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Antibacterial Activity of Essential Oil of *Aeollanthus pubescens* on Multidrug Resistant Strains of *Salmonella* and *Escherichia coli* Isolated from Laying Hens Farming in Benin

Aguidissou Oscar Nestor[1], Boko Kadoeito Cyrille[1], Sessou Philippe[1,2*], Yovo Mahudro[2], Komagbe S. Gwladys[1], Ayihou Yannick[1], Alitonou Guy Alain[2], Avlessi Felicien[2], Farougou Souaïbou[1], Sohounhloue Koko Codjo Dominique[2]

[1]Unité de Recherche sur les Maladies Transmissibles, Laboratoire de Recherche en Biologie Appliquée, Ecole Polytechnique d'Abomey-Calavi, Université Abomey-Calavi, Cotonou, Bénin
[2]Laboratoire d'Étude et de Recherche en Chimie Appliquée, Ecole Polytechnique d'Abomey-Calavi, Université Abomey-Calavi, Cotonou, Bénin
Email: *philippe.sessou@uac.bj

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Abstract

Infections due to *Escherichia coli* and *Salmonella* are of the major constraints for the laying hen's industry as they cause mortality and serious economic losses. The use of conventional antibiotics to control bacterial has shown limits because it allows multidrug-resistance. The main objective of this study was to assess the antibacterial activity of essential oil from *Aeollanthus pubescens* on multidrug resistant strains of *Salmonella* and *Escherichia coli* isolated from laying hens faming in the department of Atlantique in Benin. Altogether, 11 strains of *Salmonella* and 16 strains of *Escherichia coli* have been isolated from 101 samples of different organs including liver, spleen, lung, feces and yolk according to standardized methods and their biochemical profile using API 20E gallery. Test of sensitivity was carried out on 11 antibiotics of six different families on identified strains in order to determine their resistance profile. A sensitivity test was carried out on multi-drug resistant strains with *Aeollanthus pubescens* essential oil to determine their sensitivity with regard to this essential oil. The results showed that the majority of *Salmonella* strains presented resistance to Tetracyclines (72.7%) and Sulfonamides (63.6%) and all *Escherichia coli* strains are resistant to Sulfonamides (100%) followed by Tetracycline (93.75%) and Ampicillin (75%). *Aeollanthus pubescens* essential oil was active on all the multi-drug resistant strains investigated with Minimal inhibitory concentration varying from 0.41 ± 0 mg/ml to 0.83 ± 0 mg/ml for *Salmonella* and from 0.41 ± 0 mg/ml to 1.66 ± 0 mg/ml for *Esche-*

richia coli ($P < 0.001$). Besides, the oil can get rid of all the strains of *Salmonella* and the multi-drug resistant *Escherichia coli* investigated. Those results provide alternatives to control poultry bacterial pathologies in Republic of Benin. However, disease due to *Escherichia coli* and *Salmonella* must be taken more seriously and study on their resistance to antibiotic must be deepened as well.

Keywords

Laying Hens, *Escherichia coli*, *Salmonella*, Antibioresistance, *Aeollanthus pubescens*

1. Introduction

Poultry farming is very important for food and economy of third world countries and is a booming sector in West Africa. It occupies a prominent place in development and poverty reduction strategies in these countries. In Benin, poultry production continues to increase and there are two types of poultry farming: Traditional poultry farming and commercial poultry farming [1]. The poultry livestock is estimated in 2017 at 19,830,000 traditional poultry and 813,000 improved poultry [2]. This livestock has increased to the present day due to the boom in peri-urban poultry farming and the increase need for animal protein [3]. However, the current production methods, introduced many risk factors considerably and the poultry industry performance is hampered by several health obstacles, among which the avian pathologies of which the dominant ones are: Newcastle disease, Gumboro disease, chronic respiratory disease, coccidiosis, colibacillosis, and salmonellosis [4]. The latter two are the main bacterial pathologies affecting laying hens farming and are one of mortality causes and economic losses in the poultry industry [5]. Faced with these pathologies, the means of the struggle of farmers rely essentially on antibiotic therapy which consists of the use synthetic antibiotics. However, the excessive use of Antibiotics has led to the emergence of multi-resistant strains of *Salmonella* and *Escherichia coli* in poultry farms [6] [7]. This multidrug resistance phenomenon is becoming a public health problem because previous studies indicate that among pathogenic microorganisms most commonly found in food, poultry products (eggs, chicken meat, etc.) include *Salmonella* and *Escherichia coli* strains [8] [9]. Thus, these strains gradually acquired the major antibiotic resistance genes used both in veterinary and human medicine, leaving the prospect of a therapeutic impasse for the most severe infections [10]. As a result, antimicrobial resistance has become a major concern worldwide and it's important to find new molecules who can ensure satisfactory substitution of synthetic antibiotics. Thus, medicinal plants represent a significant source of new drugs, especially since they have fewer side effects [11]. The present study aim is to evaluate the antibacterial activity of the essential oil of *Aeollanthus pubescens* on multi-resistant strains of

Salmonella and *Escherichia coli* isolated in laying hen farming in Department of Atlantique.

2. Material and Methods

2.1. Study Field

This study was conducted from July to December 2018 in the department of Atlantique located in southern Benin. Department of Atlantique is located between 6˚66'0" North Latitude and 2˚22'0" East Longitude and covers an area of 3.233 km^2. It stretches nearly 100 km from the coast to the interior of the country. Limited to the North by Zou, south by the Atlantic Ocean to the west by the Mono and Couffo and east by the department of Ouémé, this department has 8 towns: Ouidah, Abomey, Allada, Kpomassè, Toffo, Tori-bossito, Zè and Sô-Ava (Figure 1).

2.2. Survey Material

As part of the field work, a survey form was developed. It mainly includes information relating to the identification of the farm, its status on the dominant bacterial pathologies and therapeutic practices implemented on farms.

