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# The antibiotic resistance study of Enterobacteriaceae, Yersiniaceae and Morganellaceae bacteria isolated from broilers (outside veterinary control) in western Algeria

**Merazi Yahya [1]*, Kheira Hammadi [1], Fedoul Firdaous Faiza [2]**

[1] Laboratory Educational Microbiology, Abdelhamid Ibn Badis University, Mostaganem, B.P. 188, 27000, Algeria

[2] Department of Biology, Faculty of Natural and Life Sciences, Djillali Liabes University, Sidi Bel Abbes, B.P. 89, 22000, Algeria

* Corresponding author: E-mail: meraziyahya@hotmail.fr

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**ABSTRACT:** In the context of our study of the animal side, especially in broiler chickens, the breeding of the latter requires the use of antibiotics for treatment and prophylaxis purpose; to give a closer look at the antibiotic resistance status of broiler chickens in Western Algeria. The bacteria *Enterobacter*, *Escherichia coli*, *Proteus*, *Salmonella*, *Serratia* were present and showed a high multidrug-resistance at a percentage of 67.14%, 63.64%, 60%, 57.27%, 50% respectively. The forty-nine bacteria identified belong to different families of Enterobacteriaceae, Yersiniaceae and Morganellaceae have shown an overall resistance of 61.22%. Presented resistance to cefazolin 91.84%, flumequine 89.80%, neomycin 83.67%, ceftiofur 79.59%, ampicillin 73.47%, trimethoprim 73.47%, aztreonam 57.14%, colistin 48.98 %, nadilixic acid 32.65%, streptomycin 30.61%, gentamicin 12.24%. To study the sensitivity, critical values to antibiotics a statistical Student's t-test was used. All bacteria were significantly resistant ($P \leq 0.05$) to the following antibiotics: flumequine, neomycin, cefazolin, trimethoprim, ampicillin, and ceftiofur. However, a low sensitivity was also noted to gentamicin, nalidixic acid, streptomycin ($P \leq 0.05$). Some isolated bacteria were resistant to many antibiotics, with resistance from 3 to 10 antibiotics simultaneously. The highest percentage of all bacteria (28.57%) were resistant to 8 antibiotics, while the lowest percentage of all bacteria (2.04%) were resistant to 3 and 10 antibiotics.

**Keywords:** Antibiogram; Antibiotic resistance; Enterobacteriaceae; Yersiniaceae; Morganellaceae.

## 1. INTRODUCTION

After independence, poultry production became dependent mainly on family farming as well as on small farms and units [1]. Poultry production in Algeria has also experienced real development over the past twenty years, largely with the intervention of the private and public sectors. Despite this, poultry farming is not without problems. Most poultry farmers are not professionals and do not master the application of basic hygiene rules, which promotes the emergence of many diseases that affect the health of poultry [2]. Poultry farming relies on the use of antibiotics to prevent infection, treat infection, promote growth, and improve the

production of farm animals [3]. But in fact, the excessive use of antibiotics in veterinary medicine has led to the emergence of resistance to pathogenic bacteria and treatments have become ineffective [4]. The percentage of resistant bacteria increases and at the same time strains increase resistance to infection with antibiotics [5]. In general, when used irrationally, antibiotics kill susceptible bacterial strains that leave behind those whose characteristics can resist the drug. These resistant bacteria then multiply and become the dominant group, they are thus able to transmit (horizontally and vertically) the genes responsible for their resistance to other bacteria [6]. In recent decades, new types of antibiotics have not been produced and most of the known antibiotics are increasingly losing their activity against pathogenic microorganisms [7]. To answer this problem related to antibiotic resistance. We conducted an experimental study on the sensitivity of harmful bacteria to antibiotics in a flock of broilers during their rearing period.

The present work aimed to verify and confirm what had been previously mentioned in previous studies, about the existence of a problem of antibiotic resistance in pathogenic strains of broilers. It also aims to draw attention to the existing danger to the authorities concerned with animal health and that she takes measures to enable the current problem to be stopped and prevented in the future.

## 2. MATERIALS AND METHODS

### 2.1. Bacterial isolates

The broilers used in our study where from informal production units that were not under the control of veterinary services in the west of Algeria. Samples are taken according to the recommendations of the International Organization of Epizootics (O.I.E), the samples are sent to the laboratory for a diagnosis [8]. A necropsy is a surgical examination to find out why the poultry died or to clarify a particular disease. The necropsy was practised under aseptic conditions, it consisted of the external examination of the dead or morbid subjects, then washing and drying of the corpses, after a median skin incision, we made the opening of the abdominal cavity [9]. The internal organs were removed (liver, spleen, intestine, and heart) and placed in sterile physiological water. The samples were collected and kept at 4°C until arrival at the laboratory.

The bacteria were isolated from sick and dead animals. The media were chosen according to the bacterial groups sought [10]. The bacteria were isolated by inoculating the samples in Petri dishes containing MacConkey agar (Bio Lab, Algeria) [11]. MacConkey Agar was used to isolating the Enterobacteriaceae based on the ability to ferment the lactose [12]. The incubating at 37°C for 24 hours. The colonies were observed macroscopically (size, shape, color, consistency, opacity, the appearance of the outline). The Gram stain coloration was used of all the strains.

