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MASTER

Topic

Search for Carbapenemase-Producing Enterobacterales strains isolated from different ecological niches

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In front of the jury composed of:

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Finelly, I would like to show my gratitude to all the farmers and breeders who helped me to get the different samples.

Dedication

Praise God

All the letters can't find the words

That are needed...

All words cannot express gratitude, love, respect and

appreciation …

Also, it is simply that

I dedicate this dissertation…

To my dearest and most cherished mother

Source of tenderness, strength and patience, my eyes through which I have seen and see this world, to you who was and will always be my example in life, my source of inspiration.

To my father

Who helped me in the realization of this work, I am grateful to you for the time you gave me, for your unfailing support.

"Have in your veins the sweet milk of your mother, and the generous spirit of your father; be good, be strong, be honest, be just! And receive, in the kiss of your grandmother, the blessing of your grandfather." Victor Hugo

To my dearest sisters Lyna and Basma

To the rays of sunshine that illuminate my life, no expression, no dedication can express to its true value, the extent of the Love, the Attachment, the Gratitude that I feel for you

To all my friends

Amine, Karim, Katia, Lyticia, Mohamed, Redouane, Slimane, Yasmine ...

From the beginning to the end you were present, you were an example of support.

I dedicate this work to you as a tribute to all the pleasant, unforgettable moments that we lived together. I consider you as my second family.

"Friends: a family whose members have been chosen." Alphonse Karr

I want to tell you to believe in yourself, in your dreams, never give up.

With all my love, I wish you a bright future

This reflects friendship and good understanding.

"One of the greatest joys of this life is friendship; and one of the joys of friendship is having someone to confide a secret to." Alessandro Manzoni / The Count of Carmagnola

List of abbreviations

3GC	3rd generation cephalosporins
4GC	4th generation cephalosporins
AMC	Amoxicillin clavulanic acid
ATCC	American type culture collection
ATM	Aztreonam
CAZ	Ceftazidime
CPE	Carbapeneùase-Producing Enterobacterales
CRE	Carbapenemase-Resistant Enterobacterales
CR	Carbapenemase-Resistant
CTX	Cefotaxime
DD-test	Double disc synergy
ERT	Ertapenem
ESBL	Extended Spectrum β-Lactamases
EUCAST	European Committee on Antimicrobial Susptibility Testing
FEP	Cefepime
FOX	Cefoxitin
MDR	Multi Drug Resistance
MEM	Meropenem
OXA	Oxacillinase
TSB	Trypticase Soy Broth
TSI	Tripel sugar iron
WHO	World Health Organization

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Appendices	Title
S1	Results of identification tests of Enterobacterales strains.

Summary

INTRODUCTION

Over seventy years the antimicrobial era has been marked by successive discoveries of a wide range of antibiotics and the subsequent emergence of antibiotic resistance (Codjoe., 2016). The β-lactams are a class of antibiotics that interrupt peptidoglycan synthesis in the bacterial cell wall. They include penicillin derivative, cephalosporins (cephamycin and ceftazidime), monobactam (aztreonam) and carbapenems (meropenem, ertapenem, doripenem, imipenem) (Bush et Bradford., 2016).

Carbapenems are the most potent β-lactam agents against Gram-negative bacilli. Unfortunately, even carbapenems were not spared to the remarkable ability of *Enterobacteriaceae* to adapt to selective pressure and the first carbapenem-resistant *Enterobacteriaceae* (CRE) emerged in Japan in 1990 (Ivolva et al., 2017). Bacteria employ multiple mechanisms to resist to carbapenems. These resistance mechanisms include principally carbapenemase enzymes that hydrolyze carbapenems. They are included in the Ambler class A (Ex. KPC for *Klebsiella pneumoniae* carbapenemase), class B metallo-β-lactamases (Ex. NDM for New Delhi metallo-β-lactamase), and class D oxacillinases (Ex. OXA-48) (Robert et al., 2016).

These carbapenemases have been described worldwide, some of them are typically associated with specific regions or countries. OXA-48-like carbapenemases were first detected in *K. pneumoniae* in Turkey in 2003. Since then, these enzymes have been frequently reported in several countries, particularly in North Africa (HyunjinK., 2016). In Algeria, OXA-48 carbapenemases have been endemic and reported in cases of nosocomial and community-acquired infections, as well as in animals and environment compartments (Mairi et al., 2019).

In this dissertation, we aimed to provide an update of the current prevalence of carbapenemase-producing Enterobacterales strains isolated from different ecological niches.

1. Collection of samples

From 1st May to 2nd July, 2021, different randomly and prospectively selected niches were enrolled, which are distributed over five Algerian provinces (Bejaia, Tizi Ouzou, Bouira, Jijel, and Setif). A total of 1729 samples were collected, including wild animals (n= 418), farm animals (n= 494), pets (n= 94), aquatic environment (n= 26), and food products (n= 697) (Figure 1 and Appendices).

Rectal swabs were performed to screen animal species, excepted for wild animals (fresh stool samples) and broilers (intestinal samples). Food products were randomly obtained from different markets, stores and farms. Samples of aquatic environment were collected from waste waters (n= 6), rivers (n= 8), lakes (n= 8), fountains (n=2), and caves water (n=2). One hundred milliliters of water samples were taken in sterile flasks.

All samples were transported under refrigeration temperature (4°C) to the Microbiological Ecology Laboratory at the University of Bejaia (Algeria) and were analyzed within the day of sampling.

2. Isolation and Identification

Samples from animal were enriched in 10 mL of Trypticase Soy Broth (TSB) (Fluka, St Louis, USA). For food products, 25g (except a volume of 25 mL for milk) of each food items were enriched in 225 mL of peptone water (Fluka, St Louis, USA). For aquatic environment, 1 mL of water was cultured in 9 mL of TSB. All of these samples were incubated overnight at 37°C.

For CPE screening, 50 µL of culture was introduced into 1 mL of the in house Carba MTL-broth containing ertapenem (0.5 mg/L), cloxacillin (250 mg/L), vancomycin (64 mg/L) and amphotericin B (2 mg/L). Paraffin was added to create anaerobiosis. After incubation at 37°C/12-18 hours, tubes of Carba MTL-broth showing a color change from green to yellow were considered as positive for CPE (Mairi et al., 2019a). A volume of 100 µL of positive culture was streaked on chromID media (Titan Biotech, India) and was incubated at 37°C for 24 hours. Suspected colonies were subcultured onto Mac Conkey (Fluka, St Louis, MO, USA) plates.