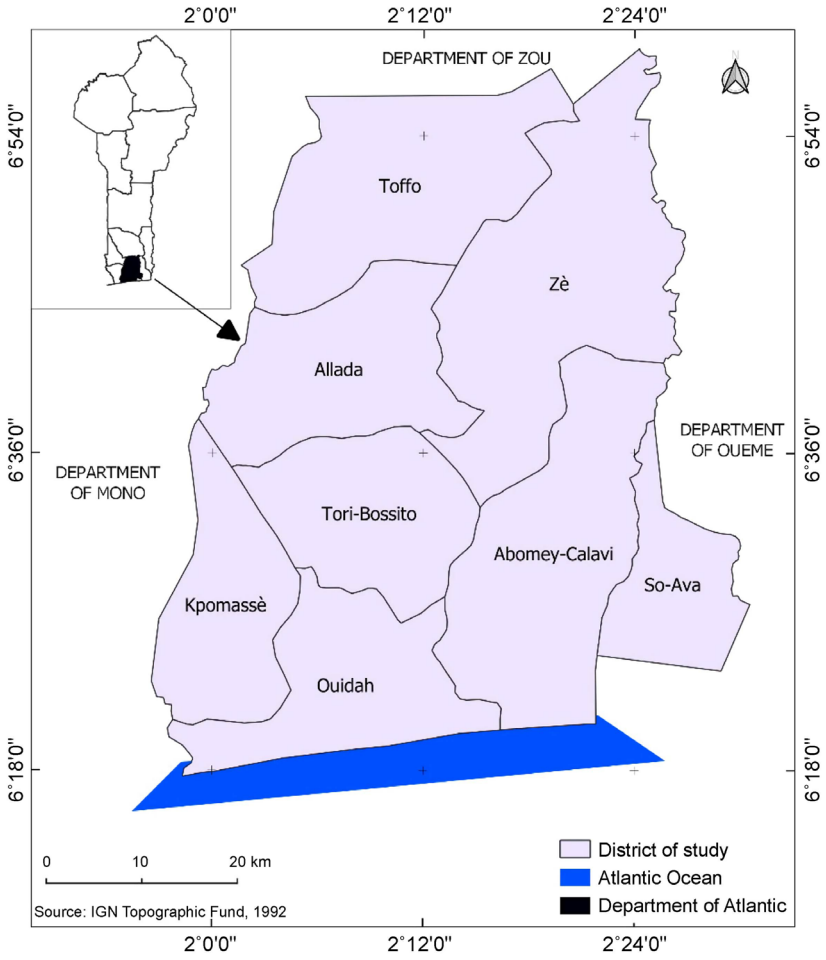

Figure 1. Map of the Atlantique department and of the 8 towns of the study area.

2.2.1. Biological Material

Biological material is made of chicken corps and freshly collected dead chicks on farms of laying hens farming visited.

2.2.2. Essential Oil Used

The essential oil of *Aeollanthus pubescens* used in this study in previously extracted by hydro distillation and analyzed chemically using GC and GC-MS by Alitonou *et al.* [12].

2.3. Methodology

This study was conducted in three phases namely survey, sampling and laboratory.

2.3.1. Survey

A retrospective study was conducted in 26 laying hens farming in the department of Atlantique to identify the dominant bacterial diseases and highlight antibiotics used by farmers.

2.3.2. Sampling

86 corpses freshly dead chickens were collected randomly from 30 poultry farms where mortality cases have been observed and reported during the study period. The collected samples were immediately transported to the laboratory of Research Unit on Communicable Diseases in Benin for bacteriological analysis.

2.3.3. Laboratory Tests

Laboratory analysis consisted firstly to autopsy of corpses collected in order to organ harvesting for the isolation and identification of *Salmonella* and *Escherichia coli* strains and sensitivity test of isolated strains bolt-to-bolt synthetic antibiotics, in order to determine their resistance profile and finally to test the action of *Aeollanthus pubescens* essential oil on antibiotic-resistant strains tested.

1) Autopsy and Sampling

Autopsy was performed on the collected corps (86 dead) in laying hens farming visited by the conventional procedure poultry autopsy. Indeed, it was to the external examination of the corps, skin incisions, open abdominal and thoracic cavities and evisceration. These steps were followed by gross examination itself of tissues and organs in order to detect any lesion changes. Of the 86 corps collected 50 corpses had lesions that can be investigated. Thus, samples such as the liver, spleen, lungs, caeca and yolk were collected from damaged organs and tissues. A total of 101 samples were investigated for the isolation and identification of *Escherichia coli* and *Salmonella* strains.

2) Bacteriological Analysis

The analyses were performed according to standard isolation techniques, identification and antibiotic susceptibility.

Research and identification of *Salmonella*

The *Salmonella* research was carried out in four stages, following the ISO 6579

(2002) standard adapted to our studies.

Pre-Enrichment

One gram (1 g) of each sample was removed aseptically and added to 9 ml of sterile buffered peptone water (EPT). The whole was incubated at 37˚C for 24 hours.

Enrichment

After 24 hours of incubation, 0.1 ml of the pre-enriched was placed in 10 ml of Rappaport-Vassiliadis broth (RV) for selective enrichment and then incubated at 41.5˚C for 24 hours. Also, 1 ml of the medium was added to 10 ml of Muller Kauffman broth with tetrathionate and novobiocin always for selective enrichment.

Isolation

A loopful of the RV-enriched was seeded on XLD (Xylose-Lysine-Desoxicholate) and Hecktoen agar and incubated at 37˚C for 24 hours. Characteristic colonies on XLD agar were removed and subcultured on nutrient agar to obtain pure colonies. Incubation was performed at 37˚C for 24 h.

Identification

Gram stain, catalase and oxidase tests were firstly performed, and the identification of *Salmonella* strains was confirmed using API 20E gallery. In fact, 24-hour colonies obtained on nutritive agar were used to prepare the microbial suspension (a colony in 5 ml of 0.85% NaCl solution). The API 20E plate was then inoculated according to the manufacturer's instructions and incubated at 37˚C for 24 hours. The reading and the interpretation were made by referring to the API 20E analytical catalog and from Abis online software.

Isolation and identification of *Escherichia coli*

For the isolation of *E. coli*, a frank incision samples was made and the contents were then inoculated directly and simultaneously on blood agar and MacConkey agar. All plates were incubated at 37˚C for 24 hours. After 24 hours of incubation, characteristic colonies were transplanted on nutrient agar for pure colonies. The identification has been confirmed by API 20E gallery.

3) Determination of Sensitivity and Resistance Profile of Strains to the Antibiotics

After biochemical identification, all *Escherichia coli* and *Salmonella* isolates identified were selected for carrying out antibiogram to determine their resistance profile. Thus, 11 antibiotics belonging to 6 families were tested: Ampicillin (10 µg), Cefotaxime (30 µg), Cephalotine (30 µg), Cefoxitin (30 µg), Aztreonam (30 µg), Amoxicillin + Clavulanic acid (20/10) µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), Sulfonamide (300 µg) and Tetracycline (30 µg). The method used is agar diffusion on Muller-Hinton agar medium of antibiotic discs according to Clinical and Laboratory Standards Institute [13]. In fact, a colony of 24 hours pure strain was inoculated in 5 ml of Mueller Hinton Broth and incubated for 2 hours to obtain an exponential growth phase suspension (0.5 on the scale McFarland, approximately 1.5×10^8 cells/ml). 0.1 ml of this suspension was inoculated by spreading on the surface of the MHA agar previously poured into Petri dishes. Tested antibiotic disks were deposited on the surface of the inoculated plates using sterile forceps and all plates were incu-

bated at 37˚C for 24 hours. After 24 hours incubation, the inhibition diameters in Table 1 observed were measured using calipers and the values obtained were compared to the standards specified by CLSI [13] and EUCAST [14]. *Escherichia coli* and *Salmonella* strains showing resistance patterns were selected for the sensitivity test to the essential oil of *Aeollanthus pubescens*.

