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Theme

Study of antibiotic susceptibility of enterohemorrhagic Escherichia coli strains isolated from bovine feces

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Thank you, to you who are reading this modest work that I hope will be useful to you.

I dedicate this modest work, which is the fruit, harvested after so many years of efforts:

- *To you dad: you are the man I love the most in the world, you have always been there for me, no dedication can express the love, the esteem and the respect that I have for you.*
- *To Mama: you gave me so much that I don't have enough words to express my gratitude, I love you.*
- *To my grandparents: I thank you for all the support and the exceptional love that you have given me since my childhood.*
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- *To you my husband Sofiane: who is always present near me.*
- *To the Mansouri family; my new family: thank you for your encouragement.*
- *To all my friends.*

Summary

List of abbreviations

ADH: arginine dihydrolase

AMC: Ampicillin clavulanic

AMY: amygdalin

ARA: arabinose

ATB: antibiotic

ATM: aztreonam

CEF: Cefazolin

CIP: ciprofloxacin

CIT: citrate

CTX: cefoxitin

EHEC: *Enterohemorrhagic Escherichia coli*

EPEC: Enteropathogenic Escherichia coli

ERT: ertapenem

EU: union Euro

FOX: cefotaxime

Gb3: globotriosyl céramide 3

GEL: gelatin

GEN: gentamicin

GLU: glucose

GNB: gram-negative bacilli

HUS: hemolytic uremic syndrome

H2S: thiosulfate

IND: indole

INO: inositol

LDC: lysine decarboxylase

MAN: mannose

MEL: melibiosis

MIC: minimum inhibitory concentrations

N: number

ODC: ornithine decarboxylase

ONPG: o-nitrophenyl-bD-galactopyranoside

Pbp: penicillin

R: resistant

Rfb: region functional B-glucuronidase

RHA: rhamnose

S: sensitive

SAC: sucrose

SOR: sorbitol

UM: Micrometer

UL: micro-liter

URE: urease

TDA: Tryptophan deasaminase

VP: voges proskauer

+: positive

- : negative

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Introduction

Beef is an integral part of the diet of a large part of the world's population, ranks first among the meats traded in the world of the world's population, and is the most traded meat in value. Animal products can be expressed in terms of protein quantity, which allows comparisons between species and products. East and Southeast Asia, with about 19 million tons of protein, is the leading region for the production of animal products, mainly based on monogastric farming. Western Europe, North America, Latin America and the Caribbean, and South Asia have comparable production levels, between 12 and 10 million tons of protein. However, their profiles are different: while beef and milk play a major role in Latin and North America, the dairy sector dominates in Western Europe and buffalo production plays an important role in South Asia. The Middle East, North and Sub-Saharan Africa, Eastern Europe, Oceania and the Russian Federation, with a production of between 4 and 1.6 million tons of protein, each have a smaller individual share on the world scale[1]. Faced with the increase in world beef consumption and the production difficulties encountered in many countries, the international beef trade is growing, with the arrival of new export players such as India. Although there are significant flows of live cattle and beef between its Member States, the EU remains a modest player in the world market, both for imports and exports [2].

The Enterobacteriaceae family is heterogeneous, it includes many bacterial numerous bacterial genera that are grouped according to their common bacteriological characteristics. They are Gram-negative bacilli, not spore-forming, they are aerobic-anaerobic facultative and develop in an ordinary medium (18 to 24 hours at neutral pH at 37°C). They lack oxidase, have catalase, and can ferment glucose into acids with or without gas production, but also reduce nitrates to nitrites. They have variable mobility depending on the presence or not of flagella. They have a characteristic composition of the bases constituting their DNA (GC % generally between 50% and 60%), which allows them to be differentiated from Pseudomonas and Vibrionaceae. The differences between the many genera and species come from more precise criteria, such as the fermentation of different sugars, the production or not of sulfide, the production of indole, the production of urease, the presence or absence of metabolic enzymes (deaminases, decarboxylases)[3]

In animals, *Escherichia coli* is responsible for many diseases that can cause intestinal infections. Gastroenteritis can be caused by various strains of *Escherichia coli*. It is a bacterium

that resides in the digestive tract of humans and warm-blooded animals, only a few are pathogenic to humans[4], such as "*Escherichia coli Enterohaemorrhagic*" bacteria, which eliminated by the animals' feces, can contaminate the environment (water, soil, etc.