Enterobacterales species identification was performed using a set of biochemical tests including Urea Indole media, Triple Sugar Iron Agar (TSI), and Citrate (Fluka, St Louis, USA).

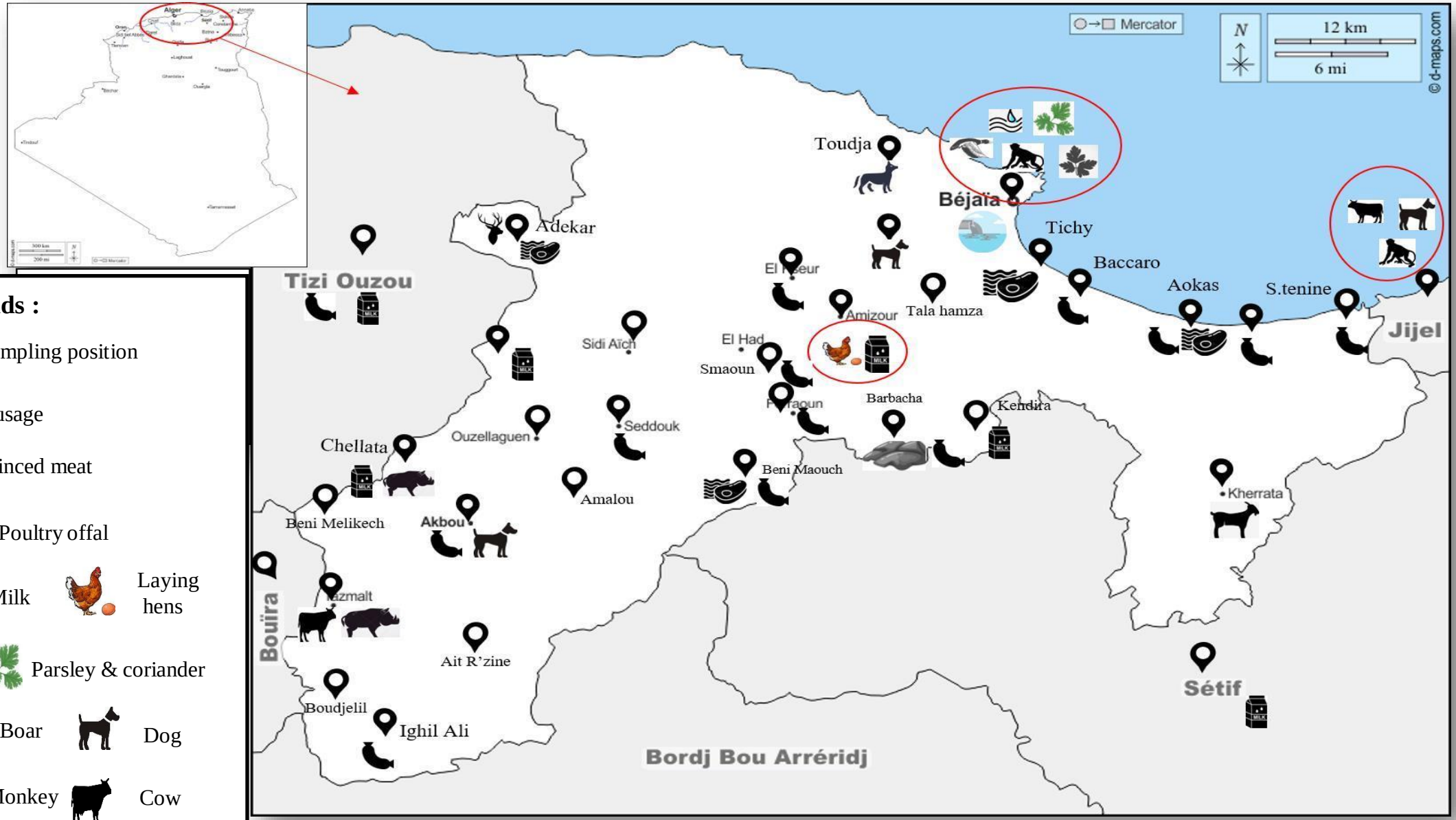

Figure 1: Sampling position in different ecological niches in Algerian provinces.

3. Antibiotic Susceptibility Testing

The susceptibility of Enterobacterales strains to antibiotics was determined on Mueller Hinton agar (Liofilchem, Italie) using the standard disc diffusion procedure as described by the European Committee on Antimicrobial Susceptibility Testing EUCAST 2021. Mueller Hinton agar plates were inoculated by swabbing from a bacterial suspension of approximately 10^8 bacteria/mL. After depositing the antibiotic discs (Biorad, France), the plates were incubated at 37°C for 18-24 hours. The inhibition diameters were interpreted based on the recommendations of EUCAST 2021 (Table 1).

Table 1: Antibiotics tested.

Antibiotic	Abbreviation	Disk load (µg)	Critical diameters	
			S≥	R<
Amoxicillin + clavulanic acid	AMC	20+10	19	19
Cefotaxime	CTX	5	20	17
Ceftazidime	CAZ	10	22	19
Cefepime	FEP	30	27	24
Aztreonam	ATM	30	26	21
Cefoxitin	FOX	30	19	19
Ertapenem	ERT	10	25	25
Meropenem	MEM	10	22	16

4. Detection of Extended Spectrum β-Lactamases (ESBLs)

The strains were further processed for double disc synergy test (DDST). A disc of amoxicillin-clavulanic acid (AMC) was placed in the center of Mueller-Hinton agar plate (90mm) at 20mm distance to ceftazidime (CAZ, 30µg), cefotaxime (CTX, 30µg), aztreonam (ATM, 30 µg), and cefepime (FEP, 30µg). ESBL production is detected by the appearance of synergy image between ceftazidime, cefotaxime, aztreonam and/or cefepime with clavulanic acid (Ejaz et al., 2013).