The purification of the strains was carried out by repeated cultures until a pure culture was obtained [13]. Each pure culture was Gram stained. The bacilli are then subjected to the oxidase test performed with oxidase discs (HiMedia, India). The oxidase-negative a bacilli (presumed Enterobacteriaceae) were subjected to other biochemical tests allowing the search for family characteristics [14]. All the strains were identified using classical bacteriological methods (Gram staining, catalase production, oxidase production and by biochemical characters) [15]. The catalase is an enzyme that converts $H_2O_2$ to water and molecular oxygen [16]. After contact of an isolated colony with a drop of hydrogen peroxide (Saidal, Algeria), immediate observation of the bubbles ($O_2$ release) indicates that the bacteria are catalase positive. The identification of Enterobacteriaceae based on the fermentation of glucose, lactose, sucrose and the production of gas, $H_2S$. This is done by a Triple Sugar Iron test (Institut Pasteur, Algeria). The respiratory type of bacteria is determined by

the culture on meat-liver medium (Institut Pasteur, Algeria), facultative aero-anaerobic bacteria thrive with or without air, this is case with the majority of Enterobacteriaceae.

### 2.2. Antibiogram

The sensitivity of the isolated strains to antibiotics was determined by the Mueller-Hinton agar (HiMedia, India) diffusion method as recommended by the European Committee on Antimicrobial Susceptibility Testing (CASFM-EUCAST) [17].

The families of antibiotics selected for the sensitivity tests are those used in avians according to the Algerian Network for the Surveillance of Bacterial Resistance to Antibiotics (RA-SRBA). Antibiotics and their charges are as follows: Ampicillin (AM, 10 µg), Aztreonam (ATM, 30 µg), Cefazolin (CEZ, 30 µg), Ceftiofur (TIO, 30 µg), Colistin (CS, 10 µg), Erythromycin (E, 15 µg), Flumequine (UB, 30 µg), Gentamycin (GM, 10 µg), Nadilixic Acid (NA, 30 µg), Neomycin (N, 30 µg), Spiramycin (SP, 100 µg), Streptomycin (S, 10 µg) and Trimethoprim (TMP, 5 µg) (HiMedia, India and Bio-Rad, France). The results were read after 16-24 hours.

The diameters of the zones of inhibition are measured to the nearest millimeter with a ruler, the Petri dishes being placed 30 cm from the eye. Interpretation of the diameters of the zones of inhibition was done by categorizing them according to the critical values. The strains are defined as sensitive (S), resistant (R) or intermediate (I) according to the value of their diameter of inhibition compared to the limits of the critical diameters top (D) and bottom (d) of the zones of inhibition on antibiograms as recommended by EUCAST in the Antibiogram Committee of the French Society of Microbiology [17, 18].

### 2.3. Statistical analysis of the sensitivity of all bacteria to different antibiotics

The inhibitions diameters of all bacteria were compared with critical diameters in veterinary medicine, using the Student's t-test. The different diameters were determined with significance at $p \leq 0.05$ with a 95% confidence interval. We used SPSS v19.0 for windows.

## 3. RESULTS

### 3.1. Bacterial isolates

The organs of the chicken, which exhibits an alteration was selected for the bacterial test. A total of one hundred and forty-six samples of the liver, heart, spleen, and intestine were collected from broiler farms. A total of one hundred and three non-duplicate (70.55% of the total organ samples) strains of bacteria were isolated in this study. Seventy-five resistant strains were detected from different families (72.81% of the total resistant strains), among which forty-nine of Enterobacteriaceae, Yersiniaceae, and Morganellaceae, were chosen for this study.

The bacterial strains (Figure 1) tested were as follows: out of forty-nine strains from Enterobacteriaceae, Yersiniaceae and Morganellaceae: the *Escherichia coli* 48.98% (n = 24), *Enterobacter* 14.29% (n = 7), *Proteus* 12.24% (n = 6), *Salmonella* 22% (n = 11), and *Serratia* 2.0% (n = 1).

The sensitivity of the subject makes it difficult to obtain samples and to contribute comfortably with the breeder, as chicken farms are beyond the control of the veterinarian.

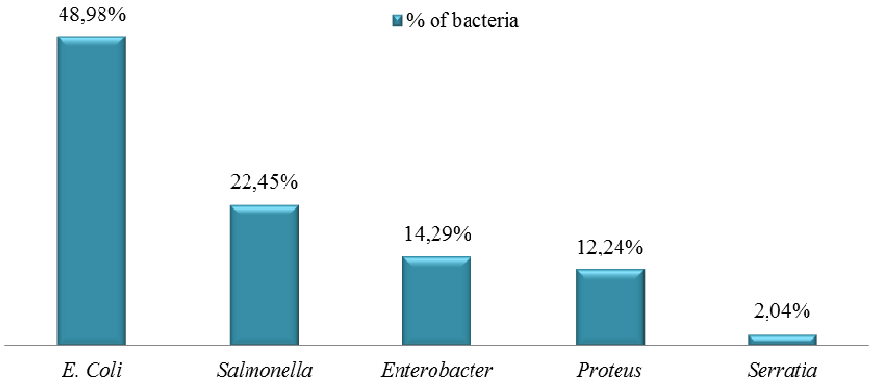


**Figure 1.** Distribution of strains of different families.

### 3.2. The profile of the sensitivity of Enterobacteriaceae to different antibiotics

#### *3.2.1. Escherichia coli*

A total of 24 strains of *Escherichia coli* (Figure 2) were isolated from sick and dead broilers. All the bacteria identified, presented multidrug resistance of 63.64%, against eleven antibiotics tested. This percentage is broken down as follows: Ceftiofur 91.67%, Flumequine 91.67%, Neomycin 83.33%, Trimethoprim 83.33%, Cefazolin 83.33%, Aztreonam (70.83%), Ampicillin 62.50%, Colistin 62.50%, Streptomycin 37.50%, Nadilixic Acid 29.17%, and Gentamicin 4.17%.