Since the 80s, *Enterohemorrhagic Escherichia coli (EHEC*) infections have been a public health problem in North America and Europe. Particular strains of pathogenic *Escherichia coli* were first described in 1982 in two outbreaks in the United States (in Oregon and three months later in Michigan) following the consumption three months later in Michigan) following the consumption of contaminated and undercooked contaminated and undercooked hamburgers[5]. The affected patients had hemorrhagic colitis, these particular strains were named *Enterohaemorrhagic Escherichia coli (EHEC)*. It was first described in 1885 in infant stools by the German Theodor Escherich. Its current name, however, was proposed in 1919 to reclassify a species previously known as "Common Bacterium coli", "Bacillus coli" or "Bacterium coli [5].

EHEC, one of the intestinal pathovars of *E. coli* species Gram-negative, facultative aero-anaerobic, oxidase-negative bacillus, 2-4 µm long and approximately 0.6 µm in diameter. *Enterohemorrhagic Escherichia coli* strains are currently considered emerging pathogens in public health. They are responsible for foodborne illnesses that result in diarrhea but also in syndromes that are more serious for humans such as hemolytic uremic syndrome (HUS) which can lead to death. Surveillance data also show that serotype O157:H7 is predominant in cases of HUS. The main reservoir of these strains is the digestive tract of cattle, in which they are carried asymptomatically. The main modes of transmission of *EHEC* infections to humans are the consumption of contaminated food (undercooked beef, unpasteurized dairy products), the contaminated food (undercooked beef, unpasteurized dairy products), person-to-person ingestion of contaminated water and contact with animals (especially cattle) and (especially cattle) and their environment[6].

EHEC are characterized by the production and release of toxins, shigatoxins (also called verotoxins). In the patient, these toxins pass through the intestinal epithelium before entering the bloodstream and reaching specific receptors, the glycolipid receptors Gb3 (globotriosyl ceramide 3) which are found on the surface of endothelial cells. They lead to the death of the target cells by stopping protein synthesis and induce lesions of the vascular endothelium, mainly intestinal, renal and cerebral, which explains the clinical manifestations with renal or neurological complications. The ingestion of *Escherichia coli* causes, 3 to 4 days later, digestive symptoms: diarrhea,

abdominal pain and vomiting. Within 15 days, infected people may show contaminated people can present signs of great fatigue, pallor, and a decrease in the volume of urine[7]

A model for the emergence of the O157:H7 clone has been proposed by Feng et al. This model is based on events that would have occurred from a genetically closest ancestor of *EHEC* O157:H7: the sorbitol-fermenting, β-glucuronidase-positive *EPEC* O55:H7 serotype. These two lineages would have emerged from a common ancestor possessing the LEE pathogenicity island at least thirty thousand years ago. According to this model, the first step in the separation of the two lineages would have been the acquisition of the stx2 gene probably by transduction with phages; this results in the emergence of an stx2-positive O55: H7 stx2-positive clone and later the acquisition of a plasmid encoding hemolysins and the *rfb* region (required for O157 antigen this clone lost the ability to ferment sorbitol and produce functional B-glucuronidase activity through a T->G mutation at position 92 of the *uidA* gene. This clone is also thought to have acquired the virulence plasmid pO157 and to have given rise to the O157:H7 "sorbitol-" clone with a worldwide distribution[8]

Antimicrobial agents or antibiotics are substances that interfere with the growth of microbes. Strictly speaking, the term "antibiotic" should only be used to refer to antimicrobial agents produced (or derived) by microorganisms. Although some antimicrobial agents act on protozoa, fungi-fungi, and even viruses, in general, it can be said that antibiotics act only on bacteria. In animals, antibiotics are used to prevent disease as well as to stimulate growth. It is estimated that 70% of antibiotics used in animals are not used to treat sick animals and that half of this is used to stimulate growth or increase feed conversion. It should be noted that in the European Community, all antibiotics related to those used in humans have been banned for two years as growth promoters. Antibiotics specifically block the vital metabolic processes of susceptible bacteria processes of sensitive bacteria and thus stop their development, usually only temporarily (bacteriostatic effect) (bacteriostatic effect) but sometimes permanently (bactericidal effect) [9].