5. Detection of carbapenemase producter by Hodge test

After inoculating a Mueller Hinton agar with a reference strain sensitive to carbapenems (*Escherichia coli* ATCC 25922), an imipenem disc (IMP, 10 µg) was applied on the center of the plate. After that, the strain to be tested, the negative control (*E. coli* ATCC 25922) and the positive control (*E. coli* NDM-5) were inoculated on the agar in the form of streaks deposited from the imipenem disc to the periphery of the Mueller Hinton plate. After 18-24 hours of incubation at 37°C, the production of a carbapenemase results in a distortion of the zone of inhibition around the imipenem disc (Lee et al., 2010a).

1. Bacterial isolates

A total of 62 CPE isolates from 61 samples of which one individual carried 2 strains (PP66A/PP66B) were identified to be carbapenemase producers by phenotypic methods, giving an overall prevalence of 3.58% (62/1729). The isolates were recovered from farm animals (n = 16; 3.2%), pets (n = 5; 5.3%), wild animals (n = 9; 2.1%), food products (n = 28; 4%), and aquatic environment (n = 4; 15.3%) (Figure 2 and Table 2). The isolates were recovered only in Bejaia (n=59) and Jijel (n=3) (Figure 2).

These isolates were identified as *E. coli* (n=45), *K. pneumoniae* (n=12), *K. oxytoca* (n=4), and *Enterobacter sp* (n=1) (Figure 3 to 7, Table S1).

Legends :

- Sampling position
- Sausage
- Minced meat
- Poultry offal
- Milk
- Laying hens
- Parsley & coriander
- Boar
- Dog
- Monkey
- Cow
- Deer
- Goat
- Wolf
- River
- Bird
- Waste water

Figure 2: Distribution of CPE in different ecological niches in Algerian provinces.

Table 2: Distribution of samples and numbers of carbapenemase-producing Enterobacterales(CPE) strains obtained in different ecological niches.

Niches	Samples collected (n)	CPE (n)
Animals	1006	30
Farm animals	494	16
Cows	79	2
Sheeps	119	0
Goats	65	1
Laying hens	87	13
Broilers	111	0
Rabbits	33	0
Pets	94	5
Dogs	55	5
Cats	11	0
Horses	28	0
Wild animals	418	9
Barbary macaques	105	3
Barbary deer	102	1
Boars	102	3
Bats	35	0
Birds	69	1
Wolfs	5	1
Food products	697	28
Poultry Offals	111	4
Sausages	117	7
Minced meat	127	5
Cow milk	50	1
Goat milk	75	3
Tomatoes	76	0
Herbs	38	8
Cucumber	43	0
Table eggs	60	0

Aquatic enrivonment	26	4
Waste waters	6	3
Lakes	8	0
Caves water	2	0
Rivers	8	1
Fountains	2	0
Total	1729	62

Figure 3: Result on chromID agar pink colonies presumptive: *E. coli*

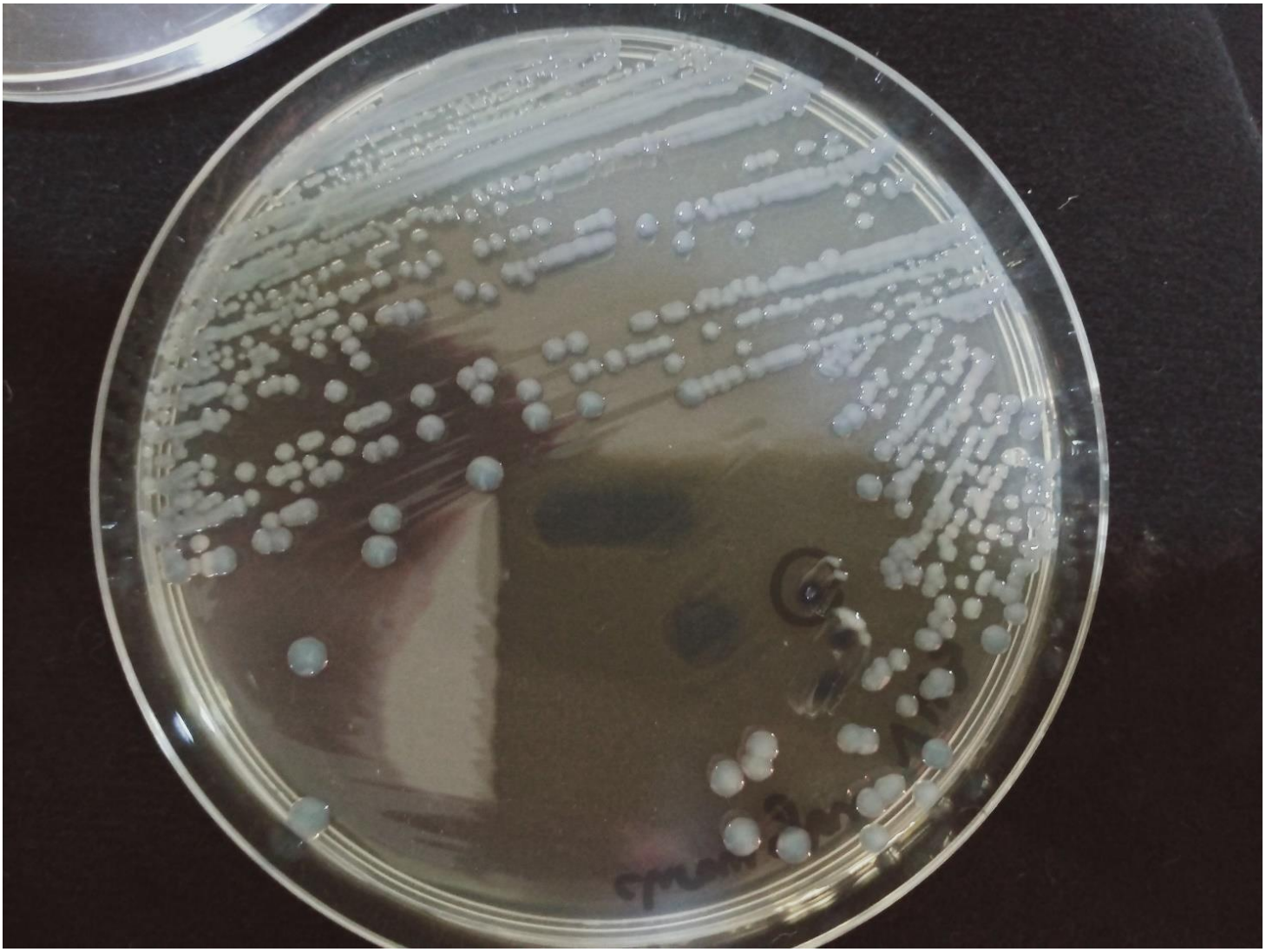

Figure 4: Result on chromID agar blue-green colonies: KESC group (*Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter***)**

