* ATCC 25922 are presented in Table 16. Almost all of the inhibition diameters of the different antibiotics are within the range of the antibiogram standards. The inhibition diameter of Amoxicillin + clavulanic acid was 4 mm larger than the standards. The inhibition diameter of this antibiotic must be interpreted with precaution.

Table 14: Result of the susceptibility profile of *E. coli* ATCC 25922.

Antibiotic	Inhibition diameter for *E. coli* ATCC 25922	Norm
CTX	35	29-35
AMC	28	18-24
CAZ	28	23-29
FOX	31	23-29
AK	21	19-26
IMP	32	26-32
FF	28	22-30
CIP	37	30-40

CTX: Cefotaxime, **AMC**: Amoxicillin + clavulanic acid, **CAZ**: Ceftazidime, **FOX**: Cefoxitin, **AK**: Amikacin, **IMP**: Imipenem, **FF**: Fosfomycin, **CIP**: Ciprofloxacin.

5.2. Antibiogram results

The antibiogram results of the different strains of *E. coli* isolated in this study are presented in Table 15, in accordance with the recommendations for national standardization of antibiotic susceptibility testing (human and veterinary medicine, 6th version, 2011). Most isolated strains were resistant to cefotaxime whereas two of them were intermediate. No strain was resistant to Imipenem, Amoxicillin-clavulanic acid or Fosfomycin.

Moreover, one strain (M 25) was resistant to 3 classes of antibiotics and is considered multidrug resistant.

Table 15: Antibiogram results of isolated *E. coli* strains.

Antibiotics	**R**		**I**		**S**	
	N	%	**N**	%	N	%
CTX	11	84,62	2	15,38	0	0
IMP	0	0	0	0	13	100
FOX	1	7,69	0	0	12	92,31
AMC	0	0	0	0	13	100
AK	1	7,69	0	0	12	0
CIP	8	61,54	3	23,08	2	15,38
FF	0	0	0	0	13	100

R: Resistant , **I**: Intermediate , **S**: Sensitive , **N**: norm.

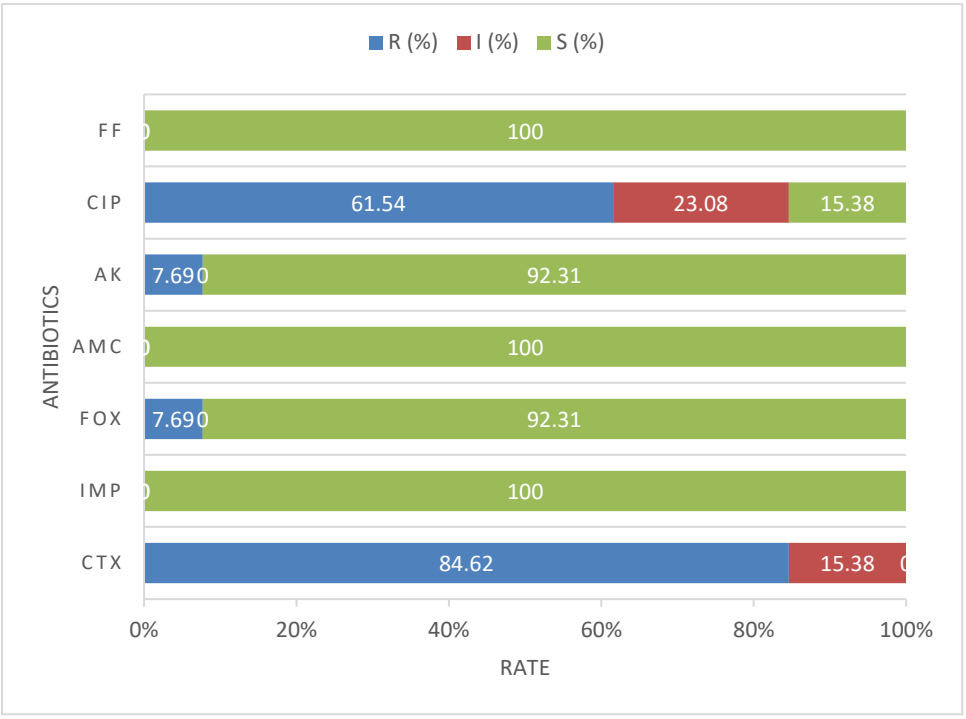

Figure 26: Repartition of Antibiogram results.

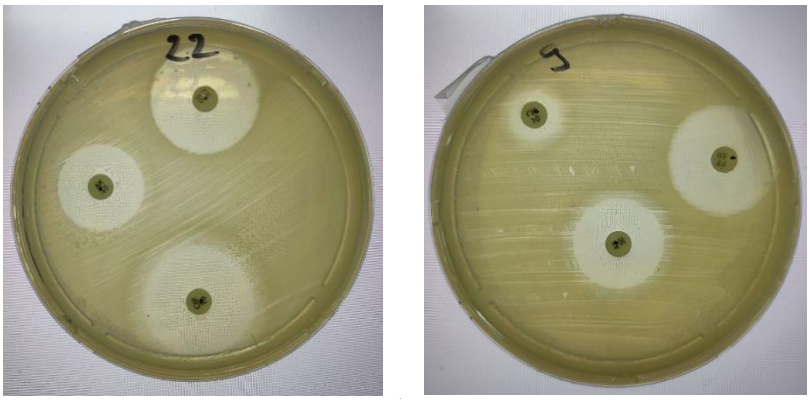

Figure 27: Antibiogram results.

6. Confirmation of ESBL production

The results of the Synergy Test Method ESBL Production Confirmation Test are presented in the following table. All strains of Escherichia coli, showed positive results for the production of ESBL.