Bacterial resistance to an antibiotic is genetic in origin. The genes of the resistance are either in the chromosome (chromosomal resistance). Or in mobile elements, such as plasmids, transposable elements, or integrons (extrachromosomal resistance). The resistance can be either natural or acquired.

Beta-lactam antibiotics remain the most widely used family of antibiotics in the world. This is mainly due to the number of specialties available covering a relatively broad bacterial spectrum.

These molecules act by inhibiting the synthesis of the bacterial wall by binding to penicillin-binding proteins (PBPs), enzymes involved in the synthesis of peptidoglycan synthesis. In Gram-negative bacilli (GNB), there are three types of mechanisms of resistance to β-lactams: low affinity for PBPs, impermeability, efflux and especially enzymatic inactivation by β-lactamases [10].

Carbapenems are last-line clinical antibiotics against infections caused by multidrug-resistant Gram-negative bacteria, which are β-lactamases with hydrolytic activity towards carbapenems. Resistance to carbapenems in enterobacteria is still a marginal phenomenon, as shown by the epidemiological data on a large number of strains, with sensitivity percentages of 99-100%[11].

The evolution of bacterial resistance to antibiotics is an increasingly worrying problem. What was only a marginal phenomenon a few years ago is becoming a major public health issue: an estimated 12,500 deaths per year in France due to multi-resistant infections (InVS and ANSM).

The objective of this work is to study the antibiotic sensitivity of *Escherichia coli* sorbitol negative in cattle feces. To develop this research, we adopted the following methodology:

- Isolation and identification of sorbitol enterobacteria strain from bovine fecal material.
- Determination of minimum inhibitory concentrations.
- Study of the susceptibility of these strains to β-lactams and other families of antibiotics.

MATERIALS AND METHODS

1-Samples

Fecal samples of bovine animals were collected, between May 2022 and June 2022 from different farms in Bejaia. These samples were transported in a cooler box to the microbiology laboratory of the University of Bejaia for analysis.

Table 1: sampling information

Place of sampling	Sampling date	Samples	Total samples
LA GAZEL LOTA	05/09/2022	cows	24
N20 AOKAS	05/16/2022	bull	8
TIBO3LAMINE TIZI N BERBER	05/22/2022	cows	6
TALA OUGHALIM AOKAS	05/22/2022	cows	8
LOUZINE OUKACHOR AOKAS	05/22/2022	cows	7
LARBE3A - BACCARO	05/28/2022	Cows (n=2) and bull (n=17)	19
AKFADOU	05/29/2022	cows	08
MAREDJ W AMAN	06/06/2022	Cows(n=13) and bull (n=7)	20

2-Isolation procedure

A ôse of feces was collected with a swab then it was introduced in 10 ml of physiology water for one hour, after that 1 ml was inoculated in 9 ml of lactose broth and incubated at 37°C for one hour. A volume of 100 ul of the culture was inoculated into sorbitol Mac Conkey agar and then incubated for twenty-four (24) hour. The next day, one to two colonies were picked and streaked on chromagar.

3-Identification

Gallery identification protocol was made by introducing the bacterial suspension into the gallery API 20E (picture 2) tubes using a micropipette. Anaerobiosis was performed in the LDC, ADH, ODC, H2S, and URE tests by filling their wells with paraffin oil and then incubating at 37°C for 18 to 24 hours. The reading was done after the tests have been developed and required the addition of reagents (TDA, JAMES and VP1 VP2), then the result was noted on the result sheet.

Figure 1: the API 20E gallery

4-Determination of Minimum Inhibitory Concentrations

The minimum inhibition concentration (MIC) of bacterial growth is the lowest concentration of an antibiotic that completely inhibits bacterial growth[12]. MICs of all strains against Cefazolin, Ampicillin clavulanic, Gentamicin, ciprofloxacin, Ertapenem, and Cefotaxime were determined by the Mueller Hinton agar dilution method according to EUCAST recommendations. The stock solution was prepared at an initial concentration of 320 mg/l using a powder for injection. The volume of the solvent (sterile distilled water) was calculated according to the following formula:

$$\text{Mass of ATB powder} = \frac{\text{Volume of solvent (ml)} \times \text{concentration (g/ml)}}{\text{Purity of the powder (Mg/g)}}$$

Table 2: Preparation of dilutions of agents for use in agar dilution susceptibility tests.