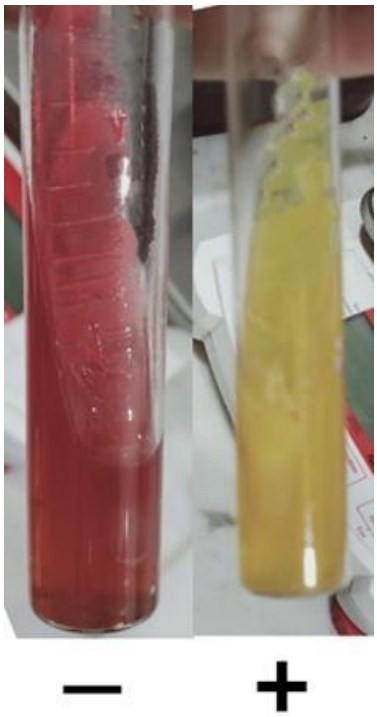

Figure 5: Negative and positive TSI test results

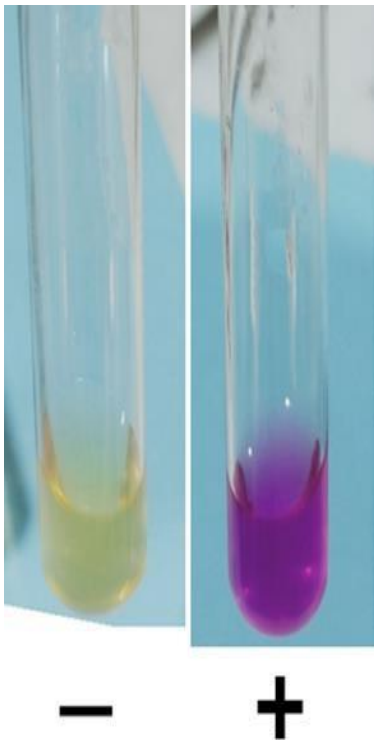

Figure 6: Negative and positive results of the Urea Indole medium

Figure 7: Positive and negative result on Simmons citrate media

2. Susceptibility to antibiotics

All CPE isolates were resistant to amoxicillin-clavulanic acid, ertapenem, and meropenem (100%). The strains were also resistant to cefotaxime, ceftazidime, cefepime (n=10; 16.1%) (Table 3 and Figures 8).

3. Analysis of resistance phenotypes

The Hodge test was positive for all strains of Enterobacterales resistant to ertapenem and meropenem indicating the probable production of a carbapenemase (Figure 9).

The DD-test performed on Mueller Hinton agar showed a synergy in 10 strains of CPE resistant to 3GC and 4GC reflecting the probable production of ESBL in these strains **(**Figure 10 and Table 3). It should be noted that the test of synergy was positive for 08 *E. coli* and 02 *K. pneumoniae* strains.

