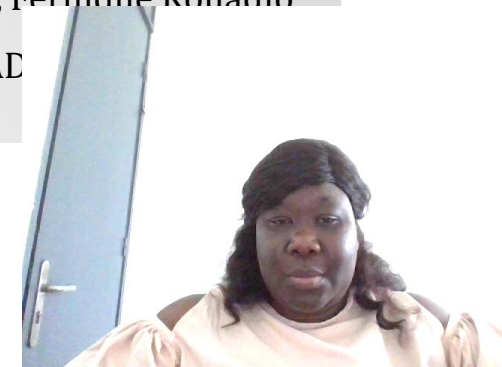




Use of an Algae-Based Feed Supplement in Pig Farming : Toward a « One Health » Strategy for Controlling Pathogenic and Antibiotic-Resistant *Escherichia coli* Strains

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PLAN

- **Introduction**
- **Materials and methods**
- **Results and discussion**
- **Conclusion**





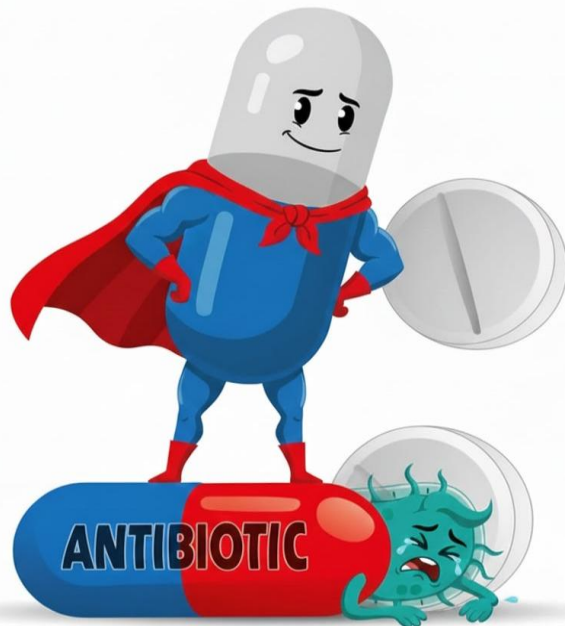
Introduction



were discovered by Alexander Fleming in 1928

(Tigaud, 2006)

Antibiotics



- This discovery marked a true medical revolution
- as these molecules made it possible to fight infectious diseases
- and to increase life expectancy worldwide





In animals, antibiotics are used for preventive, therapeutic purposes, and as growth promoters





Digestive and respiratory infections



Responsible for high mortality rates



Antibiotic therapy seems to be the only means of control

(Anses, 2013)

In Côte d'Ivoire, in weaned piglets



Preventive use of antibiotics

Sometimes without a veterinarian's advice

5

(Madec, 2012)

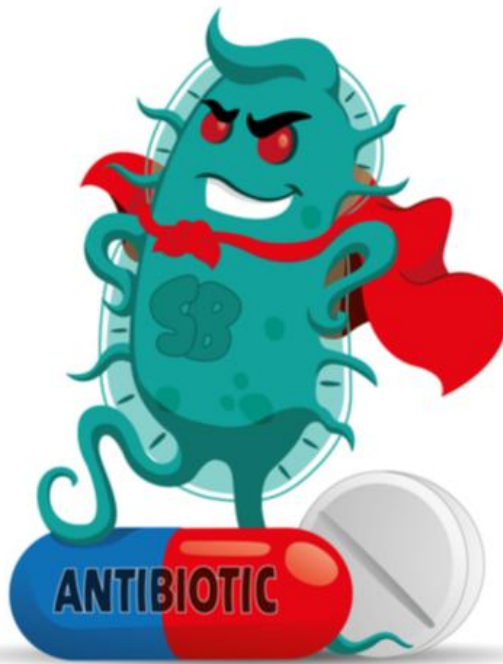




However, repeated use
of antibiotics



exerts a selective pressure
that favors the survival of
resistant bacteria in the
animal's microbiota



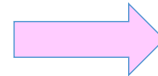
BACTERIAL RESISTANCE





Counter the phenomenon of bacterial resistance to antibiotics

Alternative solutions

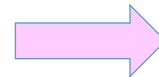


Improve gut health



Without promoting bacterial resistance

Among these alternative strategies, seaweed could represent a natural option.



(Ulva americana)





GENERAL OBJECTIVE

Contribute to food safety of pork produced in Côte d'Ivoire through the use of a seaweed-based feed supplement in pig farming





SPECIFIC OBJECTIVES

- ❖ Evaluate the effect of the seaweed-based feed supplement on the resistance of *Escherichia coli* strains isolated from piglets
- ❖ Determine the impact of the feed supplement on the virulence of *Escherichia coli* strains





Materials and methods

1. Biological material



Figure 1: Piglet fecal matter

Table 1 : Reference bacterial strains

Positive control strains	Characteristics	Techniques	References
<i>E. coli</i> ATCC 25922	Susceptible to all antibiotics	Antibiogramm	Institut Pasteur Paris
<i>E. coli</i> U2A2118 <i>E. coli</i> U2A2119 <i>E. coli</i> U2A2120	qnrA1 qnrB1 qnrS1	PCR	
<i>Salmonella</i> spp U2A1446	TEM-1 SHV-12	PCR	
<i>E. coli</i> U2A1790	CTX-M 1	PCR	
<i>E. coli</i> CIP RP4	tet A	PCR	
<i>E. coli</i> CIP Pip 69	tet B	PCR	
<i>E. coli</i> M19 <i>E. coli</i> EA47	eae-stx1 eae-stx2	PCR	
<i>E. coli</i> ATCC 35401	lt-st	PCR	Dadié et al., 2000
<i>E. coli</i> Pb71	Bfp	PCR	Dadié et al., 2010
<i>E. coli</i> He104 <i>E. coli</i> He2 <i>E. coli</i> He96	lal aggA ipaH	PCR PCR PCR	Dadié et al., 2014