Antimicrobial concentration+A17 +A1:E19+A1:E20+A 1:E19	Volume stock solution (mL)	Volume distilled water (mL)	Antimicrobial concentration obtained (mg/L)	Final concentration in medium after addition of 19 mL of agar
10 240	1	0	10 240	512
10 240	1	1	5120	256
10 240	1	3	2560	128
2560	1	1	1280	64
2560	1	3	640	32
2560	1	7	320	16
320	1	1	160	8
320	1	3	80	4
320	1	7	40	2
40	1	1	20	1
40	1	3	10	0,5
40	1	7	5	0,25
5	1	1	2.5	0,125
5	1	3	1.25	0,06
5	1	7	0.625	0,03
0.625	1	1	0.3125	0,015
0.625	1	3	0.1562	0,008
0.625	1	7	0.0781	0,004

A series of Petri dishes were prepared by adding 1ml of each antibiotic dilution to 19ml of Mueller Hinton agar. Three to five colonies were taken from a pure culture to prepare a bacterial suspension, and then a 6µl volume of this dilution was spotted on the surface of the agar plates (picture1). After that, the plates were incubated at 37°C/18-24H.

5-Antibiotic susceptibility testing

All suspected EHEC strains were characterized for antibiotic susceptibility by the disk diffusion method using Mueller-Hinton agar. Zones of inhibition were measured after 18 and 48 hours using a caliper; susceptible, intermediate resistant, or resistant, depending on the diameter of the zone of inhibition. The antibiotics used in the test were: are Aztreonem, Cefoxitin, Cefotaxime, and Ertapenem

RESULTS

During the period of the study, 100 samples were collected. 10 strains were isolated and identified as *Escherichia coli* sorbitol negative with a prevalence of 10 %. The strains were isolated from stools taken from the farms of Louzine Okchour Aokas, Akfadou and Lareb3a Baccaro.

1- Gallery results

The gallery identified 10 strains that are *Escherichia Coli* sorbitol negative (table 3 appendix 2)