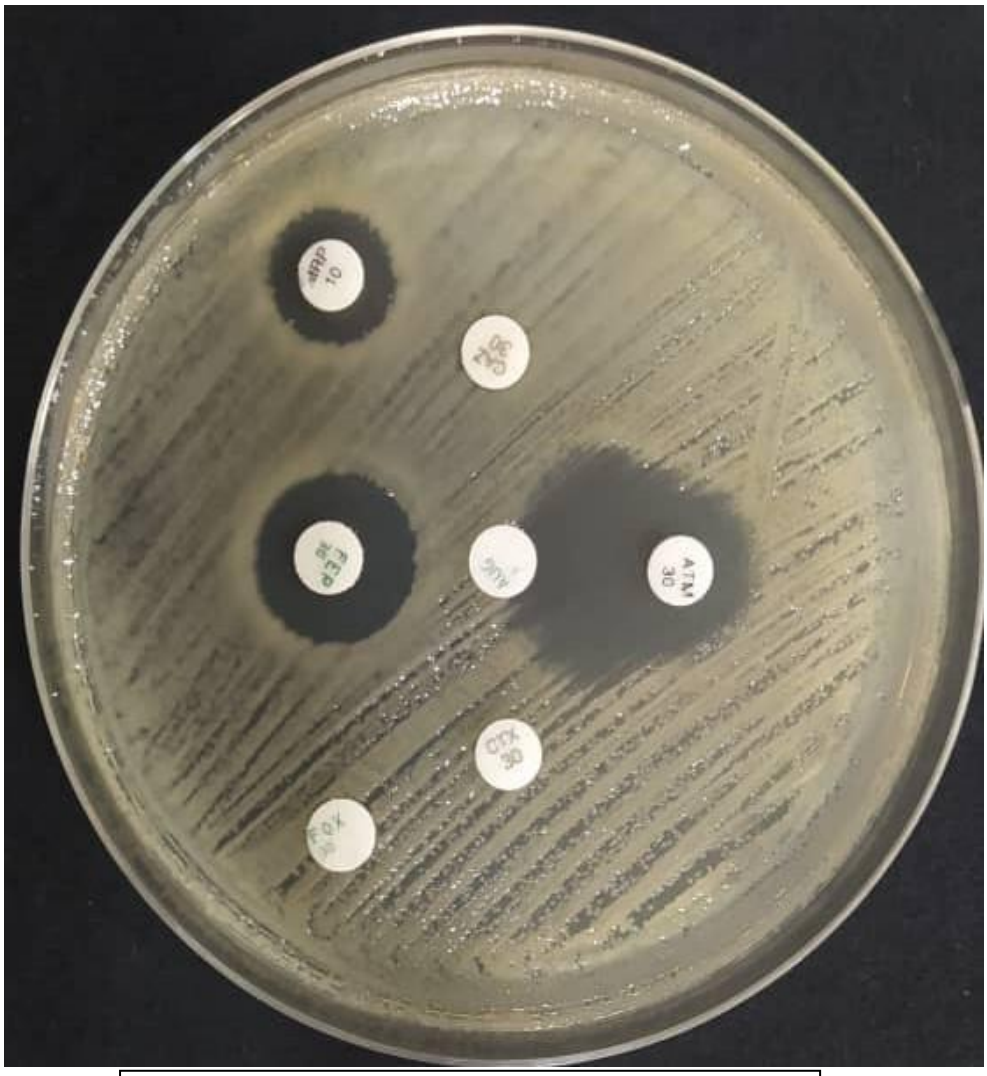

Figure 8: Results of Antibiotic Susceptibility

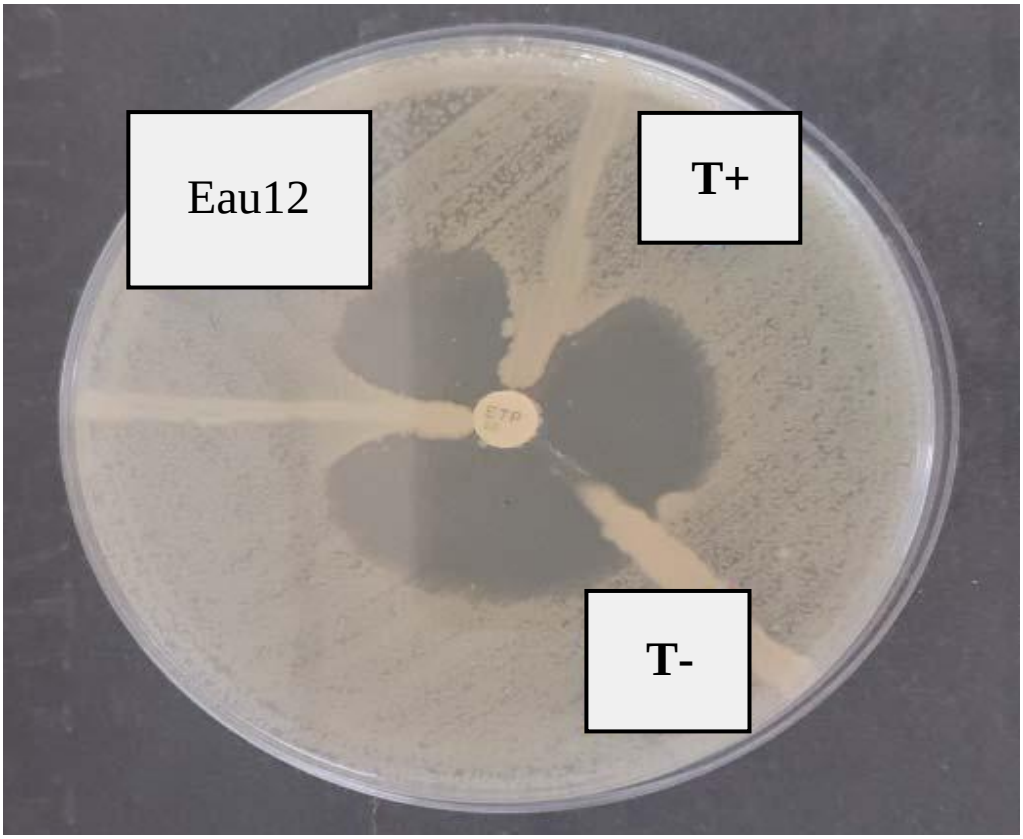

Figure 9: Distortion obtained in the Hodge test of EAU12 strain.
T+: Positive control, **T-:** Negative witness, **Eau12**: Strain

Figure 10: Synergy picture obtained in the DD-test of EAU12 strain

Table 3: Characteristics of CPE isolates detected from different ecological niches in Algerian provinces.

Strain	Species	Date of sampling	Origin	CTX		CAZ		FEP		ATM		FOX		MEM		ERT		DD-test	Localisation
AB105	*E. coli*	20/06/2021	Poultry offal	20	S	23	S	27	S	27	S	22	S	19	I	22	R	(-)	Bejaia (Barbacha)
AB106	*E. coli*	20/06/2021	Poultry offal	21	S	24	S	28	S	27	S	22	S	17	I	20	R	(-)	Bejaia (Barbacha)
AB108	*E. coli*	20/06/2021	Poultry offal	18	S	22	S	27	S	26	S	24	S	19	I	24	R	(-)	Bejaia (Barbacha)
AB110	*E. coli*	20/06/2021	Poultry offal	21	S	22	S	28	S	26	S	22	S	18	I	23	R	(-)	Bejaia (Barbacha)
CF7	*K. pneumoniae*	31/05/2021	Barbary deer	28	S	26	S	32	S	34	S	24	S	21	I	22	R	(-)	Bejaia (Adekar)
CH7	*E. coli*	23/06/2021	Goat	21	S	23	S	28	S	28	S	21	S	16	R	24	R	(-)	Bejaia (Kherrata)
CN1	*E. coli*	13/06/2021	Dog	21	S	24	S	28	S	27	S	21	S	16	R	24	R	(-)	Bejaia (Akbou)
CN25	*E. coli*	21/06/2021	Dog	22	S	23	S	27	S	27	S	24	S	20	I	23	R	(-)	Bejaia (Oued Ghir)
CN31	*E. coli*	22/06/2021	Dog	23	S	23	S	29	S	27	S	23	S	18	I	24	R	(-)	Bejaia (Sidi Ahmed)
CN33	*E. coli*	27/06/2021	Dog	25	S	24	S	29	S	29	S	25	S	21	I	19	R	(-)	Jijel
CN35	*E. coli*	27/06/2021	Dog	22	S	23	S	29	S	26	S	23	S	19	I	21	R	(-)	Jijel
EAU12	*K. pneumoniae*	15/06/2021	Aquatic environment (waste water)	6	R	6	R	15	R	19	R	24	S	11	R	19	R	(+)	Bejaia (17 october)
EAU13	*K. pneumoniae*	15/06/2021	Aquatic environment (waste water)	24	S	24	S	30	S	32	S	24	S	19	I	22	R	(-)	Bejaia (Stadium)
EAU16	*K. pneumoniae*	19/06/2021	Aquatic environment (river)	26	S	24	S	30	S	31	S	25	S	21	I	22	R	(-)	Bejaia (Tala Ntziwin)
EAU17	*E. coli*	19/06/2021	Aquatic environment (waste water)	22	S	23	S	28	S	27	S	23	S	17	I	24	R	(-)	Bejaia (Chriaa)
H14	*E. coli*	29/06/2021	Raw vegeteble (coriander)	22	S	23	S	27	S	28	S	22	S	18	I	22	R	(-)	Bejaia (Edimco)
H15	*E. coli*	29/06/2021	Raw vegeteble (perseley)	21	S	22	S	28	S	28	S	20	S	18	I	21	R	(-)	Bejaia (Edimco)
H16	*E. coli*	29/06/2021	Raw vegeteble (perseley)	6	R	9	R	20	R	17	R	23	S	19	I	21	R	(+)	Bejaia (Edimco)
H17	*E. coli*	29/06/2021	Raw vegeteble (perseley)	22	S	23	S	28	S	27	S	22	S	17	I	21	R	(-)	Bejaia (Edimco)