2. List of antibiotics

Penicillin

Amoxicillin (25 µg)

Cephalosporin

Cefotaxim (5 µg)

Cefoxitin (30 µg)

Carbapenem

Imipenem (10 µg)

Fluoroquinolone

Ciprofloxacin (5 µg)

Nalidixic Acid (30 µg)

Aminoside

Amikacin (30 µg)

Streptomycine (10 µg)

Tetracycline

Tetracycline (30 µg)

Chloramphenicol (30 µg)

Others

Colistine (50 µg)

3. Technical equipment





□ The experimental setup consisted of

6 healthy recently weaned piglets (divided into 3 groups)

(Aged 8 weeks / 7-10 kg)



A



B



C

Used as a controle

Treatments administered for 5 consecutive days



20mg/L of water per kg of body weight
Oral route in drinking water

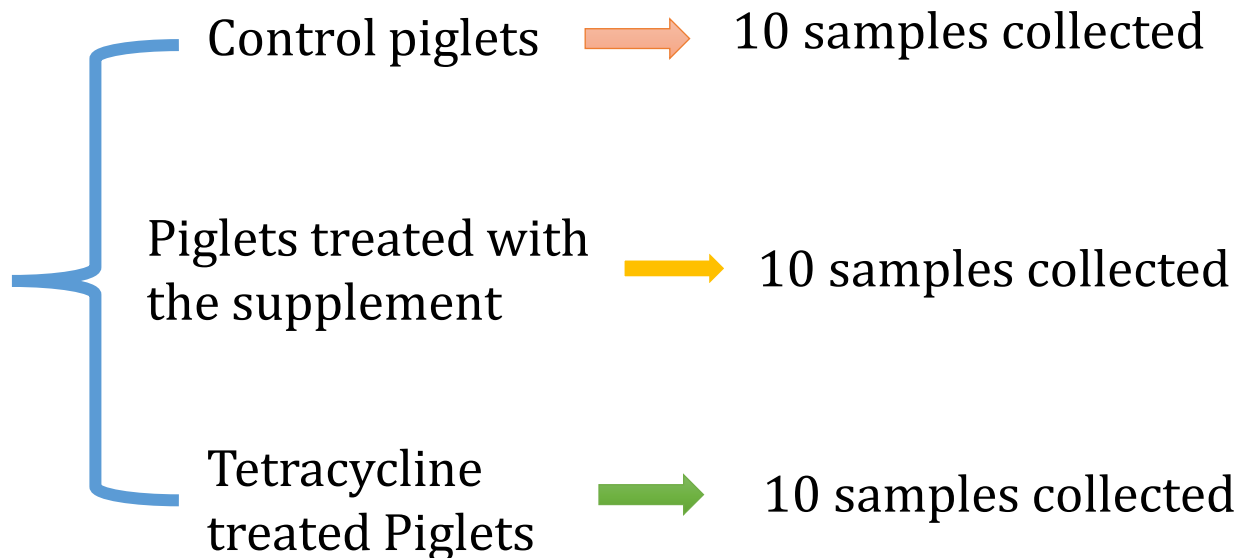
Figure 2 : Piglet treatment





❑ Sample collection

- ❑ The upper part of a fecal portion from each piglet was collected every morning



A total of 30 samples were collected





Evaluation of the impact of tetracycline and the feed supplement administration on the development of antibiotic resistance in enterobacteria