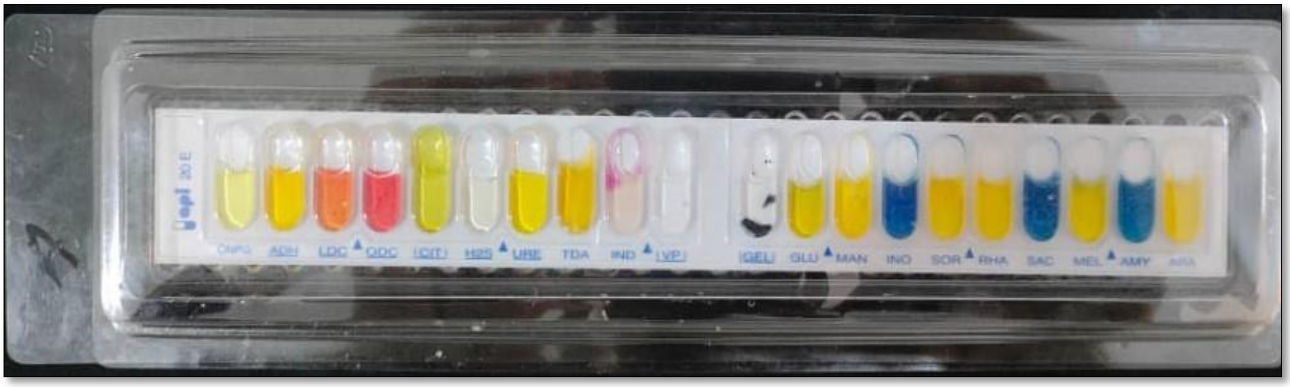

Figure2: API 20E gallery results of reference strain (*Escherichia coli)*

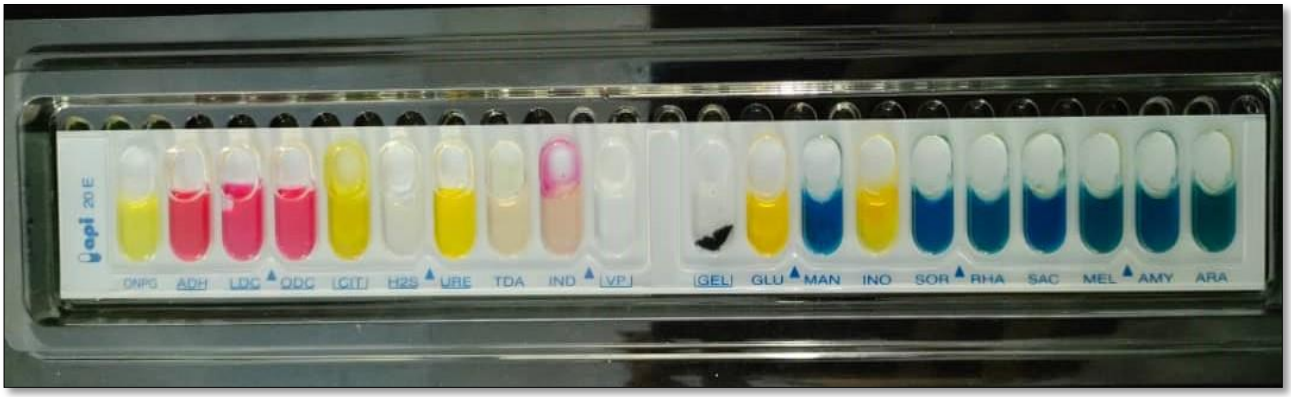

Figure 3: API 20E gallery results of strains isolated from cattle feces (ONPG+, ADH-, LDC+, CIT-,H2S-, URE-,TDA-, IND+,VP-, GEL-, GLU+, MAN+,INO-,

2. MIC results

According to the recommendations of the European Committee on Antibiotic susceptibility testing, the strains are sensitive to the antibiotics tested except for ampicillin, of which five strains (strains 5, 6, 10, 11, and 12) were resistant (MIC is above 16) (table 4).

Table 4: MIC breakpoints result.

Sample number	Sample code	CMI results
2	T38	CEF(S), AMP(S), GEN(S), CIP(S)
4	V53	CEF(S), AMP(S), GEN(S), CIP(S)
5	V50	CEF(S), AMP(R), GEN(S), CIP(S), ERT(S), CTX(S)
6	V54	CEF(S), AMP(R), GEN(S), CIP(S), ERT(S), CTX(S)
8	T22	CEF(S), AMP(S), GEN(S), CIP(S)
9	T16	CEF(S), AMP(S), GEN(S), CIP(S)
10	T14	CEF(S), AMP(R), GEN(S), CIP(S), ERT(S), CTX(S)
11	T15	CEF(S), AMP(R), GEN(S), CIP(S), ERT(S), CTX(S)
12	T11	CEF(S), AMP(R), GEN(S), CIP(S)
13	T10	CEF(S), AMP(S), GEN(S), CIP(S)

3. Antibiogram result

The diameters of the zones of inhibition (mm) and according to the recommendations of the European Committee on Antibiotic susceptibility testing [12], the strains were sensitive to the antibiotics tested in table 4.

Table 5: Antibiogram results.

Sample number	Code	results of the antibiogram
5	V50	ATM(S), FOX(S), ERT(S), CTX(S)
6	V54	ATM(S), FOX(S), ERT(S), CTX(S)
10	T14	ATM(S), FOX(S), ERT(S), CTX(S)
11	T15	ATM(S), FOX(S), ERT(S), CTX(S)

Discussion

Enterohemorrhagic Escherichia coli (*EHEC*) is a pathogen carried mainly by cattle, and responsible for food-borne infections. Numerous epidemics are regularly recorded in the world, and *EHEC* infections cause symptoms that can lead to rare but serious pathologies. HUS affects mainly young children and causes kidney damage often leaving lifelong sequels. Despite the existence of EHEC detection techniques, contaminated food is regularly found on the market[6].