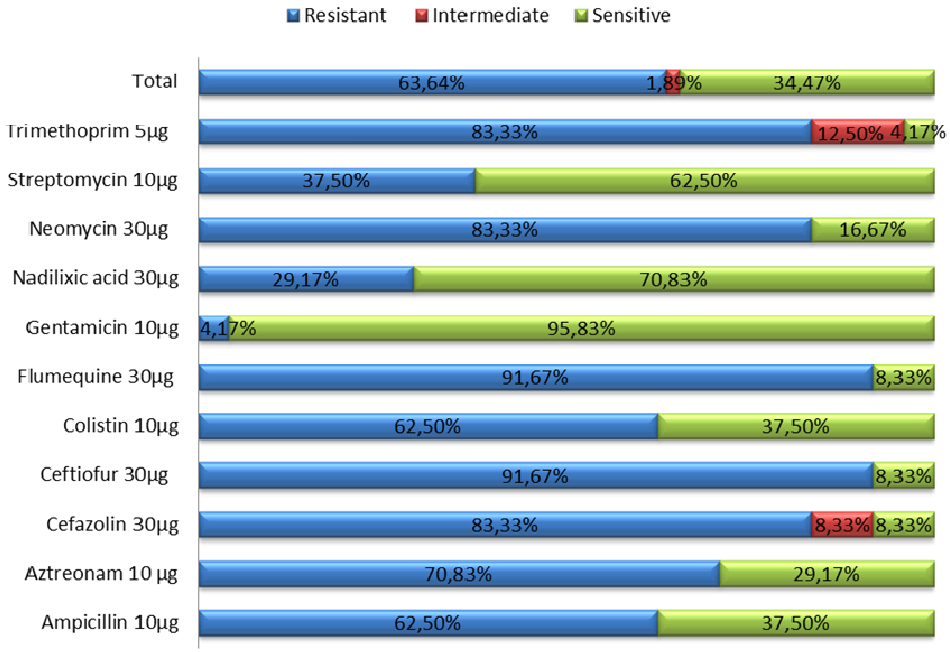


**Figure 2.** The sensitivity of *Escherichia coli* to each antibiotic used and in total.

#### *3.2.2. Enterobacter*

The percentage of resistance of the 7 pathogenic *Enterobacter* (Figure 3), presented multi-resistance of 63.64%, this percentage and distributed in descending following form: Neomycin 100%, Cefazolin 100%,

Ampicillin 85.71%, Trimethoprim 85.71%, Ceftiofur 71.43%, Flumequine 71.43%, Colistin 57.14%, Nadilixic Acid 57.14%, Gentamicin 42.86%, Aztreonam 28.57%, and Streptomycin 0%.