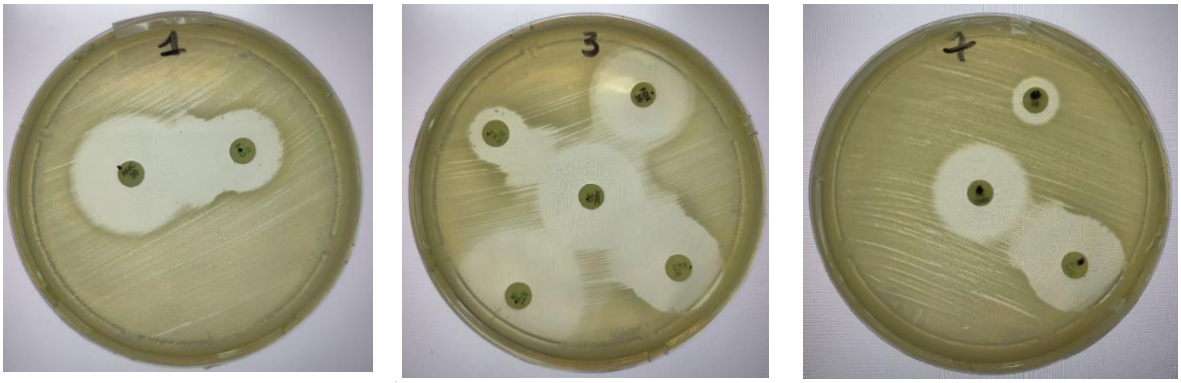

Figure 28: Confirmation test of ESBL-producing *E. coli* strains using Synergy test method.

Discussion

It is now evident that the epidemiology of multidrug resistant microorganisms has changed and is no longer restricted to the hospital environment. Gram-negative bacteria that cause ESBL are widely seen in domesticated animals, wild animals, and pets (Shen et *al.*, 2020). Important indicator bacteria that could be used to monitor antibiotic resistance in many environments include enterobacteria, especially *E. coli*.

E. coli is currently regarded in Algeria as one of the most significant sources of financial losses in the poultry industry (Halfaoui et *al.*, 2017). In this study, we aimed to investigate the presence of ESBL producing *Escherichia coli* in chicken meat in Algeria specifically in Msila.

We detected a total of 13 out of 62 strains of antibiotic-resistant *E. coli* that produce ESBL in isolated samples from the region of Msila. Several previous studies in different parts of our country have reported the detection of ESBL-producing enterobacteria in chicken samples. Among them, (Rebbah et *al.*, 2018) reported the presence ESBL producing *E. coli* in 27,5% of the sampled ground beef in Algiers, while (Chenouf et *al.*, 2021) detected it in chicken livers collected in Djelfa at a rate of 5,9%. ESBL producing *E. coli* was also detected in ready-to-eat sandwiches composed of different types of meat in a study conducted in Bejaia by (Yaici et *al.*, 2017).

Strains of ESBL-EC have also been isolated in Bejaia. For example, in one study, 20 strains of *E. coli* resistant to third-generation cephalosporins were isolated from 61 cloacal swabs collected from broiler chicken. Of these, 16 were ESBL producers and 4 were plasmid cephalosporinases producers (Belmahdi et al., 2016). In addition, other studies have reported the isolation of ESBL-producing *E. coli* from colibacillosis lesions in chicken, collected in various regions including Bouira, Bejaia, Tizi Ouzou, Boumerdes and Ain Defla (Halfaoui et *al.*, 2017); (Meguenni *et al.*, 2019).

Not only in Algeria, the presence of ESBL-producing *E. coli* is a worldwide threat. In Emirates, a recent study has found a rate of 79,68% of ESBL producing *E. coli* in chicken carcasses sampled from supermarkets (Habib et al., 2023). In another study, Prevalence of the phenotypic ESBL-producing *E. coli* isolates was 29%, and they exhibited remarkable sensitivity to carbapenems (100%) as well as to amikacin (93.10%). All ESBL-producing strains were multidrug resistant. Molecular analysis was performed as the final confirmation of the production of extended spectrum β-lactamases for 24 isolates out of 29 phenotypically ESBL-producing *E. coli* isolates (Hadžić-Hasanović et *al.*, 2020).

Additionally, a different study conducted in Ethiopia revealed the existence of a total of 35 (20.80%) biochemically confirmed *E. coli* were obtained from 168 samples. From 35 *E. coli* isolates, 7(20%) of them were confirmed as ESBL producers. Poor hygienic status of butcher shops and unhygienic practice of meat handlers were observed (Abayneh et *al.*, 2019).

Our results report the contamination of chicken meat by ESBL producing *E. coli* and highlight the risk of transmission of these bacteria to humans through the food chain. In effect, the colonization of chicken meat by ESBL producing bacteria maybe due to the fecal carriage of this strains by chicken (Rebbah et *al.*, 2018). Although, further characterization of the resistance genes and the strains is needed to better assess this concern.

Therefore, it is important to thoroughly evaluate any possible risks to one of the most important protein sources on the planet. Due to numerous studies that have revealed a connection between poultry meat consumption and the occurrence of chronic human infections, the significance of this issue for public health is all the more urgent (Apostolakos & Piccirillo, 2018).

Conclusion

Conclusion

In the animal industry, almost 80% of the essential antibiotics for human medicine are used as a therapeutic tool, mostly to encourage growth in healthy animals. Although antibiotic resistance is a natural phenomenon, it is being accelerated by the abuse of these medications in both humans and animals. The danger posed by antibiotic resistance is being amplified by the inappropriate, unchecked, and excessive use of antibiotics in both conventionally and industrially bred animals. The outcomes obtained unmistakably show the existence and spread of antibiotic resistance in bacterial strains isolated from chicken meat. Because it threatens the effectiveness of antibiotic treatments in humans, this resistance is a matter for concern.

This highlights the need for careful and responsible use of antibiotics in the poultry industry to minimize the development and spread of resistance. Furthermore, it has been shown that antibiotic resistance can be transmitted between bacteria present in food and bacteria present in the human gut, highlighting the importance of the food chain in the spread of this resistance. In addition, our current results suggest that poultry can act as a reservoir for *E. coli*.