H18	*E. coli*	29/06/2021	Raw vegeteble (perseley)	22	S	24	S	28	S	26	S	24	S	21	I	17	R	(-)	Bejaia (Edimco)
H20	*K. oxytoca*	29/06/2021	Raw vegeteble (coriander)	28	S	24	S	36	S	33	S	26	S	18	I	20	R	(-)	Bejaia (Edimco)
H22	*E. coli*	29/06/2021	Raw vegeteble (perseley)	6	R	11	R	19	R	18	R	20	S	17	I	20	R	(+)	Bejaia (Edimco)
H23	*E. coli*	29/06/2021	Raw vegeteble (perseley)	22	S	23	S	29	S	29	S	26	S	21	I	20	R	(-)	Bejaia (Edimco)
L104	*K. pneumoniae*	21/06/2021	Cow milk	24	S	23	S	28	S	29	S	23	S	17	I	24	R	(-)	Bejaia (Chemini)
L105	*Enterobacter sp.*	21/06/2021	Goat milk	25	S	24	S	30	S	34	S	6	R	18	I	24	R	(-)	Bejaia (Chemini)
L22	*K. oxytoca*	24/05/2021	Goat milk	30	S	29	S	36	S	35	S	30	S	20	I	22	R	(-)	Bejaia (Amizour)
L25	*K. oxytoca*	24/05/2021	Goat milk	28	S	30	S	36	S	38	S	27	S	20	I	25	S	(-)	Bejaia (Amizour)
LP2	*E. coli*	21/06/2021	Wolf	23	S	25	S	27	S	28	S	24	S	21	I	20	R	(-)	Bejaia (Toudja)
MZ86	*E. coli*	21/06/2021	Sausage	22	S	23	S	27	S	26	S	25	S	19	I	23	R	(-)	Bejaia (Souk El Tenin)
MZ89	*E. coli*	21/06/2021	Sausage	10	R	12	R	20	R	19	R	22	S	17	I	23	R	(+)	Bejaia (Souk El Tenin)
MZ97	*E. coli*	21/06/2021	Sausage	22	S	23	S	29	S	27	S	23	S	20	I	22	R	(-)	Bejaia (Ighil Ali)
MZ98	*E. coli*	21/06/2021	Sausage	23	S	24	S	27	S	27	S	23	S	19	I	23	R	(-)	Bejaia (Ighil Ali)
MZ100	*E. coli*	21/06/2021	Sausage	22	S	23	S	29	S	27	S	23	S	19	I	23	R	(-)	Bejaia (Seddouk)
MZ53	*K. pneumoniae*	24/05/2021	Sausage	30	S	24	S	30	S	30	S	25	S	20	I	24	R	(-)	Bejaia (El Kseur)
MZ62	*E. coli*	31/05/2021	Sausage	25	S	23	S	30	S	28	S	23	S	19	I	23	R	(-)	Bejaia (Baccaro)
OI21	*K. pneumoniae*	20/06/2021	Wild bird	26	S	24	S	31	S	30	S	25	S	20	I	20	R	(-)	Bejaia (Mezaia)
PP55	*E. coli*	29/06/2021	Laying hens	22	S	23	S	27	S	28	S	23	S	20	I	17	R	(-)	Bejaia (Amizour)
PP57	*E. coli*	29/06/2021	Laying hens	24	S	25	S	28	S	28	S	24	S	21	I	20	R	(-)	Bejaia (Amizour)
PP58	*K. pneumoniae*	29/06/2021	Laying hens	13	R	15	R	22	R	20	R	25	S	17	I	19	R	(+)	Bejaia (Amizour)
PP60	*E. coli*	29/06/2021	Laying hens	9	R	12	R	19	R	19	R	23	S	18	I	23	R	(+)	Bejaia (Amizour)
PP65	*E. coli*	29/06/2021	Laying hens	22	S	24	S	28	S	28	S	23	S	19	I	23	R	(-)	Bejaia (Amizour)