In our study, 10 *EHEC* strains were isolated from 100 fecal samples of slaughterhouse cattle and dairy cows. The prevalence of *EHEC* in the samples was 10%. This observation reinforces the results of a previous preliminary Algerian study of 230 samples of which 18 yielded *EHEC*[13]. This percentage found in this study can be compared to other studies, the prevalence is very variable from one study to another. For example, some percentages were higher and some percentages were lower However, these results are sometimes difficult to compare because the number of samples, the area sampled, and the detection method was different.

The highest number (53) of studies (n = 88,643) was reported from Europe covering 16 countries. In Europe, 14 studies were from the United Kingdom, seven from each Ireland and Italy, four from each France and Turkey, two from each Norway, Serbia, Spain, Sweden, Switzerland, and the Netherlands, and one from each Belgium, Czech Republic, Denmark, Finland, and Germany. The second highest number (46) of studies (n = 110,641) was from Northern America. Among the Northern American studies, 40 were from the USA, five were from Canada and one was from Mexico. A total of 22 studies (n = 14,916) were identified in Asia, from 11 countries: eight were from Japan, three from India, two from each South Korea and Thailand, and one from each Bangladesh, China, Hong Kong, Iran, Jordan, Taiwan, and Vietnam. In total, 11 studies (n = 4,313) were reported from Latin America and the Caribbean representing five countries. Among them, five were found from Argentina, three from Brazil, and one from each Chile, Peru and Venezuela. Only four studies were identified from each Africa (n = 626) and Oceania (n = 1,288) representing two and one country, respectively. In Africa, two studies were from each Nigeria and South Africa. In Oceania, all four studies were reported from Australia[14].

Comparing our prevalence to some of the countries mentioned above. Those with higher percentages: 49% in Brazil[15], 47.7% in Nigeria[16], 40% in India[17], 31.3% in Vietnam[18],

26% in Australia[19], 18% in Italy[20], 16.6% in Japan[21], 13.6% in Turkey[22] and 12.14% in Iran[23]. And some percentages were lower: 8.6% in South Africa[24], 3.8% in Argentina[25], 2.8% in France[26], 1.7% in China[27], 0.66% in Ohio[28] and 0.3% in the USA[29].

Our results showed that there is a rate of sensitivity to B-lactam antibiotics except for AMC and the family of aminoglycosides and fluoroquinolone. In our study, strains isolated from fecal samples are more than 50% resistant to clavulanic ampicillin and sensitive to CTX, FOX and ATM, however, in comparison with our results, low levels of resistance have been reported in previous studies [30]. The ß-lactams have long been commonly used in the treatment of Enterobacteriaceae infections. Concerning the carbapenem family, the strains are sensitive to ertapenem, this reinforces the results of a previous study in April 2022 in Iraq [31].

The sensitivity rates of our strains to aminoglycosides exactly gentamicin are 99%, these results are close to those reported in Morocco 2008 [32] with a very high rate, unlike the results obtained in Italy[33] strains are resistant to gentamicin. The same results were obtained for the fluoroquinolone family, with a high sensitivity to ciprofloxacin of 99%. However, in comparison with our results, several studies have the same results for example in IRAQ, 2022[31], Morocco [32], and Italy[33].

Most antibiotics are not recommended for treating *EHEC* infections. By killing the bacteria, they cause the release of Shigatoxins into the body, which can worsen HUS. However, treatments based on certain antibiotics, such as azithromycin, which do not cause the release of these toxins are being evaluated. While awaiting their results, the therapeutic strategy for HUS consists of compensating for the deficiencies caused by Shiga-toxins (fall in red blood cells and platelets, renal damage) by transfusion, dialysis, and plasma exchange. Diarrheal episodes are treated symptomatically: patients are rehydrated but do not take anti-diarrheal drugs, to allow the elimination of the bacteria and its toxins in the stools[34].

The interest of our work is the detection of sorbitol-negative *EHEC* responsible for severe hemolytic syndrome in humans. This highlights the role of the reservoir that play the cattle in this human pathology and should encourage better knowing and controlling the risk to public health. To effectively prevent these infections, strict hygiene practices must be followed throughout the

food chain, from producer to consumer. Personnel involved in the production and preparation of raw plant and animal products must be trained in good hygiene practices.