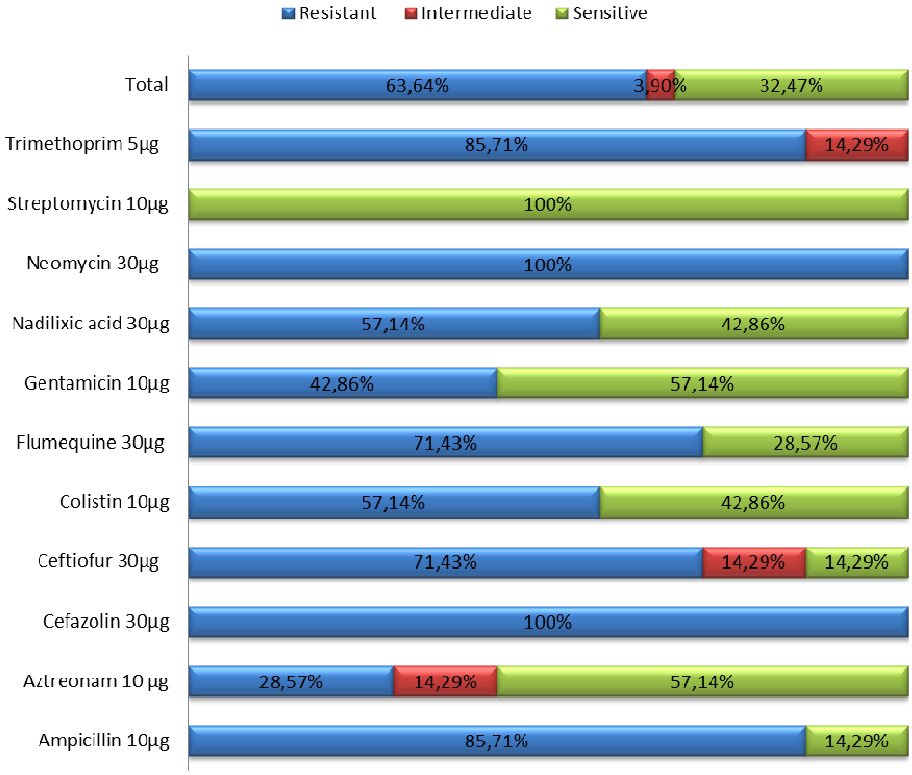


**Figure 3.** The sensitivity of *Entérobacter* to each antibiotic used and in total.

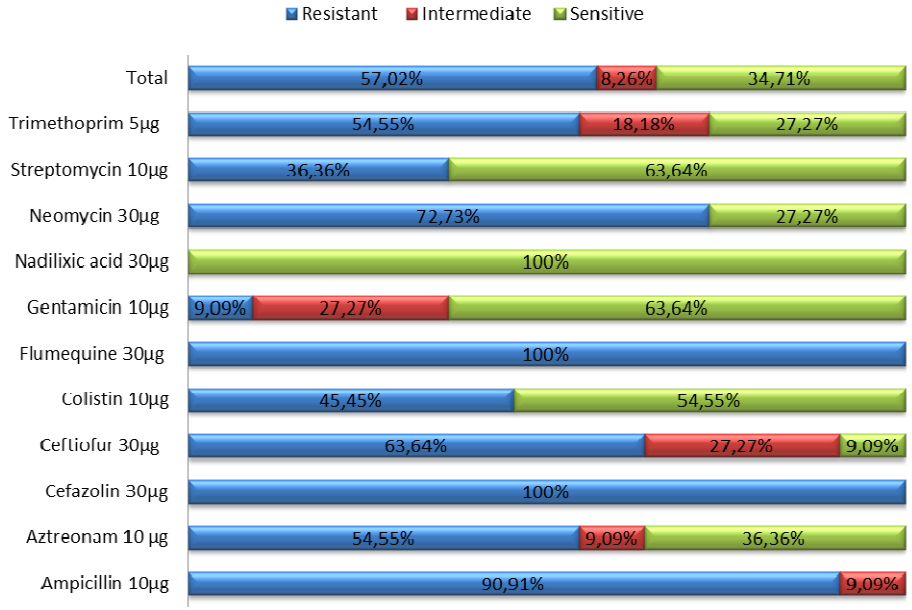


**Figure 4.** The sensitivity of *Salmonella* to each antibiotic used and in total.

### *3.2.3. Salmonella*

The percentages of resistance of the 11 pathogenic *Salmonella* (Figure 4), presented multi-resistance of 57.02%, this percentage and distributed as follows: Flumequine 100%, Cefazolin 100%, Ampicillin 90.91%,

Neomycin 72.73%, Ceftiofur 63.64%, Aztreonam 54.55%, Trimethoprim 54.55%, Colistin 45.45%, Streptomycin 36.36%, Gentamicin 9.09%, and Nadilixic Acid 0%.

### 3.3. The profile of the sensitivity of Morganellaceae and Yersiniaceae to different antibiotics

#### *3.3.1. Proteus*

The resistance percentage of the 6 pathogenic *Proteus* (Figure 5), presented multi-resistance of 62.12%, this percentage and distributed in the following form: Neomycin 100%, Flumequine 100%, Colistin 100%, Cefazolin 100%, Ampicillin 83.33%, Ceftiofur 83.33%, Nadilixic Acid 66.67%, Aztreonam 50%, Trimethoprim 50%, Streptomycin 16.67%, and Gentamicin 0%.

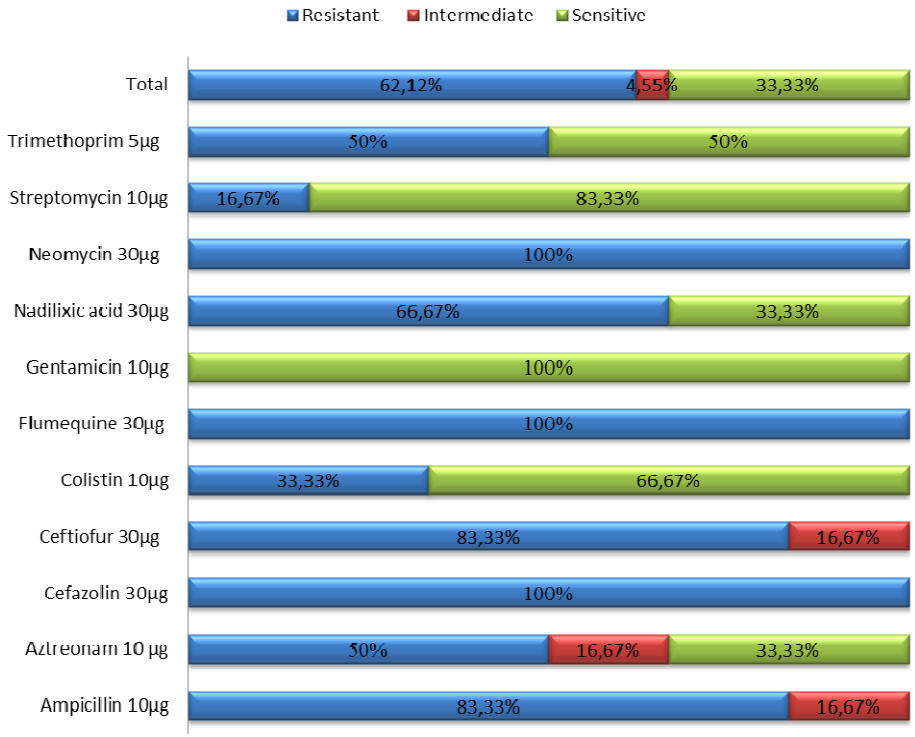


**Figure 5.** The sensitivity of *Proteus* to each antibiotic used and in total.

#### *3.3.2. Serratia*

The percentage resistance of one pathogenic *Serratia* (Figure 6). These identified bacteria are presented with multi-resistance of 45.45%, this percentage and distributed in the following form: Gentamicin 100%, Streptomycin 100%, Trimethoprim 100%, Nadilixic Acid 100%, Cefazolin 100%, Ampicillin 0%, Aztreonam 0%, Ceftiofur 0%, Neomycin 0%, Flumequine 0%, and Colistin 0%.

### 3.4. The bacterial group

All of the forty-nine all bacteria (Figure 7) identified exhibited multidrug-resistance of 61.22%, this percentage being distributed as follows: Cefazolin 91.84%, Flumequine 89.80%, Neomycin 83.67%, Ceftiofur 79.59%, Ampicillin 73.47%, Trimethoprim 73.47%, Aztreonam 57.14%, Colistin 48.98%, Nadilixic Acid 32.65%, Streptomycin 30.61%, and Gentamicin 12.24%. Results are displayed in a large group of bacteria to provide a clear aspect of antibiotic resistance within the group of bacteria.