The results obtained revealed the presence of ESBLs in the chicken meat from (13/62) samples isolated from the region of Msila. This indicates that ESBL-carrying bacteria may be present in chicken meat, potentially exposing consumers to antibiotic-resistant strains.

The detection of ESBL in chicken meat highlights the need for increased surveillance of antibiotic resistance in food production. It is essential to understand the extent of this problem in order to take appropriate measures to minimize the spread of resistance.

These results further highlight the significance of judicious antibiotic usage in poultry. To reduce the prevalence of resistant strains, sustainable husbandry practices, appropriate biosecurity protocols, adoption of improved hygiene and animal welfare standards, and the development of new antibiotic alternatives must all be implemented. This requires the prudent use of antibiotics in animal husbandry. Additionally, it is essential to raise awareness among poultry farmers, consumers, and healthcare professionals on the dangers of antibiotic resistance and the proper usage of antibiotics.

In summary, antibiotic resistance in poultry meat strains is a worrying problem that requires a multidisciplinary and cooperative approach to resolve. It is imperative to take preventive measures to minimize the spread of resistance, in order to ensure the continued efficacy of antibiotics in the treatment of bacterial infections in humans.

Taken together, these results pave the way for several research perspectives, which can be developed as follows:

- ✓ We could be interested in the molecular identification of multi-resistant-bacteria and their resistance genes by applying a PCR method.
- ✓ And to cover as many places as possible in the wilaya of Msila, We'll have to increase the number of samples taken from each area. So that the total result of the report will represent the whole region.
- ✓ This will also enable us to carry out surveys aimed to assessing the health, technological and economic consequences that the persistence of the possible increase in bacterial resistance.

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Appendix

Appendix

Appendix 1: Types of antibiotics according to their mechanism of action (Tab **16, 17, 18, 19 and 20**).

Table 16: Antibiotics acting on the wall (Nauciel & Vildé, 2005).

Family	Group	Example of antibiotics	Mechanism of action
Beta-lactamins	**Penams**	Ampicillin Amoxicillin Carbenicillin	They work by preventing penicillin-binding proteins (PLPs) from synthesizing peptidoglycans. PLPs are capable of transglycolasic, transpeptidasic, and carboxypeptidasic action. Pentacyclic bridge production, which is responsible for the wall's cross-linked structure, is inhibited by PLP inhibition. Round or filamentous forms are produced as a result, which cause bacterial lysis.
	Penems	Imipenem Meropenem Ertapenem	
	Oxapenams or clavams (clavulanic acid)	Amoxicillin + Clavulanic acid Ticarcillin + Clavulanic acid	
	Cephems	Cefazolin Cefoxitin Ceftriaxon	
	Monobactams	Aztreonam	
Glycopeptide		Vancomycin Teicoplanin	By adhering to the peptides involved in the polymerization phase of peptidoglycan, they prevent the polymerization of peptidoglycan.
Not classified		Fosfomycin	It binds to an enzyme that produces N-acetyl muramic acid, one of the building blocks of peptidoglycan.

Table 17: Antibiotics acting on protein synthesis.

Family	**Antibiotics**	**Mechanism of action**
Aminoglycoside	Streptomycin Kanamycin Gentamicin	They either bind to the ribosome's 30S subunit or they interfere with protein synthesis (Singleton, 2005).
Macrolides-Lincosamides-Streptogramins(MLS)	Spiramycin Lincomycin Pristinamycin	They function at the S/unit 50S ribosome level. They prevent the development of new polypeptide chains from growing (Yala et *al.*, 2001).
Tetracycline	Oxytetracyclin Doxycyclin Glycylcyclins	They work by blocking the polypeptide chain's elongation phase at the 30S subunit of the ribosome, which prevents the fixing of aminoacyl-RNAt (Bryskier, 1999).
Phenicols	Chloramphenicol Thiamphenicol	Inhibition of peptidyl transferase and attachment to the bacterial ribosome's 50S subunit (Wareham & Wilson, 2002).
Oxazolidinones	Linezolid	They adhere to the ribosomal subunit 50S and block the subunit 30S from connecting to it (Nauciel & Vildé, 2005).

Table 18: Different antibiotics acting on nucleic acid synthesis.

Family	**Antibiotics**	**Mechanism of action**
Quinolones **And** **Fluoroquinolones**	Nalidixic acid, Pipemidic acid, Oxolinic acid Flumequin	They act on two enzymes involved in this synthesis: **DNA gyrase** (main target of GNB) it forms a complex ADNgyrase- Quinolones that will block the progression of DNA bacterial polymerase during replication **DNA topo- isomerase IV** The interaction between DNA, quinolone and topoisomerase stimulates DNA cutting and inhibits relegation (Hooper, 2001).
	Pefloxacin Ofloxacin Norfloxacin Ciprofloxacin	
Rifamycins	Rifamycin Rifamycin SV	Inhibition de la transcription de l'ADN en ARNm par inhibition de l'ARN polymérase (Nauciel & Vildé, 2005).
Nitrofurans	Nitrofurantoin Furazolidon Nifuroxazid	They act directly on the DNA causing various lesions (cuts and substitution of bases) (Nauciel & Vildé, 2005).