4) Strain Sensitivity Test with Essential Oil of *Aeollanthus pubescens*

Strain susceptibility testing of this essential oil was performed by the disk diffusion method on Muller-Hinton Agar medium according to National Committee for Clinical Laboratory Standards [16] reported by Lakhdar [17] and by Sessou *et al.* [7] Thus, sterile disks of 6 mm diameter impregnated with 5 µl of *Aeollanthus pubescens* essential oil were deposited on the MHA agar previously inoculated with a microbial suspension of approximately 1.5×10^8 cells/ml. All plates were then incubated at 37˚C for 24 hours. After 24 hours incubation, inhibitions diameters observed were measured using a vernier caliper. Indeed, the germ sensitivity is zero for a diameter less than or equal to 8 mm. It is limited to a diameter between 8 and 14 mm, and average diameters between 14 and 20 mm. To a diameter greater than or equal to 20 mm the germ is sensitive [7] [17]. Thus, all the strains for which an inhibition diameter greater than or equal to 14 mm was observed were retained for the determination of MIC, CMB and antibiotical power.

5) Determination of Minimum Inhibitory Concentration (MIC), Minimal Bactericidal Concentration (CMB) and the Antibiotic Power of *Aeollanthus pubescens* Essential Oil against Strains Investigated

MIC, CMB and antibiotic power (PA) of *Aeollathus pubescens* essential oil has been made following the technique of dilution in liquid medium on microplate

Table 1. Normative values of inhibition diameters [13] [14] [15].

	Salmonella			*Escherichia coli*		
Antibiotics	Sensible	Intermediate	Resistant	Sensible	Intermediate	Resistant
	(S)	(I)	(R)	(S)	(I)	(R)
AMC 10 µg	≥18	14 - 17	≤13	≥18	14 - 17	≤13
AMP 10 µg	≥17	14 - 16	≤13	≥17	14 - 16	≤13
ATM 30 µg	≥21	18 - 20	≤17	≥21	18 - 20	≤17
CTX 30 µg*	≥26	23 - 25	≤22	≥26	23 - 25	≤22
FOX 30 µg	≥18	15 - 17	≤14	≥18	15 - 17	≤14
CEF 30 µg	≥18	15 - 17	≤14	≥18	15 - 17	≤14
CHL 30 µg	≥18	13 - 17	≤12	≥18	13 - 17	≤12
CIP 5 µg	≥31	21 - 30	≤20	≥21	16 - 20	≤15
GMN 10 µg	≥15	13 - 14	≤12	≥15	13 - 14	≤12
TET 30 µg	≥15	12 - 14	≤11	≥15	12 - 14	≤11
SSS 300 µg	≥17	13 - 16	≤12	≥17	13 - 16	≤12

AMP: ampicillin; AMC: Amoxicillin + clavulanic acid; ATM: Aztreonam; CTX: Cefotaxime; FOX: Cefoxitine; CEF: Cephalotin; GMN: Gentamicin; TET: Tetracycline; SSS: Sulfonamide.

96 wells coupled to the plating on solid medium as described by Chabert *et al.* [18] and reported by Sessou *et al.* [7] For this, a solution was obtained by mixing 40 µl of the essential oil with 2 ml of Mueller Hinton broth (MHB) and a drop of Tween 80 to emulsify the mixture. Then, 100 µl of the Mueller Hinton broth (MHB) were distributed into the wells of a microplate and 100 µl of the oil extract (suspension prepared from 40 µl of the pure essential oil diluted in 2000 µl of MHB) were added to each of the wells in the first column. Successive dilutions of reason 2, wells by well until to the last well in each row were made and 100 µl of the last well were discarded. All the wells were inoculated except those serving as a negative control with 100 µl of the bacterial suspension at 10^6 germs/ml (density equal to the scale 2 of MCFarland). The negative control consisted of the oil-based Mueller Hinton broth and the positive control consisted of inoculum and Muller Hinton broth. The microplates were covered with parafilm and incubated at 37˚C for approximately 24 hours. On reading, the well corresponding to the lowest concentration of essential oil extract for which we did not observe turbidity or visible growth with the naked eye was taken as the minimum inhibitory concentration (MIC) oil on the strains tested. The CMB, which is the lowest concentration for which we do not have germs growth, was determined following the MIC by dissolving dilutions with a concentration greater than or equal to the MIC on the Mueller Hinton Agar medium. poured into sterile petri dishes and the antibiotic power (pa) of the oil was calculated by the formula: p.a = CMB/CMI. When p.a is less than 4, it is said that the oil tested has an antibiotical power.

2.3.4. Statistical Analysis

The data were coded and stored in the database designed Excel software. For data relating to the investigation, frequencies and confidence intervals were calculated with respect to the status of the dominant bacterial diseases and therapeutic practices. Then, these frequencies were compared with each other by the bilateral Z test (Test Z). For the results of microbiological analysis, descriptive analysis was made, and the means and standard deviations were calculated. These averages were compared with each other by an ANOVA one way analysis. In addition, the Turkey test was conducted for structuring averages. All analysis were performed with R3.4.4. Software

3. Results and Discussion

3.1. Results

3.1.1. Farms Status on Dominant Bacterial Pathologies and Therapeutic Practices

The counting of the 26 survey sheets made it possible to gather various information from poultry farmers in department of Atlantique, particularly in town of Abomey-Calavi, Allada, Ouidah, Tori-bossito and Zè (**Table 2**). At the end of this survey, all the farms visited are private. The dominant bacterial diseases commonly encountered by its farmers were Chronic Respiratory Disease (46.14%) followed by Salmonellosis (38.46%) and Colibacillosis (15.37%). All farmers

Table 2. Farms status on dominant bacterial pathologies and therapeutic practices.

Variable		Frequency (%)	IC	Test de Z
Dominant bacterial diseases	Salmonellosis	38.46 ab	18.70	*
	Colibacillosis	15.37 a	13.87	*
	MRC	46.14 b	19.16	*
Medical Prophylaxis Plan	Yes	100 a	0	***
	No	0 b	0	***
Prophylaxis application	Yes	76.92 a	16.20	***
	No	23.08 b	16.20	***
Reason for non-compliance with prophylaxis	Financial constraint	83.33 a	32.67	NS
	Other	16.67 a	32.67	NS
Salmonellosis Vaccination	Yes	57.69 a	18.99	NS
	No	42.31 a	18.99	NS
Colibacillosis vaccination	Yes	19.23 a	15.15	***
	No	80.77 b	15.15	***
Clinical Diagnosis	Veterinary	30.76 ab	17.74	**
	Poultry farmer	11.55 a	12.28	**
	Technician	57.69 b	18.99	**
Clinical Diagnosis confirmation	Autopsy	53.8 b	19.16	***
	Laboratory Analysis and Autopsy	23.07 c	16.20	***
	Other	23.07 c	16.20	***
Prescription	Veterinary	30.76 a	17.74	NS
	Poultry Advisor	30.76 a	17.74	NS
	Technician	23.07 a	16.20	NS
	Veterinary; Technician	11.54 a	12.28	NS
	Other	3.84 a	7.39	NS
Mode of antibiotic use	Preventive	3.84 a	7.39	***
	Curative	76.92 b	16.20	***
	Preventive and Curative	19.24 a	15.15	***
frequency of antibiotic use	Each month	15.4 a	13.87	***
	Presence of mortality	84.6 b	13.87	***
Obtaining the expected result	Yes	26.93 a	17.05	***
	No	3.84 a	7.39	***
	Sometimes	69.23b	17.74	***