PP66B	*K. oxytoca*	29/06/2021	Laying hens	30	S	30	S	38	S	38	S	28	S	21	I	23	R	(-)	Bejaia (Amizour)
PP66R	*E. coli*	29/06/2021	Laying hens	22	S	23	S	27	S	26	S	22	S	17	I	21	R	(-)	Bejaia (Amizour)
PP67	*E. coli*	29/06/2021	Laying hens	24	S	23	S	28	S	26	S	23	S	18	I	22	R	(-)	Bejaia (Amizour)
PP68	*E. coli*	29/06/2021	Laying hens	21	S	23	S	28	S	30	S	22	S	18	I	19	R	(-)	Bejaia (Amizour)
PP70	*E. coli*	29/06/2021	Laying hens	23	S	24	S	29	S	26	S	23	S	19	I	25	S	(-)	Bejaia (Amizour)
PP71	*E. coli*	29/06/2021	Laying hens	22	S	24	S	27	S	26	S	22	S	19	I	21	R	(-)	Bejaia (Amizour)
PP72	*E. coli*	29/06/2021	Laying hens	22	S	23	S	28	S	26	S	23	S	18	I	22	R	(-)	Bejaia (Amizour)
PP75	*E. coli*	29/06/2021	Laying hens	16	R	18	R	27	S	24	I	19	S	19	I	23	R	(+)	Bejaia (Amizour)
SG100	*E. coli*	20/06/2021	Barbary macaque	22	S	23	S	28	S	29	S	24	S	18	I	18	R	(-)	Jijel
SG42	*E. coli*	13/06/2021	Barbary macaque	6	R	6	R	17	R	15	R	19	S	17	I	15	R	(+)	Bejaia (Cap Carbon)
SG44	*E. coli*	13/06/2021	Barbary macaque	6	R	10	R	19	R	17	R	24	S	19	I	20	R	(+)	Bejaia (Cap Carbon)
SL46	*E. coli*	20/05/2021	Boar	24	S	25	S	29	S	28	S	22	S	19	I	20	R	(-)	Bejaia (Chellata)
SL57	*E. coli*	20/05/2021	Boar	22	S	26	S	27	S	26	S	24	S	19	I	20	R	(-)	Bejaia (Chellata)
SL76	*E. coli*	27/06/2021	Boar	24	S	22	S	27	S	28	S	23	S	20	I	20	R	(-)	Bejaia (Tazmalt)
V43	*K. pneumoniae*	27/06/2021	Cow	28	S	24	S	31	S	31	S	26	S	20	I	17	R	(-)	Jijel
V71	*E. coli*	27/06/2021	Cow	24	S	29	S	28	S	27	S	25	S	19	I	21	R	(-)	Bejaia (Tazmalt)
VH98	*E. coli*	21/06/2021	Minced meat	24	S	30	S	29	S	30	S	23	S	20	I	24	R	(-)	Bejaia (Adekar)
VH50	*K. pneumoniae*	25/05/2021	Minced meat	31	S	24	S	32	S	32	S	24	S	20	I	20	R	(-)	Bejaia (Tichy)
VH57	*K. pneumoniae*	25/05/2021	Minced meat	30	S	28	S	34	S	32	S	26	S	21	I	20	R	(-)	Bejaia (Aokas)
VH58	*K. pneumoniae*	25/05/2021	Minced meat	28	S	24	S	32	S	33	S	27	S	21	I	21	R	(-)	Bejaia (Aokas)
VH88	*E. coli*	21/05/2021	Minced meat	8	R	9	R	18	R	16	R	21	S	17	I	19	R	(+)	Bejaia (Beni Maouch)

Disscussion

Over the last decade, there has been a dramatic increase in carbapenemases in *Enterobacteriaceae* (KohTH., 2013), which are the most common human pathogens isolated in medical bacteriology both in hospital and in the community setting (Bachiri et al., 2017). Carbapenem resistance (CR) is a serious growing threat that is spreading in Algeria and worldwide, as carbapenems are important antibiotics of last resort.

This study highlighted a high prevalence of CPE isolates in different niches the five Algerian provinces examined. From 1729 samples collected from different ecological niches, 62 carbapenem resistant strains were selected giving an overall prevalence of 3.58% (62/1729). This result is similar to 2.4% reported in Algeria by Mairi and *al* (Mairi et al., 2019). In a recent systematic review Kock et *al.* reported a prevalence varying between 0.6% and 26% in Algeria (Kock et al., 2018).

In food products, prevalence of 4% was recorded which was higher than that reported in Algeria by Mairi and *al* (Mairi et al., 2019). However, this prevalence is relatively similar to that reported by Touati and *al* in vegetables (Touati et al., 2017). Moreover, a prevalence of 3.2% was reported in livestock which is a bit higher than the prevalence of 1.2% reported by Mairi et *al*. Kock et *al*. indicated that CPE were not highly prevalent in livestock in Europe whereas they found evidence for the dissemination of CPE among livestock in China and India (Köck et al., 2018; Mairi et al., 2019a). In wild animals, prevalence of 2.1% was reported which is lower than that reported by Mairi et *al*. However, this prevalence was higher than the first report of CPE in the wild animals in Algeria by Bachiri et al (Bachiri et al., 2018; Mairi et al., 2019a). Furthermore, a prevalence of 5.3% was recorded in companion animals which is higher than the first report in Algeria by Yousfi et *al*., contrary to what was reporter by Kock et *al* (Köck et al., 2018; Yousfi et al., 2016). In the aquatic environment (river water, wastewater…) a prevalence of 15.3% was evidenced. This prevalence is similar to that reported in Switzerland by Bleichenbacher et *al.* and in Algeria by Mairi et *al*.(Bleichenbacher et al., 2020; Mairi et al., 2019a).

The most prevalent species identified from our study was *E. coli* (n=45, 72.58%), followed by *K. pneumoniae* (n=12, 19.35%), *K. oxytoca* (n=4, 6.45%) and *Enterobacter* (n=1, 1.61%). In contrast to what was reported by Touati and Mairi (Touati and Mairi, 2020a) in their systematic review, *K. pneumoniae* was the most isolated strain with 51.1%, followed by *E. coli* strains 27.1% and *E. cloacae* with 9%.

This present study reported much higher carbapenem resistance with the disc diffusion susceptibility testing. The highest carbapenem resistance was 100% to amoxicillin-clavulanic acid, ertapenem, and meropenem and 16.1% only to 3GC and 4GC, there is similarities to what was

reported by Mairi et *al.*(Mairi et al., 2019a) in Algeria except for meropenem (25.6%), cefotaxime (8.9%) and ceftazidime (6.4%).

OXA-48 carbapenemases are often associated with other beta-lactamases, in particular ESBLs, which contributes to the multidrug resistance of strains. In this study, 10 strains including 8 *E. coli* and 2 *K. pneumoniae* were found to produce ESBL enzymes.

Carbapenemase-producing microorganisms are pressing problem to public health because of the limited therapeutic options and the global spread of such strains. In recent years, more and more carbapenemases-producing isolates have been reported not only from humans but also from non-human sources (Cui and al., 2018). It has been reported by different studies a possible anthropozoonotic or zooanthroponotic transmission of CRE between animals and exposed humans (Kock et al., 2018). Thus, we could hypothesize that the emergence of CPE in companion animals is due to increasing prescription to pets of antimicrobial substance but also due to the close contact between pets and their owners.