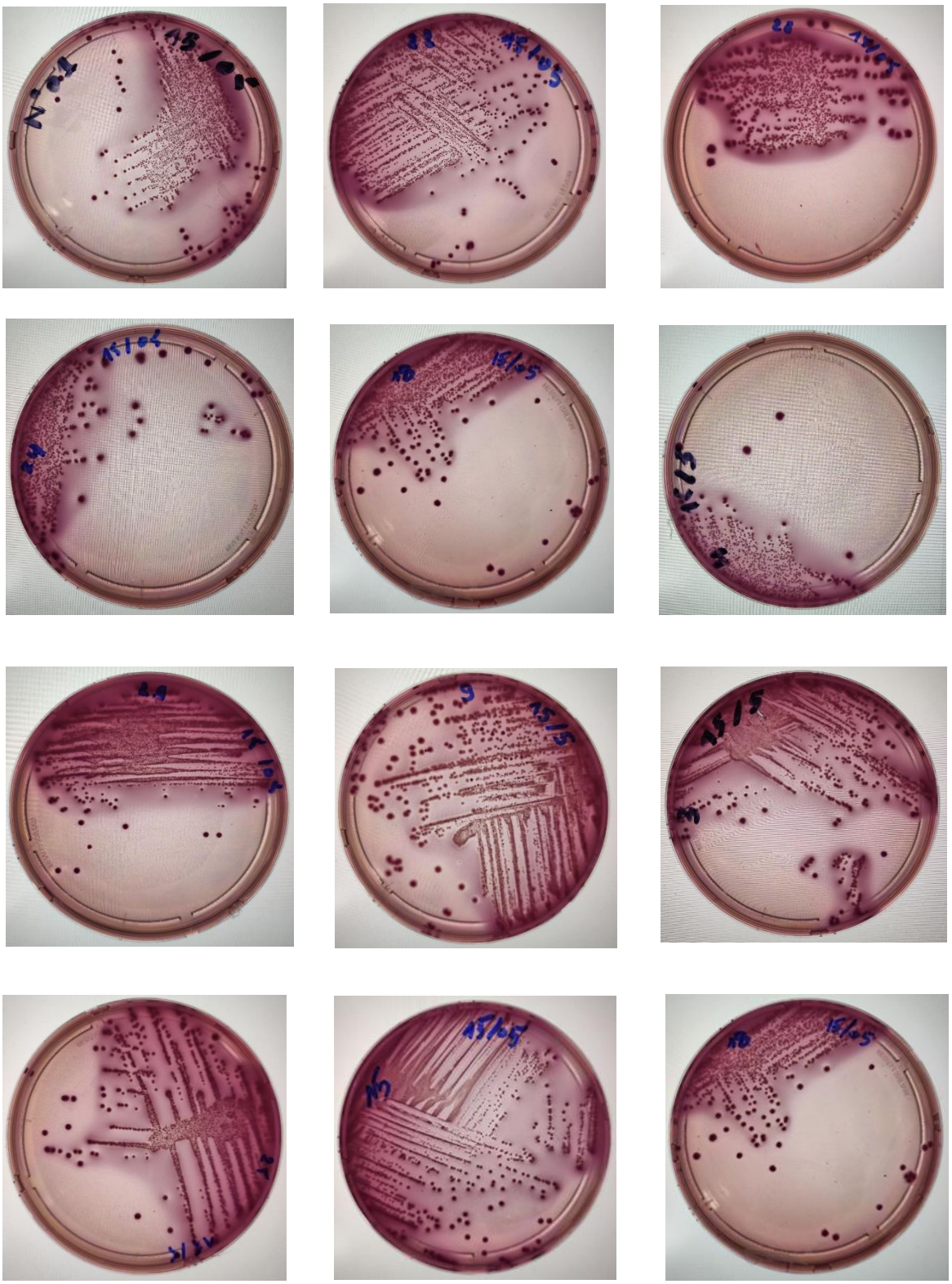
Table 19: Antibiotics acting on the cytoplasmic membrane.

Family	**Antibiotics**	**Mechanism of action**
Polymixines	Polymixin B Colistin	They attach to phospholipids, thus disrupting transmembrane transfers of nutrients and inhibiting oxidative phosphorylations of energy metabolism (Fauchère & Avril, 2002).

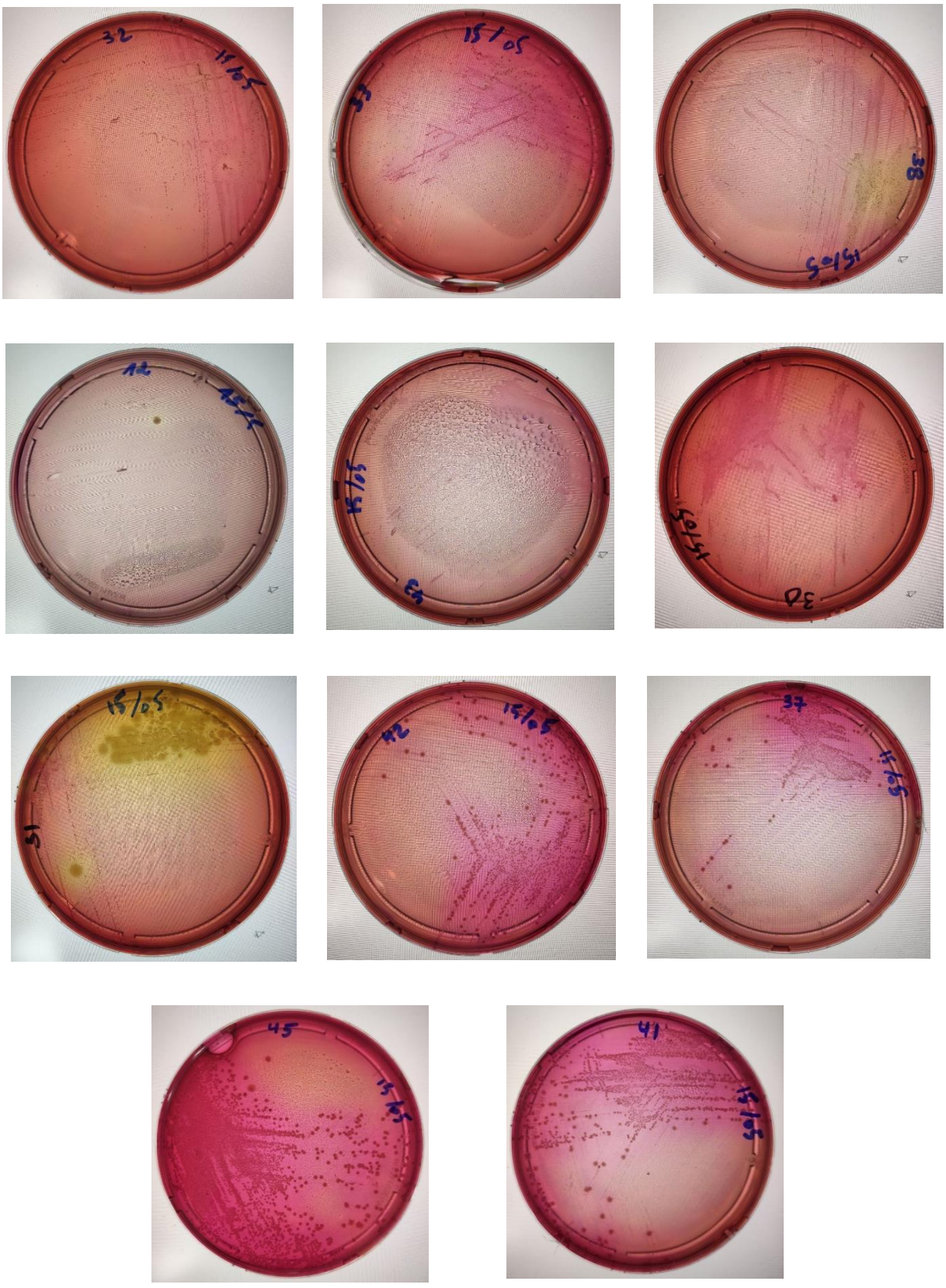
Table 20: Antibiotics acting on folic acid synthesis.