Conclusion

Enterohemorrhagic Escherichia coli (EHEC) produces Shiga toxins that cause severe bloody diarrhea and sometimes hemolytic uremic syndrome with a rapid drop in hematocrit and platelet count, elevated serum creatinine, hypertension, and possibly signs of fluid overload, hemorrhagic diathesis, and neurologic disorders. Infection can be transmitted through manure-contaminated food or water. This bacterium affects children, pregnant women or people with chronic illnesses or immune suppression much more. Most antibiotics are not recommended for treating *EHEC* infections.

Before concluding this study, it is important to present its limitations. Indeed, some elements could not be addressed in this study due to the unavailability of the material and the limited duration caused by the sanitary measures put in place in the framework of the fight against the COVID-19 pandemic. In addition to the results obtained, this study aimed to:

- The molecular study of virulence genes to determine the serotype.
- Studying the environment of the farm and determining the origin of the contamination.
- Is this an essential step for the development of new pre-slaughter strategies to reduce the risk of *EHEC* contamination of meat products?

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Annexes

Annexe 1: Composition of the culture mediums

*Lactose broth

Lactose…..……...……10g.

NaCl……...……..……... 5g.

Peptone…….……...…….3,5 g.

Tryptone……...…..……..…….3,5g.

Bromothymol blue…… ……...……..……...……..0,04g.

Ph = 7

*Sorbitol Mac Conkey agar

Peptone…….…….20g.

Agar …….…….15g.

Sorbitol ……..…….….. 10g.

NaCl……..……..……5g.

Biliary salts …….……..…….. 1,5g.

ph = 7

* Mueller Hinton agar

Beef infusion..... 3g.

Casein hydrolat..... 17,5 g.

Amidon……..... 1,5 g.

Agar..... 17g.

Annexe 2:

Table 3: API 20E gallery tests

TESTS	NEGATIVE	POSITIVE
ONPG	incolor	yellow
ADH	yellow	red
LDC	yellow	red
ODC	yellow	red
CIT	green/yellow	blue
H2S	incolor	black
URE	yellow	red
TDA	yellow/brown	
IND	brown	pink
VP	incolor	pink
GEL	incolor	black
GUL	blue	yellow
MAN	blue	yellow
INO	blue	yellow
SOR	blue	yellow
RHA	blue	yellow
SAC	blue	yellow
MEL	blue	yellow
AMY	blue	yellow
ARA	blue	yellow

Résumé

L´objectif de notre travail est d´étudier la sensibilité des souches entérobactéries responsable de grave syndrome hémolytique isolées des selles des bovins. Un total de 100 prélèvent ont été recueillis entre mai et juin 2022 dans différents fermes de Bejaia, dont soixante-huit vaches laitées et trente-deux taureaux prêtes à l´abatage. Les isolements ont été effectué sur milieux lactose modifiés puis sur milieux Mac Conkey sorbitol. Les souches ont été identifiées en utilisant des galeries API 20 E. la sensibilité aux antibiotiques a été déterminée par la méthode de diffusion sur gélose Mueller Hinton. Un taux de 10 souche de Escherichia Coli sorbitol négative (EHEC) ont été isolées chez 6 taureaux et 4 vaches d´une prévalence de 10 %. Les souches ont été sensible à toute les antibiotiques. Ces résultats indiquent la nécessité d´application de pratique d´hygiène strictes.

Mots-clés : syndrome hémolytique, bovin, EHEC, sorbitol négative, antibiotiques.

Abstract

The objective of our work is to study the sensitivity of enterobacteria strains responsible for severe hemolytic syndrome isolated from cattle feces. A total of 100 samples were collected between May and June 2022 in different farms of Bejaia, including sixty-eight milking cows and thirty-two bulls ready for slaughter. Isolations were performed on modified lactose media and then on Mac Conkey sorbitol media. Strains were identified using API 20 E galleries. Antibiotic susceptibility was determined by the diffusion method on Mueller Hinton agar. A rate of 10 sorbitol negative Escherichia Coli (EHEC) strains were isolated from 6 bulls and 4 cows of 10% prevalence. The strains were sensitive to all antibiotics. These results indicate the need for strict hygiene practices.

Keywords: hemolytic syndrome, bovine, EHEC, sorbitol negative, antibiotics.