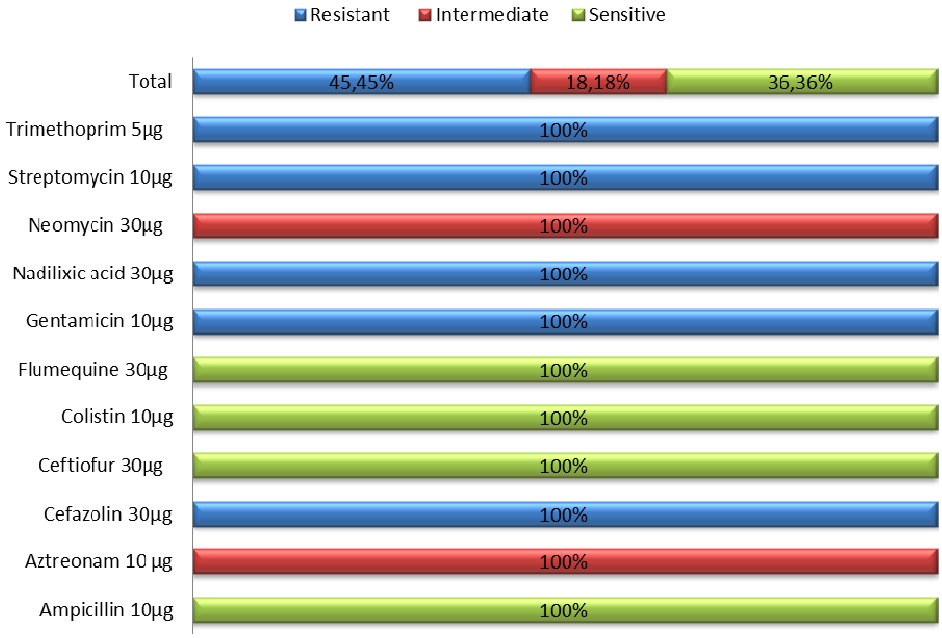


**Figure 6.** The sensitivity of *Serratia* to each antibiotic used and in total.

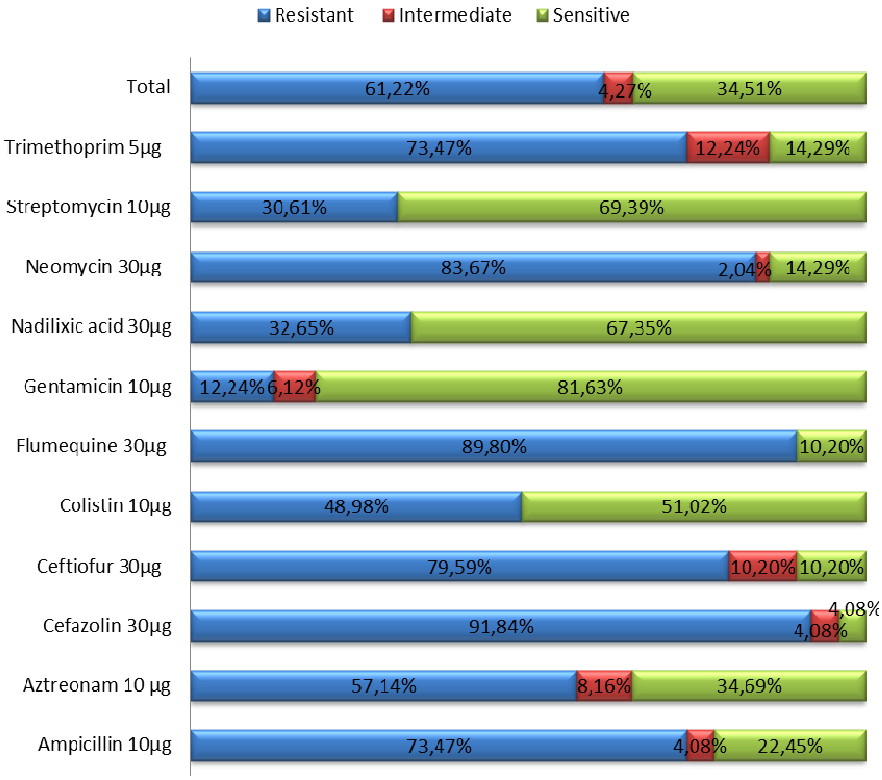


**Figure 7.** The sensitivity of all bacteria to each antibiotic used and in total.

Statistical analysis of the sensitivity of all bacteria to different antibiotics is presented in Table 1.

**Table 1.** The result of One-Sample Student's t-test.

| Antibiotics | T | Sig | Mean Difference | Value Test |
|---|---|---|---|---|
| UB | -8.683 | 0 | -13.673 | 21 |
| CEZ | -7.528 | 0 | -11.551 | 19 |
| GM | 6.798 | 0 | 6.776 | 16 |
| TMP | -6.068 | 0 | -6.49 | 12 |
| AM | -5.969 | 0 | -8.531 | 15 |
| N | -5.869 | 0 | -7.306 | 15 |
| NA | 4.422 | 0 | 6.837 | 15 |
| S | 3.889 | 0 | 4 | 13 |
| TIO | -3.713 | 0.001 | -4.49 | 18 |
| CS | -0.848 | 0.4 | -0.796 | 15 |
| ATM | 0.341 | 0.735 | 0.449 | 21 |

The diameters of inhibitions are compared to a critical value in mm.

### 3.5. Frequencies of resistance of bacteria to several antibiotics

#### *3.5.1. Escherichia coli*

The problem of multidrug-resistance to antibiotics appeared clearly in *Escherichia coli*. It was noted a resistance of 3 and 9 antibiotics simultaneously. The largest group of *Escherichia coli* (41.67%) was resistant to 8 antibiotics, while the smaller group of *Escherichia coli* (4.17%), was resistant to 3, 4 and 9 antibiotics. The resistance of 7 antibiotics was (25%). And the resistance to 6 antibiotics was (20.83%). We reported a complete absence of antibiotic resistance once, twice, five and ten times.