1. Isolation of resistant enterobacteria

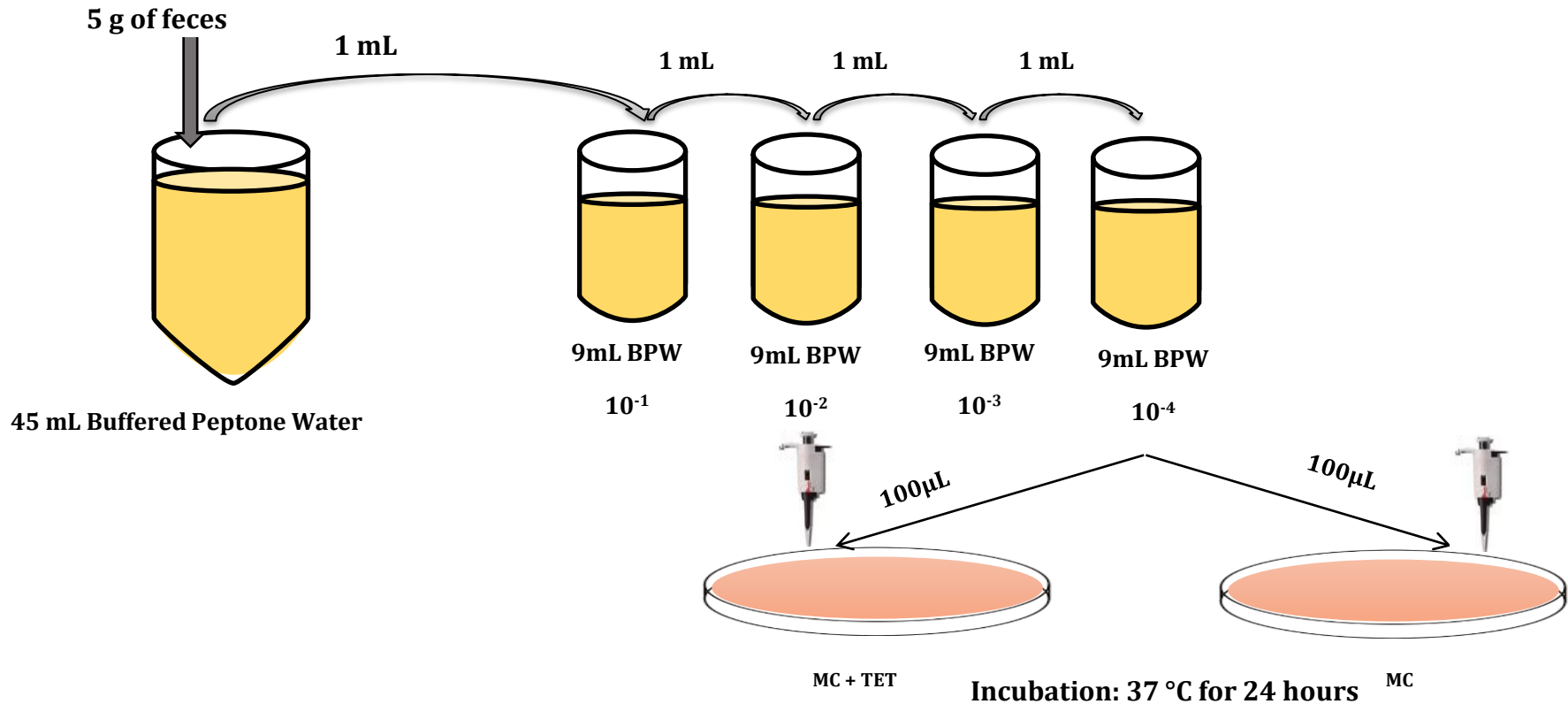


Figure 3 : Inoculations on MC agar and MC agar supplemented with tetracyclin





$$\textit{Resistance rate}(\%) = \frac{n_{\text{MC+ATB}}}{n_{\text{MC}}} \times 100$$

$n_{\text{MC+ATB}}$: Number of colonies counted on MC+ATB medium

n_{MC} : Number of colonies counted on MC medium

2. Identification of *Escherichia coli* strains

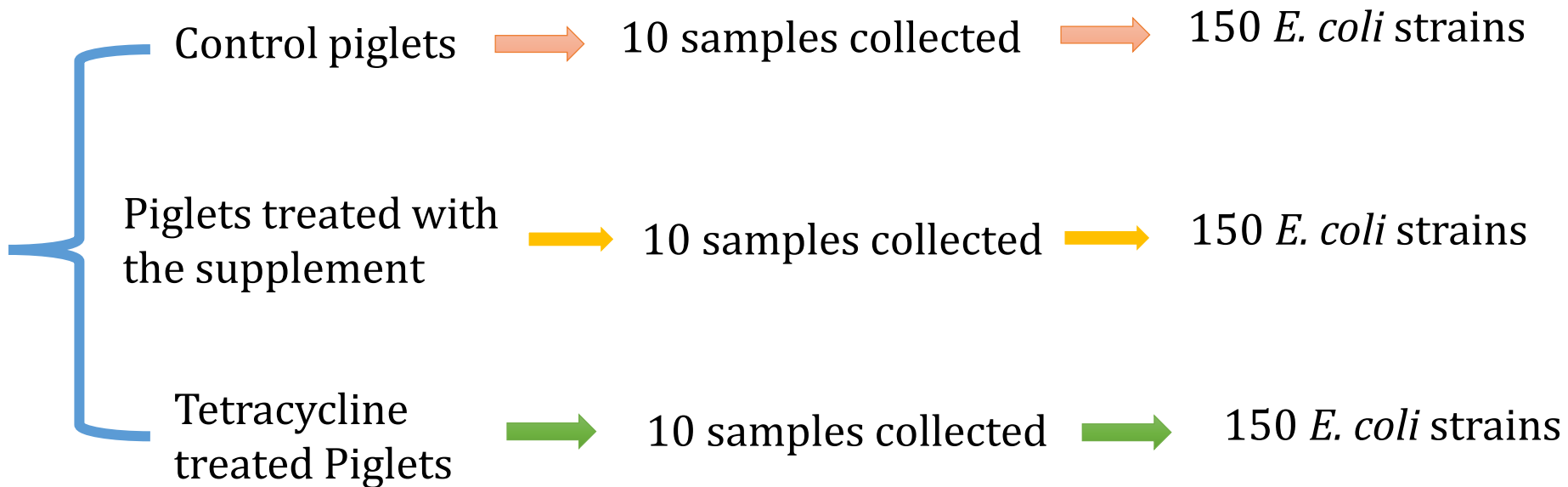
Suspected colonies  Streaked on Rapid *E. coli* 2 agar (37 °C / 24 h)

Biochemical and molecular identification by MALDI-TOF mass spectrometry





A total of 450 *E. coli* strains were identified, with 150 strains per piglet group