Family	**Antibiotics**	**Mechanism of action**
Sulfamides	Sulfamethoxazol Sulfamethizol Sulfaguanidine	Inhibit the synthesis of folates, act in competition with PABA for the active site, dihydropterate synthase (DHPS) which catalyzes an essential reaction to the synthesis of tetrahydrofolic acid (DHF) necessary for the production of purines and pyrimidines for nucleic acid synthesis (Bergogne-Bérézin & Dellamonica, 1999).
2-4 Diaminopteridine	Trimethoprime	Inhibit the synthesis of folates, fixing on dihydrofolate reductase (Veyssier, 1999).
Sulfamides + Trimethoprim	Sulfamethoxazol+ Trimethoprim (Cotrimoxazol)	Acts on the two previous enzymes (Veyssier, 1999).

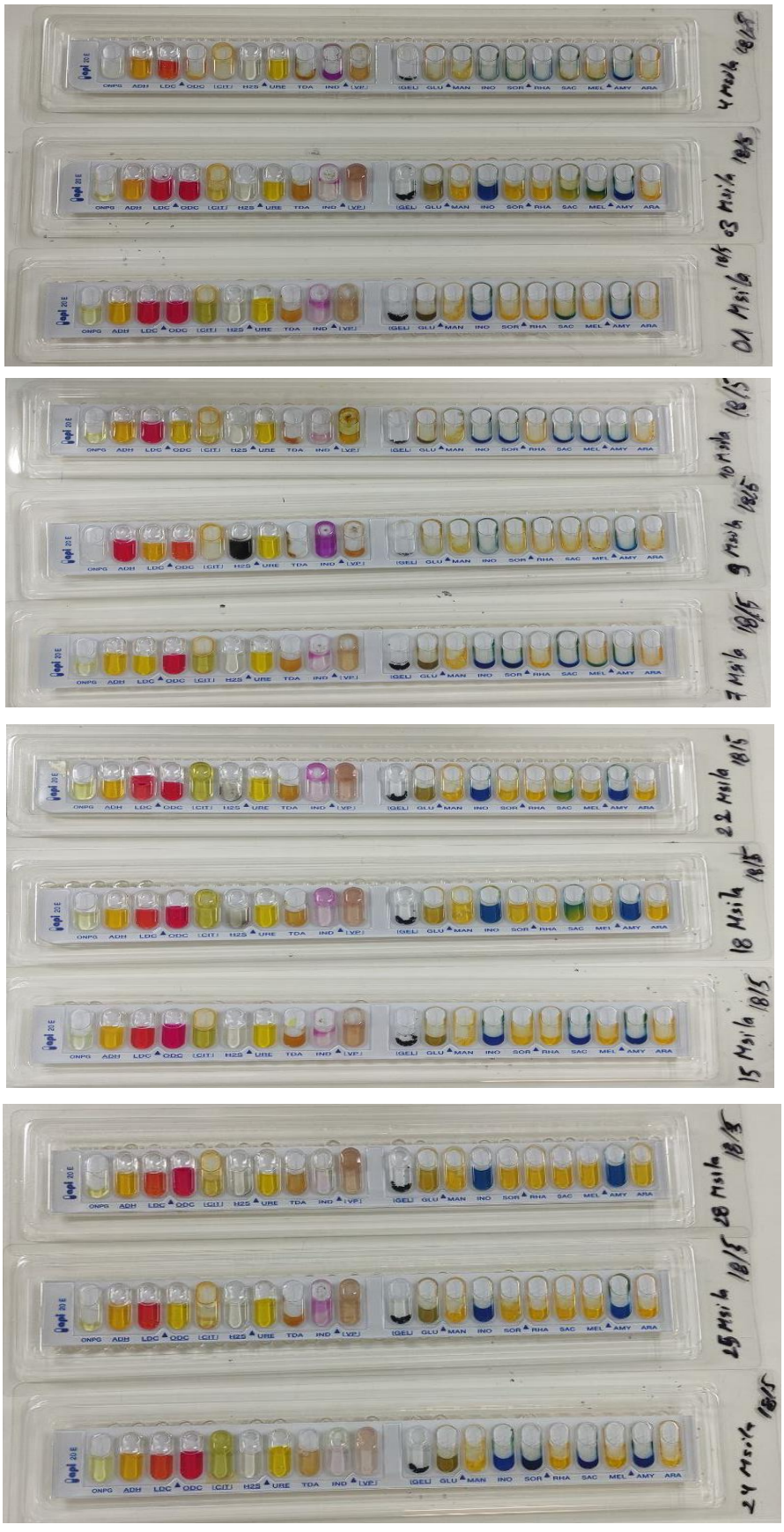
Appendix 2: Results of screening; Lactose positive.