NS: not significant ($P > 0.05$); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

(100%) have a prophylaxis plan, but 76.92% strictly respect it against 23.08% with a significant difference ($p < 0.05$). In addition, 57.69% of farmers vaccinate against salmonellosis and 42.31% do not. For vaccination against colibacillosis,

only 19.23% of farmers do it. In case of pathology, only 23.07% of farmers confirm their diagnosis by autopsy and laboratory analysis against 53.8% who confirm their diagnosis by only performing the autopsy. Regarding drugs, the most used molecules by these farmers were: oxytetracycline, colistin, enrofloxacin, tylosin, norfloxacin and the sulfadiazine-trimethoprim combination. Most of these farmers use these curative antibiotics (76.92%) and often obtain the expected result (69.23%) with a significant difference ($p < 0.05$).

3.1.2. Bacteriological Analysis

After the bacteriological analysis of 101 samples, 11 *Salmonella* strains or 10.89% and 16 *Escherichia coli* strains or 15.84% were identified (Table 3).

3.1.3. Sensitivity and Antibiotic Resistance Profile of Strains Identified

Figure 2 and Figure 3 shows respectively the sensitivity and resistance profile

Table 3. Frequencies of *Salmonella* and *Escherichia coli* strains identified in samples taken.

		Identified strains			
		Escherichia coli		*Salmonella*	
Samples	Number collected	Number of isolates	Frequency (%)	Number of isolates	Frequency (%)
Liver	34	7	20.59	4	11.76
Caeca	25	0	0	7	28
Spleen	12	3	25	0	0
Lung	20	4	20	0	0
Yolk	10	2	20	0	0
Total	101	16	15.84	11	10.89

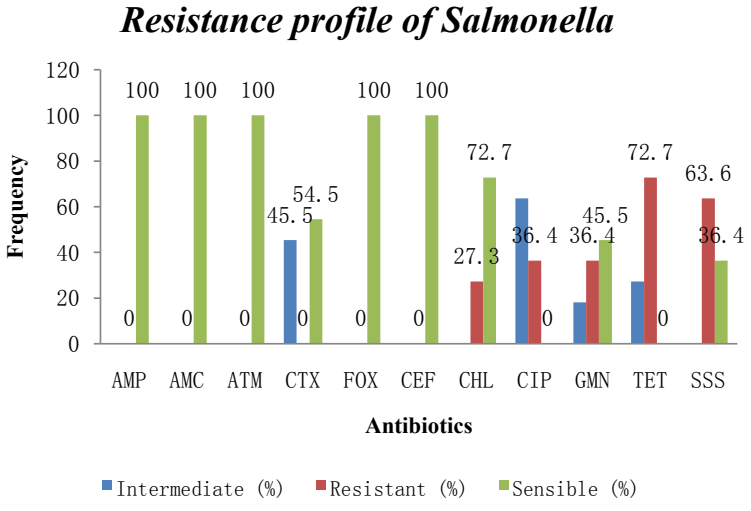

Figure 2. Sensitivity and antibiotic resistance profile of *Salmonella* strains identified. AMP: ampicillin; AMC: Amoxicillin + clavulanic acid; ATM: Aztreonam; CTX: Cefotaxime; FOX: Cefoxitine; CEF: Cephalotin; GMN: Gentamicin; TET: Tetracycline; SSS: Sulfonamide.

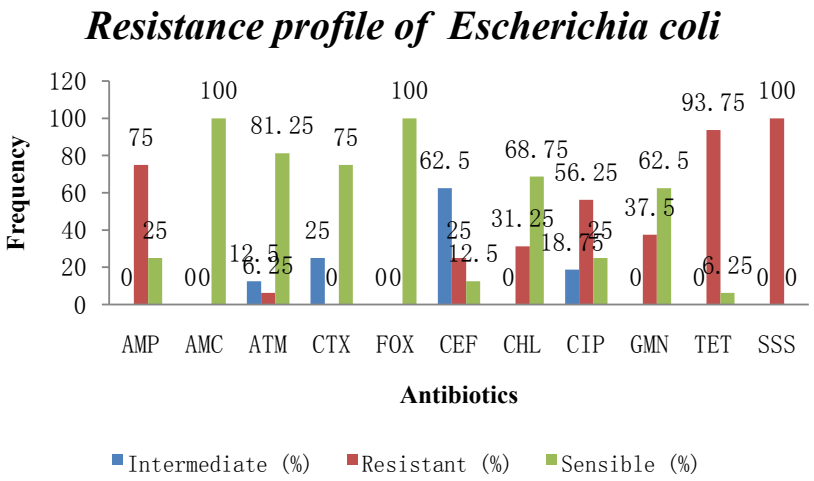

Figure 3. Sensitivity and antibiotic resistance profile of *Escherichia coli* strains identified. AMP: ampicillin; AMC: Amoxicillin + clavulanic acid; ATM: Aztreonam; CTX: Cefotaxime; FOX: Cefoxitine; CEF: Cephalotin; GMN: Gentamicin; TET: Tetracycline; SSS: Sulfonamide.

of *Salmonella* and *Escherichia coli* strains to the antibiotics tested. All *Salmonella* strains are more sensitive to Ampicillin (100%), Amoxicillin (100%), Aztreonam (100%), Cefoxitin (100%) and Cephalotin (100%); however, these strains resist the action of Tetracycline (72.7%) and Sulfonamides (63.6%). *Escherichia coli* strains are particularly sensitive to Amoxicillin (100%), Cefoxitin (100%), Aztreonam (81.25%), Cefotaxime (75%) and are more resistant to Sulfonamides (100%) followed by Tetracycline (93.75%) and Ampicillin (75%). It appears that the two categories of identified strains have developed more resistance to tetracycline and Sulfonamides belonging respectively to the family of Tetracyclines and sulfonamide.

Furthermore, Table 4 and Table 5 show respectively the average inhibition diameter of strains identified bolt-to-bolt antibiotics tested. The analysis of these tables shows that, in both *Salmonella* and *Escherichia coli* strains, the sensitivity and resistance profiles for the different antibiotics vary from one strain to another. It also appears from these tables that of the 11 strains of *Salmonella* identified, five (SB1, SB2, SC1, SC2, SC3) exhibited at least one pattern of resistance to three antibiotics of different families (Table 4). With regard to the *Escherichia coli* strains, almost all (EF1, EF2, EG1, EH1, EH2, EI1, EI2, EJ, EK, EL1, EL2, EL3, EL4, EM1, EM2) presented a resistance profile to at least three antibiotics of different families (Table 5).