**Determination of the phenotypic and genotypic resistance potential of
Escherichia coli strains**





1. Determination of the phenotypic resistance potential of *Escherichia coli* strains (CASFM, 2017)

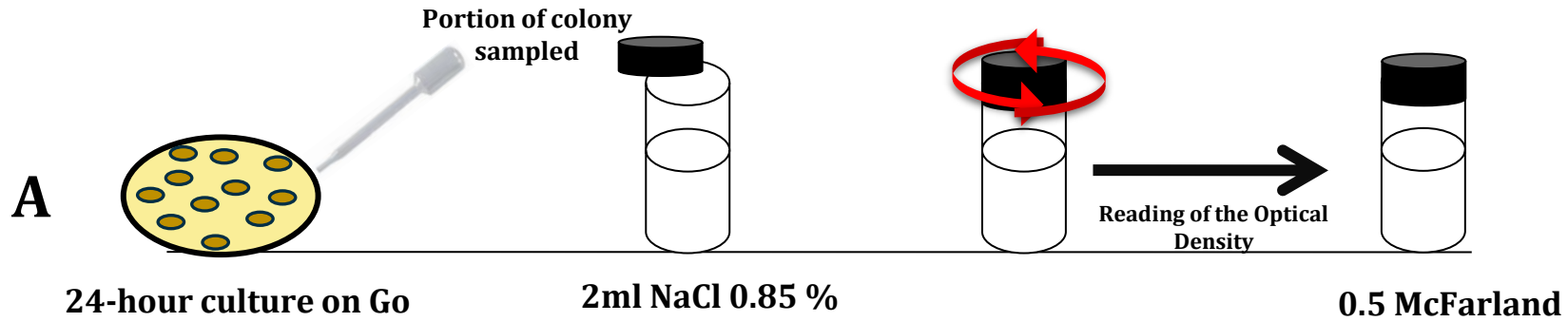
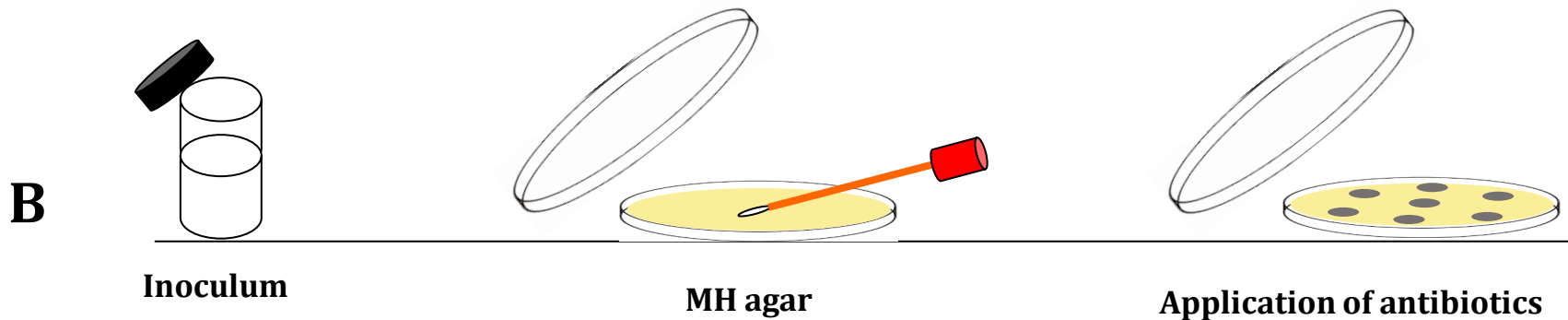


Diagram of inoculum preparation



Inoculation / Antibiotic application / Incubation / Reading

Figure 4 : Antibiotic susceptibility test





2. Determination of the genotypic resistance potential of *Escherichia coli* strains

❖ Bacterial DNA extraction (heat shock)

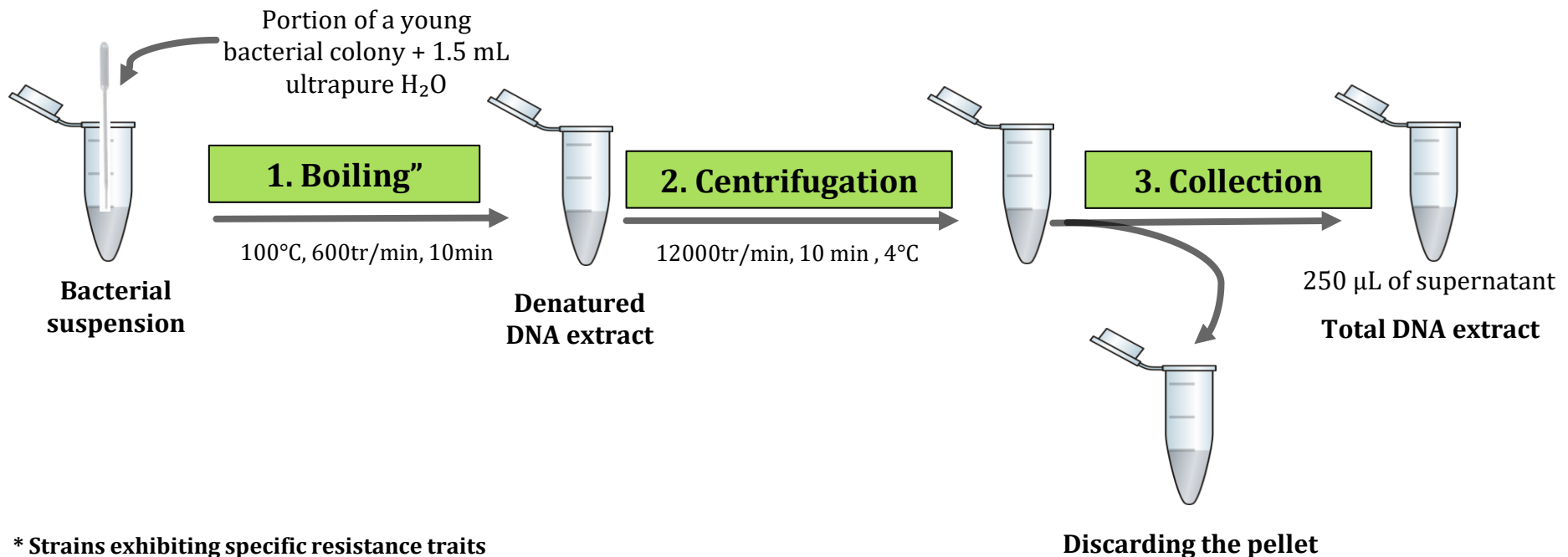


Figure 5 : Bacterial DNA extraction





❖ **Polymerase Chain Reaction (PCR)**

Simplex PCR



bla CTX-M, SHV, TEM

Amplification

Reaction mix: 50 μ L

45 μ L aliquots

5 μ L DNA

- H₂O
- BuffersMgCl₂
- dNTPs
- Specific primers
- Taq polymerase





❖ **Polymerase Chain Reaction (PCR)**

Table 2: Reaction mixture for the amplification of *Escherichia coli* resistance genes