#### *3.5.2. Enterobacter*

The problem of multidrug-resistance to antibiotics is apparent in *Enterobacter*. Resistance to 5 and 9 antibiotics was noted simultaneously. The largest group of *Enterobacter* (28.57%) was resistant to 6, 7, and 9 antibiotics, while the smaller group of *Enterobacter* (14.29%) was resistant to 5 antibiotics. We have reported a complete absence of antibiotic resistance one, two, three, four, eight and ten times.

#### *3.5.3. Proteus*

The problem of multidrug-resistance to antibiotics appears clearly in *Proteus*. It was noted a resistance of 4 and 10 antibiotics simultaneously. The largest group of *Proteus* (33.33%) was resistant to 8 antibiotics, while the smaller group of *Proteus* (16.67%), was resistant to 4,5,6 and 10 antibiotics. We reported a complete absence of antibiotic resistance once, twice, three, seven, and nine times.

#### *3.5.4. Salmonella*

The problem of multidrug-resistance to antibiotics is apparent in *Salmonella*. Resistance to 4 and 9 antibiotics was noted simultaneously. The largest group of *Salmonella* (27.27%) was resistant to 5, 6 and 8 antibiotics, while the smaller group of *Salmonella* (9.09%) was resistant to 4 and 9 antibiotics. We have reported a complete absence of antibiotic resistance one, two, three, seven, and ten times.

### *3.5.5. Serratia*

We isolated one strain of *Serratia*, and when studying antibiotic sensitivity, it was clear that *Serratia* was resistant to five antibiotics at once.

**Table 2.** Profile of the frequency of resistance of all bacteria to multiple.

| ATB | *E. coli* | *Enterobacter* | *Proteus* | *Salmonella* | *Serratia* |
|---|---|---|---|---|---|
| 1 | 0% | 0% | 0% | 0% | 0% |
| 2 | 0% | 0% | 0% | 0% | 0% |
| 3 | 4.17% | 0% | 0% | 0% | 0% |
| 4 | 4.17% | 0% | 16.67% | 9.09% | 0% |
| 5 | 0% | 14.29% | 16.67% | 27.27% | 100% |
| 6 | 20.83% | 28.57% | 16.67% | 27.27% | 0% |
| 7 | 25.00% | 28.57% | 0% | 0% | 0% |
| 8 | 41.67% | 0% | 33.33% | 27.27% | 0% |
| 9 | 4.17% | 28.57% | 0% | 9.09% | 0% |
| 10 | 0% | 0% | 16.67% | 0% | 0% |

## 3.6. Frequencies of bacterial group resistance to several antibiotics

The problem of multidrug-resistance to antibiotics appears clearly in all bacteria. It was noted a resistance of 3 and 10 antibiotics simultaneously. The largest group of all bacteria (30.61%), was resistant to 8 antibiotics, while the smaller group of all bacteria (2.04%), was resistant to 3 and 10 antibiotics.

The following groups of all bacteria: (22.45%), (16.33%), (12.24%), (8.16%), (6.12%) are multiple resistances at 6, 7, 5, 9 and 4 respectively. We reported a complete absence of antibiotic resistance once, twice, and eleven times.

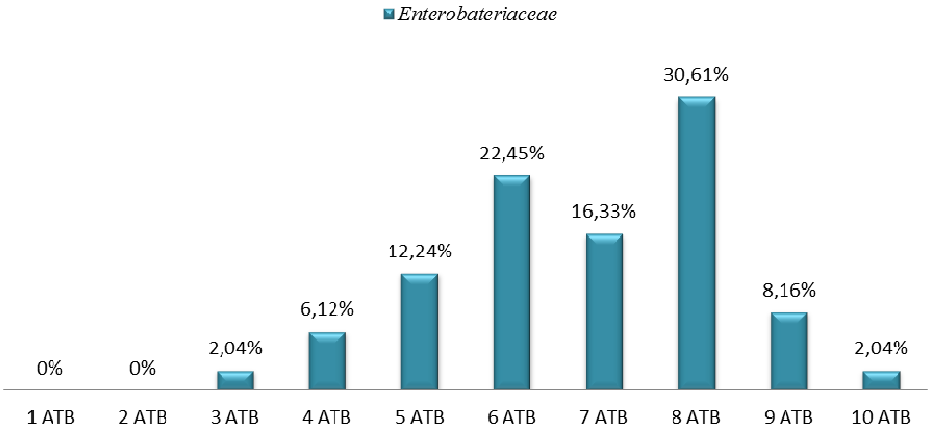


**Figure 8.** Resistance frequency profile of isolated bacterial families.

## 4. DISCUSSION

### 4.1. Bacterial isolates

The authors Benameur et al. [19] isolated the Two hundred and fifty-three Enterobacteriaceae strains from poultry samples. Where the results of the isolation were as follows: 134 *E. coli*, 55 *Enterobacter cloacae*, 42 *Klebsiella pneumoniae*, 10 *Proteus mirabilis*, 7 *Serratia marcescens*, and 5 *Providencia rettgeri*.

In another study of El-Demerdash et al. [20] showed six genera of Enterobacteriaceae as follows: *Salmonella* (45%), *E. coli* (40%), *Proteus* (10%), *Klebsiella* (8%), *Citrobacter* (4%) and *Enterobacter* species (3%).

The bacteria isolated in our study were pathogenic bacteria that cause infection, serious health problems and which sometimes cause the death of chickens. Despite the difference in the percentages of bacteria isolated, many authors agree that *E. coli*, *Enterobacter* and *Salmonella* occupy the largest share of the Enterobacteriaceae group.