3.1.4. Sensitivity of Strains to the Essential Oil of *Aeollanthus pubescens*

The sensitivity test to the essential oil of *Aeollanthus pubescens* considered only the *Salmonella* and *Escherichia coli* strains qualified as multidrug-resistant. Thus, the evaluation of the sensitivity of these different strains to the essential oil revealed that the strains were sensitive to this oil. Table 6 and Table 7 respectively show the diameters of inhibition of *Salmonella* and *Escherichia coli* multiresistants strains in contact with the essential oil of *Aeollanthus pubescens*. The

Table 4. Diameters of inhibition of *Salmonella* strains identified to the antibiotics tested.

	AMP	AMC	ATM	CTX	FOX	CEF	CHL	CIP	GMN	TET	SSS
SA	21.5 ± 0.707 a2	**25 ± 0** a3	28.5 ± 0.707 a2	25.5 ± 0.707 b4	21 ± 1.41 a1	21 ± 0 a1	**6 ± 0** b1	21.5 ± 0.707 b2	13 ± 0 b3	13 ± 0 b2	17.5 ± 0.707 a1
SB1	22.5 ± 0.707 a1	**23.5 ± 0.707** a2	27.5 ± 0.707 a2	25 ± 0 b3	20.5 ± 0.707 a1	21 ± 0 a1	**6 ± 0** b1	21.5 ± 0.707 b2	10 ± 0 b2	6 ± 0 b1	19 ± 0 a1
SB2	18.5 ± 0.707 a1	**23.5 ± 0.707** a3	25.5 ± 0.707 a2	25.5 ± 0.707 b3	19.5 ± 0.707 a1	21 ± 0 a1	9.5 ± 0.707 b2	20 ± 0 b3	14 ± 0 b3	**6 ± 0** b1	17.5 ± 0.707 a1
SC1	21.5 ± 0.707 a2	23.5 ± 0.707 a3	29 ± 0 a3	27 ± 0 a1	23 ± 0 a2	23 ± 0 a2	**28.5 ± 0.707** a4	11.5 ± 0.707 b2	10 ± 0 b3	**6 ± 0** b1	6 ± 0 b2
SC2	19.5 ± 0.707 a1	21.5 ± 0.707 a2	27.5 ± 0.707 a2	27.5 ± 0.707 a1	23 ± 0 a2	23 ± 1.41 a2	**26 ± 0** a3	10.5 ± 0.707 b1	11 ± 0 b2	**6 ± 0** b1	6 ± 0 b1
SC3	21.5 ± 0.707 a2	22.5 ± 0.707 a3	28 ± 0 a3	27.5 ± 0.707 a1	22.5 ± 0.707 a3	25 ± 0 a3	**28 ± 0** a4	11 ± 0 b3	11 ± 0 b4	**6 ± 0** b1	6 ± 0 b2
SD1	22 ± 0 a1	22 ± 0 a1	26.5 ± 0.707 a1	25 ± 0 b4	21 ± 0 a1	23 ± 0 a1	**26.5 ± 0.707** a2	21 ± 0 b3	18 ± 0 a1	**6 ± 0** b1	6 ± 0 b2
SD2	21.5 ± 0.707 a1	23 ± 0 a2	27 ± 0 a2	24.5 ± 0.707 b3	20.5 ± 0.707 a1	22.5 ± 0.707 a1	**25.5 ± 0.707** a2	20.5 ± 0.707 b2	18.5 ± 0.707 a1	**6 ± 0** b1	6 ± 0 b1
SE1	19.5 ± 0707 a1	21.5 ± 0.707 a2	27.5 ± 0.707 a2	26.5 ± 0.707 a1	23 ± 0 a2	24.5 ± 0.707 a2	26.5 ± 0.707 a3	28.5 ± 0.707 b1	18 ± 0 a2	**15.5 ± 0.707** b1	**24 ± 1.41** a3
SE2	20 ± 0 a1	21.5 ± 0.707 a2	28.5 ± 0.707 a2	29 ± 0 a2	23.5 ± 0.707 a2	25 ± 1.41 a2	**26.5 ± 0.707** a3	30 ± 0 b3	19 ± 0 a2	13 ± 0 b2	**6 ± 0** b1
SE3	23.5 ± 0.707 a2	25.5 ± 0.707 a4	31.5 ± 0.707 a4	30.5 ± 0.707 a1	23 ± 0 a2	26 ± 0 a3	**29 ± 0** a4	23.5 ± 0.707 b2	19.5 ± 0.707 a2	**6 ± 0** b1	6 ± 0 b1

The averages of the same line followed by different letters are significantly different at the 5% threshold. The letter "a" indicates the sensitivity and "b" the resistance or the intermediate profile. (a1 to a5) indicate the action of antibiotics (less sensitive to more sensitive) and b1 to b5 indicate action (more resistant to less resistant).

Table 5. Diameters of inhibition of *Escherichia coli* strains identified to the antibiotics tested.