Reagents	Initial concentrations	Volumes (μL)	Final concentrations
Ultrapure water	-	30,3	-
DNA to be amplified	-	5	-
Loading buffer	10X	5	1X
Non-colored buffer	10X	5	1X
MgCl ₂	25 mM	3	1,5 mM
dNTP	10 mM	0,5	0,1 mM
Amorce Forward	10 μM	0,5	0,1 μM
Amorce Reverse	10 μM	0,5	0,1 μM
Taq polymerase	5 U/ μL	0,2	0,02 U/50 μL
Total	-	50	-





Table 3 : Amplification conditions of the target genes

Steps	Conditions (temperature / duration)				
	<i>bla</i> _{TEM}	<i>bla</i> _{SHV/CTX-M}	<i>qnr (A-B-S)</i>	<i>tet A</i>	<i>tet B</i>
Initial denaturation	94°C/5 min	94°C/5 min	94°C/5 min	95°C/1 min	95°C/1 min
Cyclic denaturation	94°C/1 min	94°C/1 min	94°C/1 min	95°C/30 s	95°C/30 s
Hybridation	50°C/1 min	60°C/1 min	60°C/45 s	50°C/30 s	53°C/30 s
Cyclic elongation	72°C/1 min	72°C/1 min	72°C/1 min	72°C/20 s	72°C/20 s
Final elongation	72°C/7 min	72°C/7 min	72°C/7 min	72°C/7 min	72°C/7 min
Number of cycles	30	30	30	25	25





❖ Analysis of amplified products

✓ Electrophoretic migration

On 1.5% agarose gel with 8 μ L of SYBR Safe, subjected to 100 volts for 25 minutes.

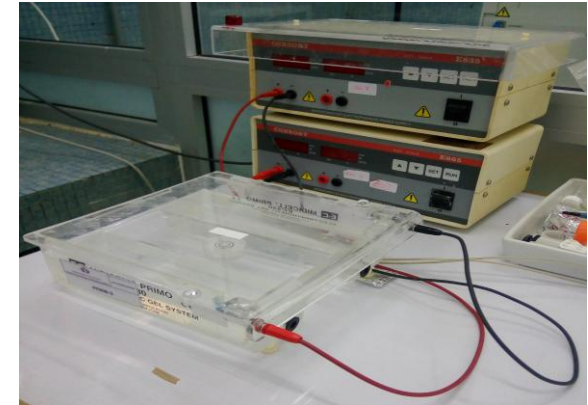


Figure 6 : Electrophoresis tank

✓ Visualization of bands

GelDoc imager (Bio-Rad Laboratories, Hertfordshire, UK).

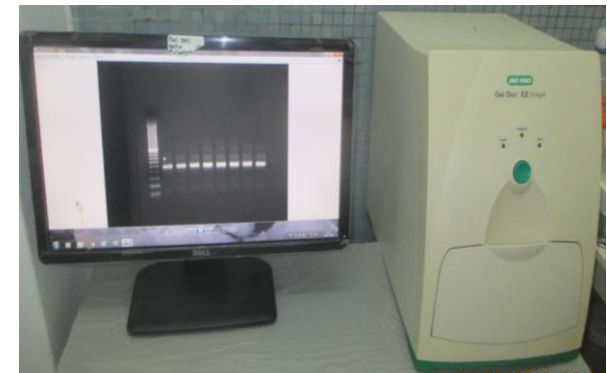


Figure 7 : Automated system





One Health
Student Conference
USAMV București



**Determination of the impact of the feed supplement on the virulence of
Escherichia coli strains**





Bacterial DNA extraction



Kit Qiagen

Virulence genes



Simplex PCR in 25 μ L

Table 4 : Amplification conditions for virulence genes

STEP	Amplification conditions/duration	
	<i>stx1</i> et <i>stx2</i>	<i>eae, bfp, ial, aggA, ipaH, st, lt</i>
Initial denaturation	94°C/3 min	94°C/3 min
Cyclic denaturation	94°C/30 s	94°C/30 s
Hybridation	57°C/45 s	56°C/20 s
Cyclique elongation	72°C/30 s	72°C/30 s
Final elongation	72°C/5 min	72°C/5 min
Cycles	35	35

Dadié et al. (2014)

Kouamé et al. (2017)





Table 5 : Reaction mixture for the amplification of *Escherichia coli* virulence genes

Reagents	Initial concentrations	Volume (μL)	Final concentrations
Ultrapure water	-	11,8	-
DNA to be amplified	-	3	-
Loading buffer	10X	2,5	1X
Non-colored buffer	10X	2,5	1X
MgCl ₂	25 mM	1,5	1,5 mM
dNTP	10 mM	0,5	0,2 mM
Amorce Forward	10 μM	1,5	0,6 μM
Amorce Reverse	10 μM	1,5	0,6 μM
Taq DNA-polymerase	5 U/ μL	0,2	0,04 U/25 μL
Total	-	25	-