### 4.2. The profile of the sensitivity of all bacteria to different antibiotics

#### *4.2.1. Escherichia coli*

Numerous studies have indicated that *E. coli* is the most numerous of the Enterobacteriaceae group and the most resistant to many antibiotics used in veterinary medicine. Regarding Ceftiofur (91.67%), our results differed with the authors Gay, et al. [21] and Benklaouz, et al. [22] whose results were as follows: 6%, 3.44%, respectively. Likewise, Flumequine (91.67%) contradicts the result of Gay, et al. [21], who obtained 44%. It is also is consistent with the results of authors Halfaoui et al. [23] and Benameur, et al. [19], who obtained 91.5%, 93.28% respectively. Our result of the sensitivity of *E. coli* to Neomycin (83.33%), is very close to the result of Benklaouz, et al. [22] 80.68%.

Regarding the two antibiotics Trimethoprim and Cefazolin, their results were (83.33%). It is considered to be in contradiction with the results of both authors, so that the Shecho, et al. [24] found resistance to Trimethoprim 7.69%, and the Asai, et al. [25] found resistance to Cefazolin 32.6%. Aztreonam (70.83%) is consistent with the result of Tansawai, et al. [26] (75.2) when he studies Extended-spectrum ß-lactamase-producing *E. coli* among backyard poultry farms, farmers, and environments in Thailand. and contradict the authors Yassin, et al. [27], He, et al. [28] whose results are 14.6%, 3.91% respectively.

Antibiotic use practices are those that make the difference between the percentage of resistance from one breeding unit to another. As in conventional farms, the regular use of antimicrobials [29]. But the too low a rate with a time-dependent antibiotic, leads to therapeutic voids at the origin of a primary underdosing, secondarily leading to the selection of antibiotic-resistant bacteria [30].

The antibiotics Ampicillin and Colistin share the same result (62.50%). Result of Ampicillin is allied with the result of several authors Halfaoui, et al. [23] 83.01%, Shecho, et al. [24] 62.50%, Benameur, et al. [19] 94.03%, Meguenni, et al. [31] 83.3%; and Benklaouz, et al. [22] 82.75%. But the result of Colistin (62.50%) contradicts the result of both Nguyen, et al. [32] 22.2% and Rahmatallah, et al. [33] 2.9%.

The low resistance of *Escherichia coli* is demonstrated against the following antibiotics: Streptomycin 37.50%, Nadilixic Acid 29.17%, and Gentamicin 4.17%. Streptomycin (37.50%) is the same result of Shecho, et al. [24] 34.61% and disagrees with the result of He, et al. [28] 84.38%; Nadilixic Acid (29.17%) agrees with the result of Shecho, et al. [24] 23.07% and disagrees with the result of several authors Halfaoui, et al. [23] 85.62%, Benameur, et al. [19] 94.03%, Meguenni, et al. [31] 83.4% and Benklaouz, et al. [22] 90.34%. While our result of Gentamicin (4.17%) is similar to the result of several authors Gay, et al. [21] 5%, Shecho, et al. [24] 7.69% and Benklaouz, et al. [22] 13.10%, and not similar to the high result of Dandachi, et al. [34] 70%.

#### *4.2.2. Enterobacter*

Our result of Neomycin (100%) is very far from the result of author Benameur, et al. [19] who has estimated resistance between 20 to 30%. While the Ampicillin (85.71%) is very close with the result of author

Nandi, et al. [35] 94.4%, And in another study among the *E. cloacae* isolates, the highest proportion of resistance was hight Benameur, et al. [19] 90.90%, Dandachi, et al. [34] 100%. The result of the Flumequine we got remains (71.43%) is similar to the result of Benameur, et al. [19] 76.36%. And in some cases, we find that antibiotic resistance is low for some and even absent for others. With a different result, record the Nadilixic Acid (57.14%), is the same result roughly of Benameur, et al. [19] 83.63%. According to Arhin, et al. [36], *Enterobacter* sp. is resistant (0.8%) to different antibiotics used as prophylaxis in poultry. The results of our study proved the effectiveness of the following antibiotics against *Enterobacter*: Gentamicin 42.86%, Aztreonam 28.57%, Streptomycin 0%. While the Gentamicin (42.86%) is consistent with the result of 37.5% Ezekiel, et al. [37] and contradicts with a minimum result Nandi, et al. [35] (5.6%) and with the greatest result Dandachi, et al. [34] 100%. Streptomycin was not completely resistant, with a result of (0%), this last one is very far from the result of Nandi, et al. [35] 55.6% when he studying the prevalence and characterization of multidrug-resistant zoonotic *Enterobacter* spp. in poultry of Bangladesh

#### *4.2.3. Salmonella*

The resistance of the Ampicillin is the greatest, is estimated at (90.91%), is consistent with the result of [38] 97.8% and contradict the result of authors [39] 26.3%, Lenchenko, et al. [40] 63.33%; the second-ranking in terms of resistance to neomycin (72.73%). In a scientific work summary on microbial resistance of broiler poultry, Sepehri, et al. [41] state that *Salmonella* strains showed resistance to neomycin (40%); Resistance of Aztreonam is (54.55%) according to Wajid, et al. [42], when studying of *Salmonella enterica* Serovars *Typhimurium* and *Enteritidis* from Poultry Farms of Faisalabad, Pakistan. Resistance prevalence *S. Typhimurium* 63.2% and *S. Enteritidis* 4.5%. Also, the result differs from Begum, et al. [43] 0%, Djeffal, et al. [44] 26.60 %, Suresh, et al. [45] 23.80%.