	AMP	AMC	ATM	CTX	FOX	CEF	CHL	CIP	GMN	TET	SSS
EF1	**6 ± 0 b1**	22.5 ± 0.707 a2	28 ± 0 a2	26.5 ± 0.707 a1	18.5 ± 0.707 a1	16.5 ± 0.707 b2	**26 ± 0 a2**	20.5 ± 0.707 b2	20.5 ± 0.707 a2	6 ± 0 b1	6 ± 0 b1
EF2	**6 ± 0 b1**	22.5 ± 0.707 a2	28 ± 0 a2	30 ± 0 a1	22.5 ± 0.707 a2	16.5 ± 0.707 b2	**25 ± 0 a2**	21 ± 0 a1	19.5 ± 0.707 a2	6 ± 0 b2	6 ± 0 b2
EG1	**6 ± 0** b1	20 ± 0 a2	25 ± 0 a2	30 ± 0 a2	23 ± 0 a2	19.5 ± 0.707 a2	**29.5 ± 0.707** a3	21.5 ± 0.707 a1	18.5 ± 0.707 a2	6 ± 0 b2	6 ± 0 b3
EG2	19 ± 0 a1	20 ± 0 a2	**6 ± 0** b1	28 ± 0 a2	23.5 ± 0.707 a3	19.5 ± 0.707 a2	**26 ± 0** a4	18.5 ± 0.707 b3	20 ± 0 a3	18 ± 0 a1	6 ± 0 b2
EH1	18 ± 0 a1	**22.5 ± 0.707** a3	24 ± 0 a2	26.5 ± 0.707 a1	21.5 ± 0.707 a2	16.5 ± 0.707 b3	23.5 ± 0.707 a3	**6 ± 0** b1	12 ± 0 b3	6 ± 0 b2	6 ± 0 b2
EH2	19 ± 0 a1	20 ± 0 a2	24.5 ± 0.707 a2	28.5 ± 0.707 a2	22.5 ± 0.707 a2	14 ± 0 b4	**26 ± 0** a3	**6 ± 0** b1	6 ± 0 b2	6 ± 0 b2	6 ± 0 b3
EI1	6 ± 0 b2	20 ± 0 a2	**26.5 ± 0.707** a2	27.5 ± 0.707 a1	20 ± 0 a2	15 ± 0 b5	6 ± 0 b3	**6 ± 0** b1	6 ± 0 b3	6 ± 0 b3	6 ± 0 b4
EI2	**6 ± 0** b1	**22.5 ± 0.707** a2	20.5 ± 0.707 b3	25.5 ± 0.707 b4	20.5 ± 0.707 a1	13 ± 0 b3	22.5 ± 0.707 a2	14 ± 0 b3	11 ± 0 b3	6 ± 0 b1	6 ± 0 b2
EJ	6 ± 0 b2	**22.5 ± 0.707** a3	25 ± 0 a2	25.5 ± 0.707 b4	23 ± 0 a3	17 ± 0 b4	6 ± 0 b2	**6 ± 0** b1	15 ± 0 a1	6 ± 0 b2	6 ± 0 b3
EK	6 ± 0 b2	20 ± 0 a2	**28 ± 0** a3	26.5 ± 0.707 a1	21.5 ± 0.707 a2	17.5 ± 0.707 b4	6 ± 0 b2	**6 ± 0** b1	6 ± 0 b2	6 ± 0 b2	6 ± 0 b3
EL1	**6 ± 0** b1	**22.5 ± 0.707** a2	22.5 ± 0.707 a1	28 ± 0 a1	23 ± 0 a2	14.5 ± 0.707 b2	6 ± 0.707 b1	22.5 ± 0.707 a2	18.5 ± 0.707 a2	6 ± 0 b1	6 ± 0 b1
EL2	6 ± 0 b1	20 ± 0 a1	25 ± 0 a1	29.5 ± 0.707 a1	21.5 ± 0.707 a1	16.5 ± 0.707 b2	**25 ± 0** a2	**6 ± 0** b1	6 ± 0 b1	6 ± 0 b1	6 ± 0 b1
EL3	**6 ± 0** b1	22.5 ± 0.707 a2	26 ± 0 a2	28.5 ± 0.707 a1	21.5 ± 0.707 a1	16 ± 0 b4	**24 ± 0** a2	15 ± 0 b4	17 ± 0 a1	6 ± 0 b2	6 ± 0 b3
EL4	20 ± 0 a1	20 ± 0 a1	26 ± 0 a1	30 ± 0 a1	21 ± 0 a1	15 ± 0 b4	**29 ± 0** a2	**6 ± 0** b1	18.5 ± 0.707 a1	6 ± 0 b2	6 ± 0 b3
EM1	**6 ± 0** b1	**22.5 ± 0.707** a2	21.5 ± 0.707 a1	25 ± 0 b3	23.5 ± 0.707 a2	11 ± 1.41 b2	6 ± 0 b1	26.5 ± 0.707 a2	15 ± 0 a1	6 ± 0 b1	6 ± 0 b1
EM2	**6 ± 0** b1	20 ± 0 a1	18 ± 0 b3	24.5 ± 0.707 b3	21.5 ± 0.707 a1	16 ± 0 b3	**25.5 ± 0.707** a2	20.5 ± 0.707 b4	16.5 ± 0.707 a1	6 ± 0 b1	6 ± 0 b2

The averages of the same line followed by different letters are significantly different at the 5% threshold. The letter "a" indicates the sensitivity and "b" the resistance or the intermediate profile. (a1 to a5) indicate the action of antibiotics (less sensitive to more sensitive) and b1 to b5 indicate action (more resistant to less resistant).

Table 6. Diameters of inhibition of *Salmonella* multiresistants strains bolt-to-bolt essential oil of *A. pubescens.*

A. Pubescens	SB1	SB2	SC1	SC2	SC3	Significance Test
	15 ± 0 mm a	22.5 ± 0.7 mm b	25.5 ± 0.7 mm c	24.5 ± 0.7 mm bc	19 ± 0 mm d	***

***: P < 0.001; the average of the same line followed by different letters, differ significantly at the threshold of 10/00.

Table 7. Diameters of inhibition of *Escherichia coli* multiresistants strains bolt-to-bolt essential oil of *A. pubescens.*

A. Pubescens	EF1	EF2	EG1	EH1	EH2	EI1	EI2	EJ	EK	EL1	EL2	EL3	EL4	EM1	EM2	Test
	16 ± 0 a	19.5 ± 0.7 b	32 ± 0 c	27.5 ± 0.7 d	30 ± 0 e	20 ± 0 b	20 ± 0 b	22.5 ± 0.7f	23.5 ± 0.7 f	23 ± 0 f	22.5 ± 0 f	29 ± 0 de	34.5 ± 0.7 g	22 ± 0 f	16.5 ± 0.7 a	***

***: P < 0.001; the average of the same line followed by different letters, differ significantly at the threshold of 10/00.

analysis of these tables shows that the essential oil of *A. pubescens* had an important activity on all the strains investigated. In fact, at 5 µl of the essential oil, the *Salmonella* strains (SB2, SC1 and SC2) showed great sensitivity with respective inhibition diameters of 22.5 ± 0.70 mm, 25.5 ± 0.70 mm. and 24.5 ± 0.70 mm while strains (SB1 and SC3) exhibited average sensitivity with respective inhibition diameters of 15 ± 0 mm and 19 ± 0 mm (Table 6). As for the *Escherichia coli* strains, with 5 µl of the essential oil, strains (EG1, EH1, EH2, EI1, EI2, EJ, EK, EL1, EL2, EL3, EL4 and EM1 also presented a high sensitivity to oil with respective inhibition diameters of 32 ± 00 mm, 27.5 ± 0.70 mm, 30 ± 00 mm, 20 ± 00 mm, 20 ± 00 mm, 22.5 ± 0.70 mm, 23.5 ± 0.70 mm, 23 ± 00 mm, 22.5 ± 00 mm, 29 ± 00 mm, 34.5 ± 0.70 mm and 22 ± 00 mm and only the strains (EF1, EF2 and EM2) showed average sensitivity with respective inhibition diameters of 16 ± These results show that, despite the resistance of these different strains to antibiotics, they showed a sensitivity towards the *Aeollanthus pubescens* essential oil with a significant difference (p < 0.001).