STATISTICAL ANALYSES

Software **XLSTAT version 2017**

◆ Comparisons

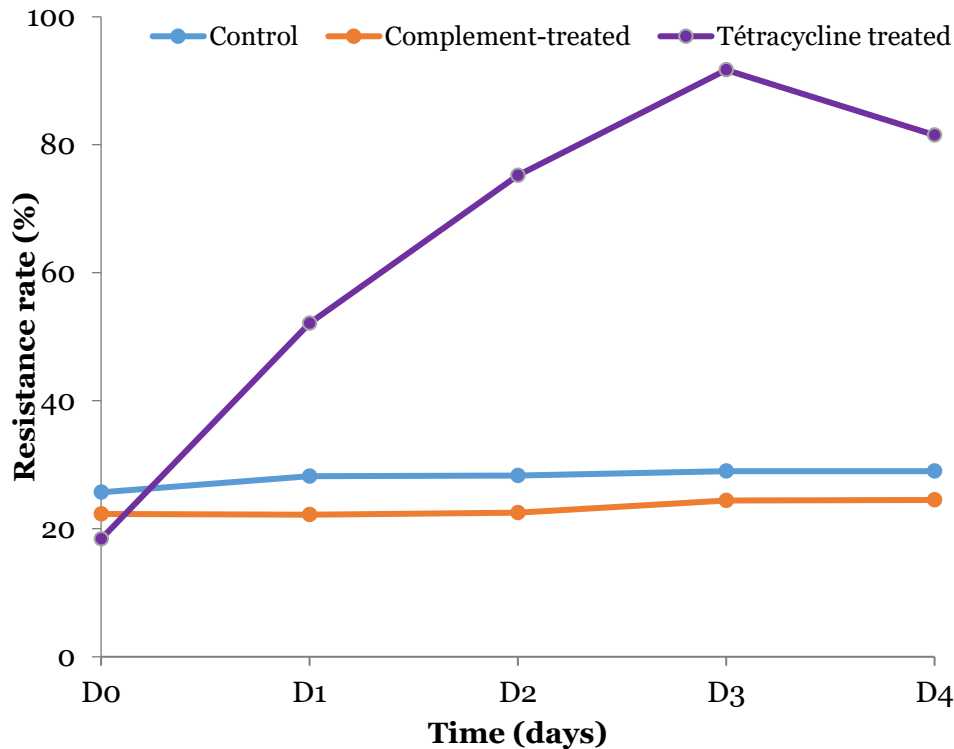
- Analysis of Variance (ANOVA) → Degree of difference between variables
- Signification statistique → 5 %





Results and discussions

1. Tetracycline-resistant Enterobacteriaceae



□ Non-significant increase in control piglets and those treated with the supplement (D0)

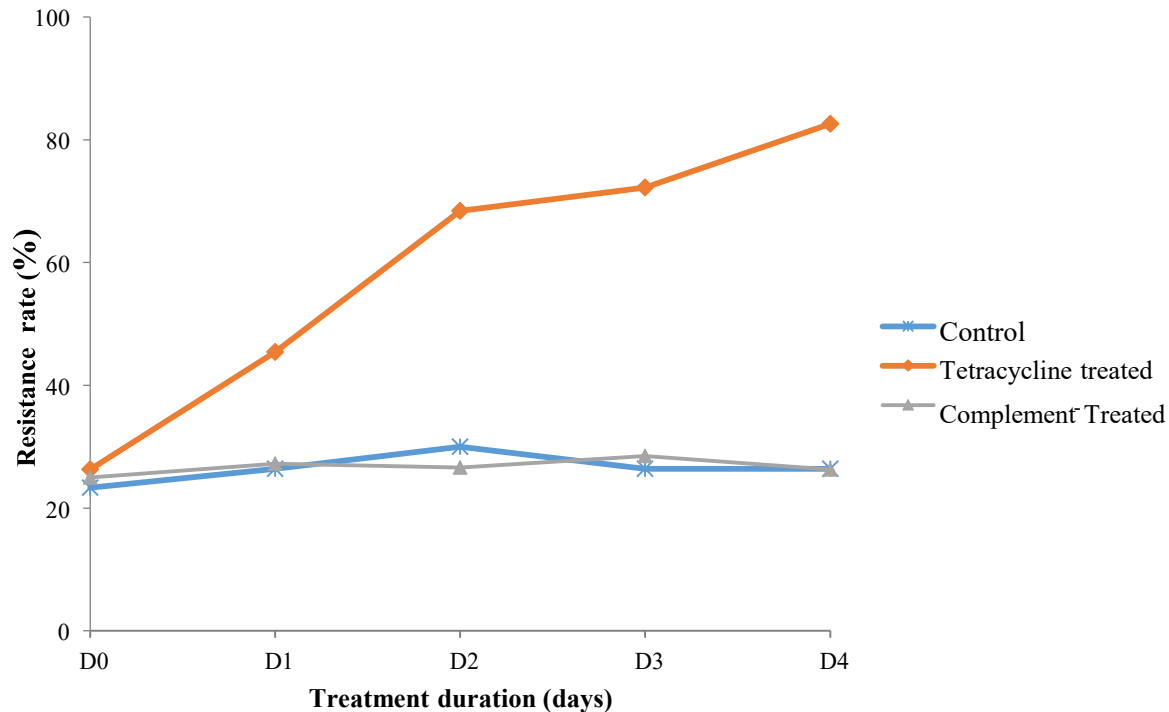
■ **Harada et al, 2008**, frequency in piglets of tetracycline-resistant Enterobacteriaceae strongly influences the prevalence of resistance

Figure 9 : Evolution of tetracycline-resistant Enterobacteriaceae





2. Tetracycline-resistant *Escherichia coli* strains



The resistance rates of *E. coli* strains to tetracycline increased in piglets that were administered this antibiotic. This could be explained by the selection pressure exerted by the administration of tetracycline.