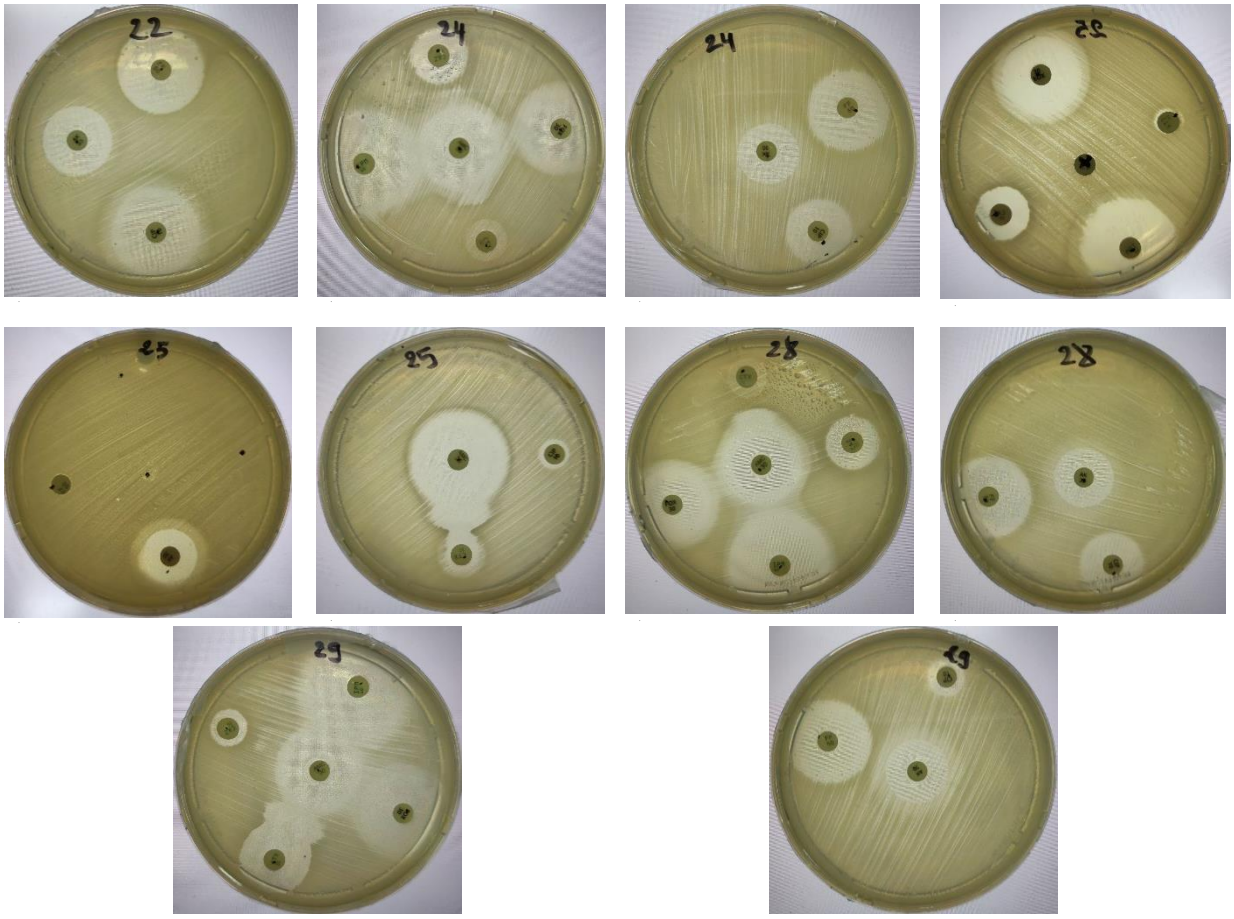
Appendix 3: Results of screening; Lactose negative.



Appendix 4: of biochemical identification by Gallery API 20E.



Appendix 5: Results of Antibiogram using Disc Diffusion (DD) method.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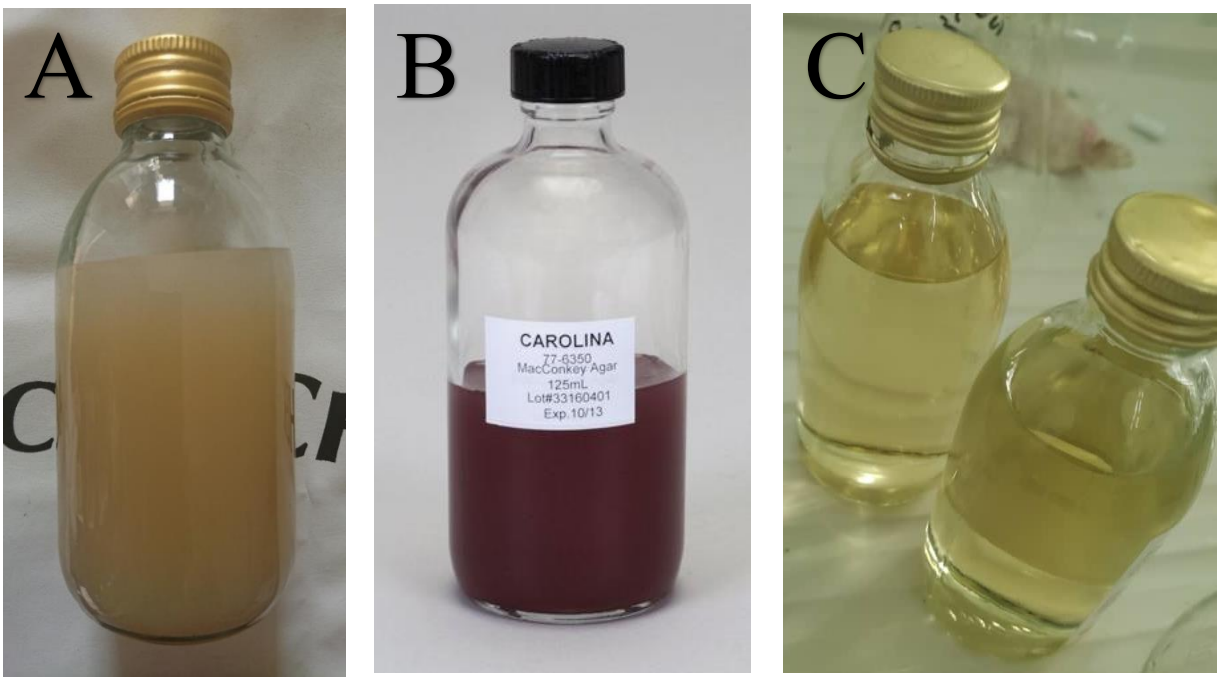
Appendix 6: Critical Values Table of Inhibition Zone Diameters for Enterobacteria.