3.1.5. Minimal Inhibitory Concentration (MIC), Bactericidal Concentration (CMB) and Antibiotic Power of *A. pubescens* Essential Oil

The determination of minimal inhibitory and bactericidal concentrations allowed us not only to confirm the activity of *A. pubescens* oil, but also to evaluate its antibiotic power on the different strains investigated. The results of the MIC, MBC and the antibiotic power of this essential oil on the *Salmonella* and *Escherichia coli* strains are presented respectively in Tables 8-13. Upon reading these results, we note that, the MIC of oil on *Salmonella* strains range from 0.41 mg/ml to 0.83 mg/ml with a non-significant difference between strains. For *Escherichia coli* strains, MIC ranged from 0.41 mg/ml to 1.66 mg/ml with a significant difference between strains (P < 0.001). It appears that the lowest concentrations of MIC are those obtained on *Salmonella* strains (Table 8 and Table 9). With regard to CMB, the same observations were made where the lowest concentrations are those obtained on *Salmonella* strains and range from 0.83 mg/ml to 1.66 mg/ml against 0.83 mg/ml to 5 mg/ml on *Escherichia coli* strains. There is no significant difference between the strains. (Table 10 and Table 11). With respect to the antibiotic power of *A. pubescens* oil, the calculation of the

Table 8. Minimal Inhibitory Concentration (MIC) of *A. pubescens* essential oil against *Salmonella* multiresistants strains.

	CMI (mg/ml)					
A. pubescens	**SB1**	**SB2**	**SC1**	**SC2**	**SC3**	**Significance Test**
	0.41 ± 0 a	0.83 ± 0 a	0.41 ± 0 a	0.62 ± 0.21 a	0.41 ± 0 a	NS

Table 9. Minimal Inhibitory Concentration (MIC) of *A. pubescens* essential oil against *Escherichia coli* multiresistants strains.

	CMI (mg/ml)															
A. pubescens	**EF1**	**EF2**	**EG1**	**EH1**	**EH2**	**EI1**	**EI2**	**EJ**	**EK**	**EL1**	**EL2**	**EL3**	**EL4**	**EM1**	**EM2**	**Test**
	1.66 ± 0 a	0.83 ± 0 b	0.41 ± 0 b	0.41 ± 0 b	0.41 ± 0 b	1.245 ± 0.5 ab	0.41 ± 0 b	0.83 ± 0 b	0.41 ± 0 b	0.83 ± 0 b	0.41 ± 0 b	0.41 ± 0 b	0.62 ± 0.21 b	1.66 ± 0 a	1.66 ± 0 a	***

***: P < 0.001; The averages of the same line followed by different letters, differ significantly at the threshold of 10/00.

Table 10. Minimal Bactericidal Concentration (CMB) of *A. pubescens* essential oil against *Salmonella* multidrug-resistant strains.

	CMB (mg/ml)					
A. Pubescens	**SB1**	**SB2**	**SC1**	**SC2**	**SC3**	**Significance Test**
	0.83 ± 0 a	1.66 ± 0 a	0.83 ± 0 a	1.25 ± 0.58 a	0.83 ± 0 a	**NS**

Table 11. Minimal Bactericidal Concentrations (CMB) of *A. pubescens* essential oil against multidrug resistant strains of *Escherichia coli.*

	CMB (mg/ml)															
A. Pubescens	**EF1**	**EF2**	**EG1**	**EH1**	**EH2**	**EI1**	**EI2**	**EJ**	**EK**	**EL1**	**EL2**	**EL3**	**EL4**	**EM1**	**EM2**	**Test**
	5 ± 2.35 a	1.66 ± 0 b	0.83 ± 0 b	0.83 ± 0 b	0.83 ± 0 b	2.5 ± 1.18 ab	0.83 ± 0 b	1.66 ± 0 b	0.83 ± 0 b	1.66 ± 0 b	0.83 ± 0 b	0.83 ± 0 b	1.25 ± 0.59 b	3.33 ± 0 a	0.83 ± 0 b	***

***: P<0.001; The averages of the same line followed by different letters, differ significantly at the threshold of 10/00.

Table 12. Antibiotic power of *A. pubescens* essential oil against *Salmonella* multidrug-resistant strains.

A. Pubescens	**SB1**	**SB2**	**SC1**	**SC2**	**SC3**	**Antibiotic Power**
	2.02	2	2.02	2.01	2.02	**Bactericide**

Table 13. Antibiotic power of *A. pubescens* essential oil against *Escherichia coli* multidrug-resistant strains.

A. Pubescens	**EF1**	**EF2**	**EG1**	**EH1**	**EH2**	**EI1**	**EI2**	**EJ**	**EK**	**EL1**	**EL2**	**EL3**	**EL4**	**EM1**	**EM2**	**Antibiotic Power**
	3.01	2	2.02	2.02	2.02	1.99	2.02	2	2.02	2	2.02	2.02	2.01	2	2.02	**Bactericide**

CMB/MIC ratio revealed that it has a bactericidal effect on *Salmonella* and *Escherichia coli* strains (Table 12 and Table 13). Indeed, according to Joubert *et al.* [19] reported by Yovo [20], when the CMB/MIC ratio is less than or equal to 4, the extract is described as bactericidal and when it is greater than 4, the extract is said to be bacteriostatic. In view of these different results, we can say that the essential oil of *Aeollanthus pubescens* has a great antibiotic power that deserves to be valued.

3.2. Discussion

The survey carried out in some towns in Department of Atlantique allowed us to identify some information on the pathological status of the various farms visited, particularly on the therapeutic practice based on antibiotics using. The choice of the study area is justified by the fact that this department has a favorable climate for poultry farming. In addition, this department has several peri-urban areas with a significant concentration of poultry farming and we meet different categories of livestock. Of the 26 farms visited, the dominant bacterial diseases commonly encountered by her farmers were Chronic Respiratory Disease followed by salmonellosis and colibacillosis. The same observation was made by Sid Nassim *et al.* [21], in Algeria who took stock of avian pathologies in some poultry farms. The frequency of occurrence of these pathologies in poultry farming could be explained by the health practices of farmers on these farms based on the non-compliance with hygienic and sanitary rules. According to the study by Boko *et al.* [3] on poultry farming practices in southern Benin, the manure storage location is often in the open air and is not always far enough from poultry houses or it can be a contamination source by air. This study also reported that most farmers use a lot of antibiotics, which could promote the resistance of the various germs responsible for these diseases, hence their persistence in these farms. Regarding antibiotics, the most molecules used by these farmers are: oxytetracycline, colistin, enrofloxacin, tylosin, norfloxacin and the sulfadiazine-trimethoprim combination. The same molecules were identified by Fofana [22] in a survey of 23 broiler farming in Senegal.