Figure 10 : Evolution of tetracycline-resistant *Escherichia coli* strains





3. Antibiotic susceptibility testing of *E. coli* strains isolated from piglets

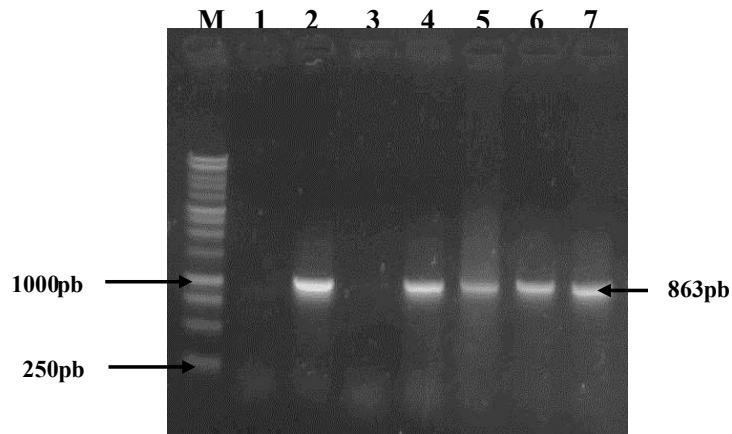
Table 6 : Antibiotic susceptibility test

	BEFORE TREATMENT (%)			AFTER TREATMENT (%)					
	Do			D2			D4		
	TM	CA	TETRA	TM	CA	TETRA	TM	CA	TETRA
AMX	17 ^a	31 ^a	19,3 ^a	31,8 ^b	30,9 ^a	20,9 ^b	31 ^c	29,6 ^a	76,9 ^c
CTX	14 ^a	14,8 ^a	18 ^a	12,4 ^b	5,9 ^b	29 ^b	7 ^c	4 ^c	38 ^c
FOX	17,8 ^a	22,3 ^a	21 ^a	17,8 ^a	18,7 ^b	36,3 ^b	17,4 ^a	15,4 ^c	46,2 ^c
NAL	18 ^a	15,7 ^a	5 ^a	13 ^b	4,8 ^b	5,9 ^a	4 ^c	3,7 ^c	8 ^b
AKM	4 ^a	11 ^a	4,9 ^a	1,9 ^{ab}	8 ^b	5,6 ^b	0 ^b	0 ^c	8 ^b
STP	33 ^a	30 ^a	17 ^a	32,2 ^a	29 ^a	48 ^b	31 ^a	27 ^a	69 ^c
TET	48 ^a	46 ^a	49 ^a	47,1 ^a	44 ^a	62,3 ^b	41 ^b	32 ^b	92,3 ^c
CHL	3,9 ^a	4,4 ^a	2,8 ^a	4 ^a	4 ^a	7,2 ^b	4 ^a	4 ^a	8 ^b
CST	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
IPM									
CIP	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	2 ^b	0 ^a	0 ^a	7,7 ^c

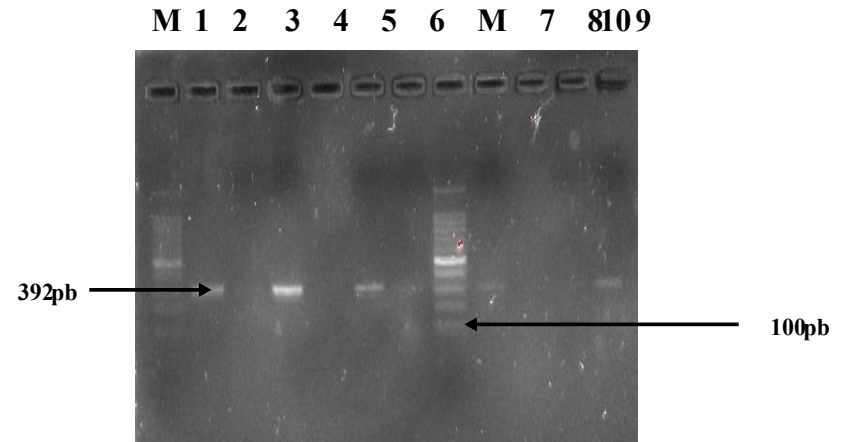




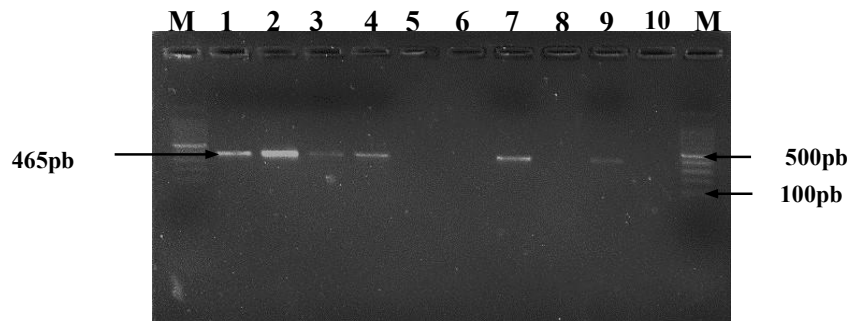
4. Genotypic potential of resistance in *Escherichia coli* strains