Antibiotiques testés	Charge des Disques	Diamètres critiques (mm)			CMI critiques (µg/ml)			Commentaires
		R	I	S	R	I	S	
Ampicilline	10µg	≤ 13	14 – 16	≥ 17	≥ 32	16	≤ 8	La réponse à l'ampicilline est valable pour l'amoxicilline
Amoxicilline +Ac.clavulanique	20/10µg	≤ 13	14 – 17	≥ 18	≥ 32/16	16/8	≤ 8/4	Les breakpoints des céphalosporines et de l'Aztréonam ont été révisés en fonction des propriétés PK-PD et des données cliniques. Ainsi, l'application de ces breakpoints dépend du respect de posologies précises : céfazoline (2g toutes les 8h), céfotaxime (1g toutes les 8h), ceftriaxone (1g toutes les 24h)…
Céfazoline	30µg	≤ 19	20 – 22	≥ 23	≥ 8	4	≤ 2	
Céfalotine	30µg	≤ 14	15 – 17	≥ 18	≥ 32	16	≤ 8	
Cefoxitine	30µg	≤ 14	15 – 17	≥ 18	≥ 32	16	≤ 8	Suite à la révision des breakpoints des céphalosporines, la lecture interprétative anciennement basée sur la détection ou non d'une BLSE, n'est plus nécessaire. La réponse R, I ou S se fait en se référant aux seuls diamètres mesurés. A souligner cependant que la détection phénotypique de la BLSE garde tout son intérêt dans les études épidémiologiques et en hygiène hospitalière. (voir chapitre recherches complémentaires).
Céfotaxime	30µg	≤ 22	23 – 25	≥ 26	≥ 4	2	≤ 1	
Ceftriaxone	30µg	≤ 19	20 – 22	≥ 23	≥ 4	2	≤ 1	
Imipénème/Méropénème	10µg	≤ 19	20 - 22	≥ 23	≥ 4	2	≤ 1	Les breakpoints des carbapénèmes ont été révisés en fonction des propriétés PK-PD et des données cliniques. L'application de ces breakpoints dépend du respect des posologies suivantes : Imipénème : 500 mg toutes les 6h ou 1 g toutes les 8h, Ertapénème : 1g toutes les 24h, Méropénème : 1g toutes les 8h. La détection phénotypique d'une carbapénémase par le test MHT est réservée aux études épidémiologiques (voir chapitre recherches complémentaires).
Ertapénème	10µg	≤ 19	20 - 22	≥ 23	≥ 1	0,5	≤ 0,25	
Amikacine	30µg	≤ 14	15 – 16	≥ 17	≥ 64	32	≤ 16	
Gentamicine	10µg	≤ 12	13 – 14	≥ 15	≥ 16	8	≤ 4	
Acide nalidixique	30µg	≤ 13	14 – 18	≥ 19	≥ 32	---	≤ 16	La sensibilité diminuée aux fluoroquinolones est détectée chez les salmonelles isolées d'infections extra-intestinales en testant l'Acide nalidixique à l'antibiogramme.
Ciprofloxacine	5µg	≤ 15	16 – 20	≥ 21	≥ 4	2	≤ 1	
Chloramphénicol	30µg	≤ 12	13 – 17	≥ 18	≥ 32	16	≤ 8	Ne pas tester en routine sauf pour les salmonelles.
Colistine	------	------	-------	-------	---------	------	--------	Ne tester à l'antibiogramme que pour un but diagnostique. (résistance si culture au contact du disque ou présence d'une cocarde).
Furanes	300µg	≤ 14	15 – 16	≥ 17	≥ 128	64	≤ 32	
Fosfomycine	200µg	≤ 12	13 – 15	≥ 16	≥ 256	128	≤ 64	Indiqué uniquement pour les souches d'E.coli isolées d'infections urinaires. La CMI est déterminée par la technique de dilution en gélose supplémentée de 25µg/ml de glucose 6-phosphate.
Triméthoprime+ Sulfaméthoxazole	1.25/23.75µg	≤ 10	11 – 15	≥ 16	≥ 4/76	------	≤ 2/38	

Appendix 7: Limit Values of the diam diameters of the inhibition zones for the reference strains used for quality control.