In farm animals we acknowledge an intensification of animal production systems leaves them vulnerable to disease outbreaks. Thus, various antimicrobial drugs have been administered as veterinary therapeutic in farmed animals. Currently livestock animals are a source of MDR Enterobacteria, and represent risks for public health associated with economic losses in livestock production (Mairi et al., 2018). Additionally, a study on broiler poultry farming in Egypt, reported that the prevalence was higher in farm workers (67%) than in veterinarians (33%), indicating that transmission could be facilitated by close contact between broilers and humans, since the workers lived in the farms during the fattening program. On the other hand, human-to-animal transmission is also possible. This suggests that the resistance genes might have been acquired from hospitals and transmitted to farms either through infected human carriers or sewage effluent (Hamza et al., 2016).

Furthermore, the food chain has recently attracted attention because it can serve as reservoir for resistance genes, partly due to the massive use of antimicrobial drugs in the livestock sectors. Vegetables may be contaminated through insufficiently treated water and fertilizers or may be compromised by the use of biocides during cultivation, this is of great concern since this produce would not necessarily be cooked and can be consumed raw (Touati et al., 2017).

Wild life acquires CPE for example by contact with sewage, manure or waste disposal site, these raise concern because they indicate that these carbapenemases are prevalent in the

environment in amounts that not only lead to colonization of wildlife, but also the migratory birds may act as a vector facilitating the spread of CPE beyond borders of farms, dwelling zones, countries or even continents (Kock et al., 2018). The occurrence in surface water highlights the potential of these pathogenic lineages to be further disseminated into nature via watering systems affecting agriculture and food-producing animals, as well as to spread carbapenem resistance (Bleichenbacher et al., 2020).

This wide dissemination in different niches other than the clinical setting may constitute a reservoir of resistant strains, which could be responsible for transmission from humans to their cohabitants and within veterinary hospitals.

Conclusion:

In conclusion, our study showed that CPE are present in different extrahuman niches.

We believe that this dissemination is due to the excessive use of antimicrobial agents and the presence of antibiotics in different environments (animals, food, wastewater).

Therefore, further studies are needed, to elucidate the transmission cycle of these bacteria between these different habitats and to explore the origin of CPE isolates circulating outside hospitals. This is important because of the high virulence potential of these isolates.

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Appendices

Table S1: ResultsofidentificationtestsofEnterobacteralesstrains.

Strain	ChromID medium	Glucose	Lactose	Urea	Indole	Simmonscitrate	Species
AB105	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
AB106	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
AB108	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
AB110	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
CF7	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
CH7	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
CN1	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
CN25	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
CN31	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
CN33	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
CN35	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
EAU12	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
EAU13	Blue colonies	+	+	+	-	+	*Klebsiella pneumoniae*
EAU16	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
EAU17	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
H14	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
H15	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
H16	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
H17	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
H18	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
H20	Bluecolonies	+	+	+	+	+	*Klebsiellaoxytoca*
H22	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
H23	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
L104	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
L105	Bluecolonies	+	+	-	-	+	*Enterobactersp.*
L22	Bluecolonies	+	+	+	+	+	*Klebsiellaoxytoca*
L25	Bluecolonies	+	+	+	+	+	*Klebsiellaoxytoca*
LP2	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
MZ86	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
MZ89	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
MZ97	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
MZ98	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
MZ100	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*

MZ50	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
MZ62	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
OI21	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
PP55	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP57	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP58	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
PP60	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP65	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP66B	Bluecolonies	+	+	+	+	+	*Klebsiellaoxytoca*
PP66R	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP67	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP68	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP70	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP71	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP72	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
PP75	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
SG100	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
SG42	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
SG44	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
SL46	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
SL57	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
SL76	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
V43	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
V71	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
VH98	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*
VH50	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
VH57	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
VH58	Bluecolonies	+	+	+	-	+	*Klebsiella pneumoniae*
VH88	Pinkcolonies	+	+	-	+	-	*Escherichiacoli*

Abstract

Objective: Provide an update of the current prevalence of CPE strains isolated from different ecological niches.

Material and methods: A total of 1729 samples were taken from different ecological niches. These samples were collected randomly, from different stores, farmers, during the period from1st May to 2nd July 2021. After the isolation and identification of the enterobacteria strains, the sensitivity of thestrainstoantibioticswasdeterminedbyantibioticswasdeterminedbyagar diffusion method. The production of carbapenemase was determined by the Hodge test.

Results: A total of 62 carbapenemase-producing Enterobacteriaceae (CPE) strains were found with a prevalence of 3.58%. The *Escherichia coli* species was dominant with a prevalence of 72.58%.

Conclusion: This study suggests a global dissemination of carbapenemases-producing Enterobacterales in different niches due mainly to the excessive use of antibiotics in the environment.

Keyswords: Enterobacteria, Carbapenemase, Resistance, Extrahuman, Algeria.

Résumé

Objectif : Fournir une mise à jour de la prévalence actuelle des souches d'ECP isolées dans différentes niches écologiques.

Matériel et méthodes : Un total de 1729 échantillons a été prélevés dans différentes niches écologiques. Ces échantillons ont été collectés de manière aléatoire, auprès de différents magasins, agriculteurs, durant la période du 1ermai au 2 juillet 2021. Après l'isolement et l'identification des souches d'entérobactéries, la sensibilité des souches aux antibiotiques a été déterminée par la méthode de diffusion en gélose. La production de carbapénémase a été déterminée par le test de Hodge.

Résultats : Un total de 62 souches d'entérobactéries productrices de carbapénémase (EPC) a été trouvé avec une prévalence de 3,58%. L'espèce Escherichia coli était dominante avec une prévalence de 72,58 %.

Conclusion : Cette étude suggère une dissémination mondiale des Enterobacterales productrices de carbapénémases dans différentes niches, principalement due à l'utilisation excessive d'antibiotiques dans l'environnement.

Mots clés : Enterobacteria, Carbapénémase, Résistance, Extra humain, Algérie.