For bacteriological analysis, samples were taken from the liver (n = 34), lungs (n = 20), caeca (n = 25), spleen (n = 12) and yolk (n = 10) from cadavers of chickens and chicks autopsied with lesions such as generalized congestion, congestion and hypertrophy, hypertrophy and inflammation. In total, 11 *Salmonella* strains and 16 *Escherichia coli* strains were identified in these different samples. *Escherichia coli* outside the caeca was isolated from the liver (n = 07), spleen (n = 03), lung (n = 04) and yolk (n = 02) and *Salmonella* was isolated from the liver (n = 04) and caeca (n = 07). Our results outside the *Salmonella* strains are close to those obtained by Rahmatallah *et al.* [6], who also isolated and identified *Escherichia coli* strains in the liver, spleen, lungs, yolk sac, but also in the bone marrow and heart on chickens and chicks in Morocco. Al Hassane [23], in Senegal, in his study on "chicken-flesh colibacillosis: anatomy-clinical study and circumstances of emergence in the peri-urban area of Dakar" also isolated and identified these *Escherichia coli* strains in the liver, spleen but also in the heart and intestine. Our study did not consider the removal from the heart and intestines although these strains can be isolated from its places in case of infection with *Escherichia coli* and *Salmonella*, can be explained by the absence of characteristic lesions may attract our attention for possible samples. Moreover, the presence of these different strains in the elements collected already poses a problem and could be explained by a superinfection of the subjects by these

germs probably at the origin of their mortality. It should also be noted that most autopsies performed revealed cases of Chronic Respiratory Diseases, Newcastle Disease and Bursal Disease. These pathologies were suspected from macroscopic lesions such as: the presence of petechiae at the level of the proventricle and under the gizzard cuticle for Newcastle disease, dehydration of carcasses and hypertrophy of the Fabricius bursa for suspicions of the Bursal disease and aerosacculitis, tracheitis, pericarditis congestion of the lungs for suspicion of Chronic Respiratory Disease. Indeed, in his manual entitled "Diseases of poultry" Didier Villate [24], veterinarian, says that infections with *Escherichia coli* can follow Chronic Respiratory Disease by complicating it most often. In addition, in case of viral diseases, the clinical and lesion is often complicated by bacterial superinfections including *Escherichia coli* and *Salmonella* generally considered as secondary pathogens. With regard to the resistance profile of these different strains, overall, the recorded resistance rates showed high levels for tetracyclines and sulfonamides. This high resistance could be explained by the excessive and unreasoned use of these molecules in the treatment of avian diseases [25]. In addition, the survey carried out in this study revealed that the most used molecules by these farmers are: oxytetracycline, colistin, enrofloxacin, tylosin, norfloxacin and the sulfadiazine-trimethoprim combination and most of these farmers use these curative antibiotics (76.92%). Most of these drugs administered belong to families of antibiotics with therapeutic representatives, such as Tetracyclines, Sulfonamides. These results could also be explained by the easiest accessibility of these molecules because of their low cost. Although bacterial resistance to oxytetracycline is high in several countries [6] [22] [23], almost all resistance of *Escherichia coli* and *Salmonella* strains isolated in this study could be of concern since this antibiotic would be of no therapeutic use against colibacillosis and salmonellosis and most probably against other avian diseases. The resistance of isolated *Escherichia coli* strains in our study, to ampicillin (75%) which is also used in the treatment of various bacterial infections in humans raises public health issue because many of these strains infect humans via avian products including chicken meat and eggs [8] [9]. However, *Salmonella* strains remained sensitive to the action of ampicillin (100%), amoxicillin (100%), aztreonam (100%), Cefoxitin (100%) and cephalothin (100%). *Escherichia coli* strains are particularly sensitive to the action of Amoxicillin (100%), Cefoxitin (100%), Aztreonam (81.25%) and Cefotaxime (75%). Our results showed that these two strains are still largely sensitive to most antibiotics belonging to the β-lactam family. The study of the antimicrobial activity of the essential oil of *Aeollanthus pubescens* revealed a sensitivity of all the multiresistants strains investigated with respect to this oil with a MIC ranging from 0.41 ± 0 mg/ml to 0.83 ± 0 mg/ml for *Salmonella* and from 0.41 ± 0 mg/ml to 1.66 ± 0 mg/ml for *Escherichia coli*. For CMB, it ranges from 0.83 ± 0 mg/ml to 1.66 ± 0 mg/ml for *Salmonella* and from 0.83 ± 0 mg/ml to 5 ± 2.35 mg/ml for *Escherichia coli*. With regard to the *Escherichia coli* strains investigated, the MIC and CMB val-

ues obtained in our work are higher than those obtained by Yovo [20] in Benin (0.27 mg/ml and 0.54 mg/ml respectively). For MIC and CMB). The same observation was made for the results of Sessou *et al.* [7] in Benin (0.81 ± 0.38 mg/ml and 1.62 ± 0.76 mg/ml respectively for CMI and CMB). These differences observed between our results and those of these authors could be explained by the fact that in our study, the investigated strains were multiresistants strains. In contrast to these authors, we determined the resistance profile of these strains and it is after the results obtained that we tested the action of this essential oil on these different strains. In addition, the essential oil of *Aeollanthus pubescens* showed a bactericidal effect on the various strains investigated, which is in accordance with the results obtained by Sessou *et al.* [7] It is important to emphasize that the work of these authors did not consider the investigation of *Salmonella* in their studies. The action of this essential oil of *A. pubescens* is due to its high concentration of thymol, α-terpinene, carvacrol and borneol [12] [26]. Indeed, these compounds are already recognized for their antibacterial activity, particularly thymol and carvacrol, which are the most bactericidal [27]. According to Zayyad *et al.* [26] reported by Yovo [20], borneol is a compound with high antimicrobial potency due to its high solubility in water, which gives it a high ability to cross bacterial cell membranes. This study showed, on the one hand, the importance of the antimicrobial resistance of *Salmonella* and *Escherichia coli* strains in our laying hens farming and, on the other hand, the effectiveness of the oil of *Aeollanthus pubescens* which deserves to be valued. Regarding the high antimicrobial activity of this oil, it is necessary to develop some strategies that will immensely boost *Aellonthus pubescens* plant production and therefore a large scale production of its essential oil in order to overcome this important bacterial problem which is a big regulator of economy in several countries.

4. Conclusion

In order to make our contribution to the endogenous fight against the bacterial resistance which is observed more and more in our farming and which constitutes a real public health problem these days, this study was interested in the activity antimicrobial effect of *Aeollanthus pubescens* essential oil on *Salmonella* and *Escherichia coli* multidrug-resistant strains isolated from laying hens farming in Department of Atlantique. At the end of this study, 11 *Salmonella* strains and 16 *Escherichia coli* strains were isolated and identified from liver, spleen, lungs, yolk and caeca collected from chickens and chicks corps collected in some laying hens farming in this department. The resistance profile of these isolated strains was determined and showed that almost all of them had a high resistance to tetracyclines, sulfonamides and ampicillin belonging to three different families of antibiotics (Tetracycline, Sulfamides and β-lactam), which are among the antibiotics commonly used in poultry farming. Faced with this resistance noted, this study has used the essential oil of *Aeollanthus pubescens* to propose an alternative therapeutic solution. Thus, this oil proved effective against the different

Salmonella and *Escherichia coli* strains investigated. There was no significant difference between the antibacterial activities of this oil on the two bacterial species. This oil offers an endogenous glimmer of hope for the control of bacterial diseases in poultry farming; it can validly replace synthetic antibiotics. For this purpose, technologies must be implemented for large scale production of this oil.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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