Antibiotiques testés	Charge des disques	*E. coli ATCC 25922*	*S. aureus ATCC 25923*	*P. aeruginosa ATCC 27853*	*S. pneumoniae ATCC 49619*	*H. influenzae ATCC 49247*	*N. gonorrhoeae ATCC 49226*
Gentamicine **	10µg	19-26	19-27	16-21	----	----	----
Imipeneme	10µg	26-32	----	20-28	----	----	----
Kanamycine	30µg	----	19-26	----	----	----	----
Levofloxacine	5µg	----	25-30	----	20-25	----	----
Nétilmicine	30µg	----	----	17-23	----	----	----
Ofloxacine	5µg	----	24-28	----	----	31-40	------
Oxacilline	1µg	----	18-24	----	≤ 12	----	----
Pénicilline	10UI	----	26-37	----	24-30	----	26-34
Pipéracilline	100µg	----	----	25-33	----	----	----
Rifampicine	5µg	----	26-34	Non déterminé	25-30	----	----
Spectinomycine	100µg	----	----	----	----	----	23-29
Tétracycline	30µg	18-25	24-30	----	27-31	14-22	30-42
Ticarcilline	75µg	----	----	21-27	----	----	----
Ticarcilline + Ac clavulanique	75/10µg	----	----	20-28	----	----	----
Tobramycine	10µg	----	----	19-25	----	----	----
Triméthoprime + sulfaméthoxazole	1.25/23.75µg	23-29	24-32	----	20-28	24-32	----
Teicoplanine	30µg	----	15-21	----	----	----	----
Vancomycine	30µg	----	17-21	----	20-27	----	----

Appendix 8: (A) Muller-Hinton medium, **(B)** MacConkey medium, **(C)** Nutrient broth.



ملخص

أصبحت العدوى الناتجة عن البكتيريا المعوية التي تنتج بيتا لاكتاماز ممتد الطيف (ESBL) أكثر شيوعًا، وهو مصدر قلق صحي عالمي كبير. وفقًا لتقارير عديدة، يمكن أن تنتقل هذه الجراثيم من الحيوانات إلى الناس عبر البيئة. كان الهدف الرئيسي من هذه الدراسة هو فحص انتشار إنتاج ESBL في الدواجن وصورة مقاومتها في مدينة المسيلة. حيث تم أخذ عينات من ثلاثة أماكن مختلفة في ولاية المسيلة (المسيلة وسط، بوسعادة، سيدي عيسى). بعد الفحص الانتقائي، تم تحديد و تأكيد وجود سلالات مقاومة منتجة للESBL للسلالات المعزولة بطريقة النمط الظاهري. ثم تم استخراج الحمض النووي عن طريق الغليان. ونتيجة لذلك، كانت جميع المناطق الثلاث تحتوي على الإشريكية القولونية المنتجة لـ ESBL. وبالتالي ، احتوت 13 (20.97٪) من العينات على للإشريكية القولونية المنتجة لـ ESBL. كانت جميع السلالات المعزولة مقاومة للجيل الثالث من السيفالوسبورين (cefotaxime) الذي تم اختباره وحساسة للأمينوسيدات وبعض بيتا لاكتامينات (imipenem، cefoxitin). في الختام، تظهر النتائج انتشارًا كبيرًا لـ *Enterobacteriacea* المنتجة لـ ESBL في مدينة المسيلة ، والمزيد من الدراسات ضرورية لتوصيف وتصنيف البكتيريا المقاومة وآلياتها.

. **الكلمات المفتاحية :** مقاومة المضادات الحيوية , بيتا لاكتاماز ذات الطيف الممتد , الإشريكية القولونية , بكتيريا الأمعاء.

Résumé

Les infections provoquées par les entérobactéries qui produisent des Bêta-Lactamases à Spectre Etendu (BLSE) sont de plus en plus fréquentes, ce qui constitue une préoccupation sanitaire mondiale majeure. Selon de nombreux rapports, ces germes peuvent se transmettre des animaux aux humains par l'environnement. L'objectif principal de cette étude était d'examiner la prévalence de la production de BLSE chez les volailles et leur profil de résistance dans la région de M'sila. Des échantillons ont été prélevés à trois endroits différents dans la wilaya de M'sila (M'sila centre, Boussaâda, Sidi-Aïssa). Après criblage sur gélose sélective BLSE, la confirmation de la résistance à la production d'BLSE des souches isolées a été déterminée par la méthode phénotypique. Ensuite, on a procédé à l'extraction de l'ADN par ébullition. Par conséquent, les trois régions abritaient des *E. coli* producteurs d'BLSE. Ainsi, 13 (20,97 %) des échantillons contenaient des *E. coli* produisant des BLSE. Tous les isolats étaient résistants aux céphalosporines de troisième génération (céfotaxime) testés et sensibles aux aminosides et à certaines de bêtalactamines (imipenème, céfoxitine). En conclusion, les résultats montrent une prévalence significative des entérobactéries productrices de BLSE dans la région de M'sila, et des recherches supplémentaires sont nécessaires pour caractériser les gènes de cette bactérie responsables de cette résistance et de leurs mécanismes

. **Mots clés :** Antibiorésistance, Bêta-lactamases à spectre étendu (BLSE) ; *Escherichia coli*, Entérobactéries)

Abstract

Infections brought on by enterobacteria that produce extended-spectrum beta-lactamases (ESBL) are becoming more common, which is a major global health concern. According to numerous reports, these germs can transmit from animals to humans through the environment. The main objective of this study was to examine the prevalence of ESBL production in poultry meat and their resistance profile in the region of **Msila, Algeria.** Samples were taken from three different places in the departement of Msila (Msila center, Bousaada, Sidi-Aissa). After screening on selective ESBL agar, confirmation of ESBL production of the isolated strains was determined by the phenotypic method. Then, DNA extraction by boiling was performed. As a result, all the three regions harbored ESBL-producing *E. coli*. Hance, 13 (20.97%) of samples contained ESBL producing *E. coli*. All the isolates were resistant to third generation cephalosporin (cefotaxime) tested and sensitive to Aminosides and some Beta lactamines (imipenem, cefoxitin) and amoxicillin clavulanic acid and Fosfomycin. In conclusion, the results show a significant prevalence of ESBL-producing *Enterobacteriaceae* in the region of Msila. Additional research is required to characterize genes of this bacteria responsible for this resistance and their mechanisms.

Key words: Antibiotic resistance, Extended-spectrum-beta-lactamase (ESBL), *Escherichia coli, Enterobacteriaceae.*