CTXM 48 %



SHV 20,7 %



TEM 70,7 %





5. Electrophoretic profiles of virulence genes of *Escherichia coli*

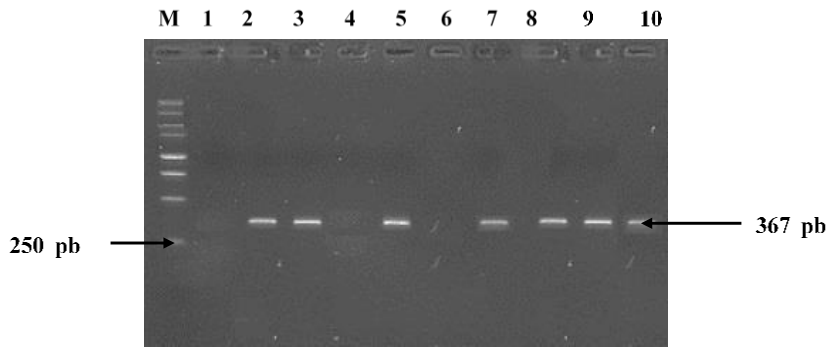


Figure 15 : Electrophoretic profile of the eaeA gene (367 bp)

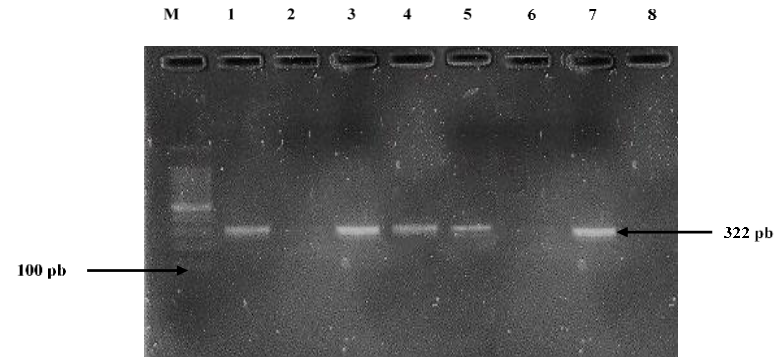


Figure 16 : Electrophoretic profile of the lt gene (322 bp)

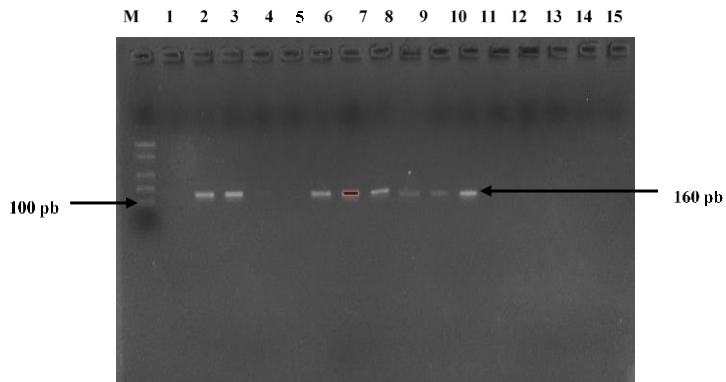


Figure 17 : Electrophoretic profile of the st gene (160 bp)

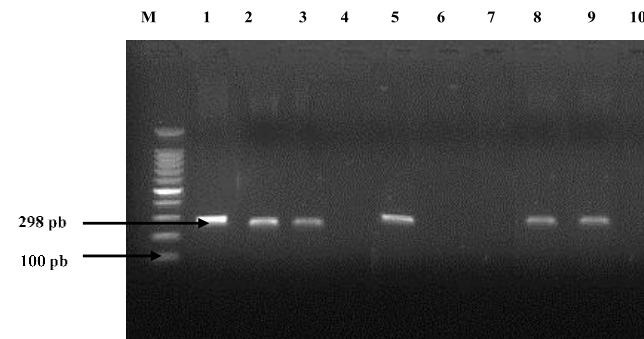


Figure 17 : Electrophoretic profile of the stx2 gene (288 bp)





5. Impact of the dietary supplement on the carriage of virulent *E. coli*

Table 7: Evolution of the carriage of virulent Escherichia coli strains

Genes	Proportion of strains harboring virulence genes(%)					
	Control		Treated with the dietary supplement		Treated with tetracycline	
	Do	D4	Do	D4	Do	D4
<i>eaeA</i>	0 ^a	14,2 ^b	50 ^a	0 ^b	75 ^a	46,6 ^b
<i>stx2</i>	66,6 ^a	85,7 ^b	75 ^a	50 ^b	50 ^a	40 ^b
<i>st</i>	33,3 ^a	71,4 ^b	75 ^a	0 ^b	25 ^a	33,3 ^b
<i>lt</i>	16,6 ^a	42,8 ^b	100 ^a	0 ^b	75 ^a	53,3 ^b

❖ Decrease in prevalence in piglets treated with the algae-based dietary supplement.

Decreasing is due to the good in vivo activity of the dietary supplement.

Good in vivo activity of the dietary supplement.





Conclusions and recommendations

- 👉 Administration of tetracycline leads to the emergence of resistant bacteria in pig farming
- 👉 The use of the dietary supplement promotes better control of antibiotic resistance risks
- 👉 Moreover, the algae-based dietary supplement exhibited antimicrobial activity against virulent *Escherichia coli* strains.
- 👉 This dietary supplement could contribute to the hygienic safety of pork produced in Côte d'Ivoire.





Conclusions and recommendations

- ✓ **Authorities:** Promote One Health, ban preventive use of antibiotics, raise awareness, and fund research on alternatives.
- ✓ **Veterinarians:** Limit and target prescriptions, prioritize alternatives and biosecurity measures.
- ✓ **Producers:** Avoid self-medication, follow prescriptions, and maintain strict hygiene.





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Thank you for your attention!

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