Our result of resistance of Trimethoprim (54.55%) is similar to the result of Lenchenko, et al. [40] 46.67%, and not similar to the result of [39] 29.8%. While the following antibiotics are poorly resistant and rank in descending order: Colistin 45.45%, Streptomycin 36.36%, Gentamicin 9.09%, Nadilixic Acid 0%.
our result of resistance of Streptomycin (36.36%) contradicts with the result of [38] 97.8%, Lenchenko, et al. [40] 96.67%; and that resistance of Gentamicin (9.09%) is very close with the result of [38] 0%, and very far with the result of Lenchenko, et al. [40] 33.33%; lastly, it is noted that Nadilixic Acid (0%) shows no resistance, remains our results far from those of Andoh, et al. [39] 89.5%, Abdi, et al. [38] 97.8%, Lenchenko, et al. [40] 36.67%.

#### *4.2.4. Proteus*

Poor effect of Ampicillin (83.33%) is consistent with the result of authors Nahar, et al. [46] 66.7% Dandachi, et al. [34] 100%, and contradict the result of Nemati [47] 22%; Another bad effect of Nadilixic Acid (66.67%) is very far from the result of Nemati [47] 93%, Nahar, et al. [46] 88.9%; while resistance of Aztreonam is (50%), it is more or less important. While our result is greater than that of He, et al. [28] 1.33%. *Proteus* has resistance to the remains of antibiotics which is very weak and/or absent.

A resistance of Streptomycin is weak (16. 67%), this result is confirmed by El-Demerdash, et al. [20] than *P. mirabilis* was resistant to Streptomycin and disagrees with the result of He, et al. [28] 90%; a resistance of Gentamicin is absent (0%) similar to the result of Nemati [47] 0% and not similar to the result of Nahar, et al. [46] 52.8%, Dandachi, et al. [34] 33%. Confirmed El-Demerdash, et al. [20] than *P. mirabilis* was resistant to Gentamicin.

#### *4.2.5. Serratia*

*Serratia* shows resistance to Streptomycin (100%). This resistance is not also shown by author Shawish, et al. [48] who declared than Serratia liquefaciens was not resistant to Streptomycin. Regarding our resistance to Nadilixic Acid (100%), such resistance is approved by Adelowo, et al. [49] for the strain *Serratia marcescens*; the resistance to cefazolin is also (100%). this same resistance is endorsed by Sala, et al. [50] when all *Serratia* strains showed multiple resistance to cefazolin in his study; *Serratia* to show sensitivity to amoxicillin and colistin (0%). According to several authors as Adelowo, et al. [49] and Sala, et al. [50] who declares that all *Serratia* strains showed multiple resistance to Ampicillin and author Adelowo, et al. [49] when he confirms that *S. marcescens* was resistant to colistin.

### 4.3. The bacterial group

Regarding the results of antimicrobial susceptibility testing of all bacteria in our study for Ampicillin our result is (73.47%); it is considered high, and that's what the authors said as Kilonzo-Nthenge, et al. [51] 53.6% Arhin, et al. [36] 54%, Faife, et al. [52] 100% and He, et al. [28] 63.23%. Also, the resistance results in our study on Aztreonam are estimated at 57.14%, the latter is very incompatible with the results of the author He, L et al.i [28] who found a severe weakness of the estimated resistance 4.23%.

The following antibiotics are not very resistant, which explains their effectiveness in eliminating bacteria harmful to poultry. Among these antibiotics we mention Nadilixic Acid, Streptomycin, Gentamicin, the percentages of which are (32. 65%), (30. 61%), and (12. 24%) respectively. Kilonzo-Nthenge, et al. [51], have mentioned that streptomycin and gentamicin, the percentages of which are respectively 42.9% and 7.1%. This is relatively consistent with our results. While streptomycin and gentamicin were very high, compared with our study, for author He, et al. [28], with a percentage of 87.83% and 24.34 respectively. The authors Arhin, et al. [36] also confirm the weak resistance of gentamicin 22%. While than author Faife, et al. [52] confirms the opposite 78%.

### 4.4. Statistical analysis of the sensitivity of all bacteria to different antibiotics

Our study group of Enterobacteriaceae, Morganellaceae and Yersiniaceae was significantly resistant ($p \leq 0.05$) to the following antibiotics: Flumequine, Neomycin, Cefazolin, Trimethoprim, Ampicillin, and Ceftiofur, with the T value equal to, -8.683, -5.869, -7.528, -6.068, -5.969 and -3.713 respectively. A significant sensitivity was also noted to Gentamicin, Nalidixic Acid, Streptomycin ($p \leq 0.05$).With the value of T equal to 6.798, 4.422, and 3.889 respectively.

The resistance of all bacteria to colistin is not significant ($p > 0.05$). With a T is equal to -0.848. And also the sensitivity of all bacteria to Aztreonam is not significant ($p > 0.05$). With a T is equal to 0.341. It was noted that despite 28/49 all bacteria resistant to Aztreonam, but this does not show a large mean difference in inhibition diameter 0.449 mm.

### 4.5. Frequencies of resistance to multiple antibiotics

#### *4.5.1. Escherichia coli*

All *E. coli* isolates from chicks of hatcheries B, C, and E, 80%, and 93.3% of those from hatcheries A and D, respectively, exhibited resistance to least a drug in three or more classes of antibacterial agents and thus MDR [53]. Yulistiani, et al. [54] confirmed that 16.98% and 33.96% of *E. coli* is resistant to only one and

two antibiotics respectively. While 15.09% and 20.75% of *E. coli* are resistant to only three and four antibiotics respectively. And also that 0% of *E. coli* is resistant to only five and six antibiotics respectively.

#### *4.5.2. Enterobacter*

Yulistiani, et al. [54] confirmed that 14.28% of *Enterobacter* is resistant to only one to four antibiotics.

#### *4.5.3. Proteus*

The results of antimicrobial susceptibility testing reported by Arhin, et al. [36], Nahar, et al. [46], Nemati [47], Lei, et al. [55], and Pan, et al. [56] showed that *Proteus mirabilis* and *P. vulgaris* strain was resistant to at least to two or more antibiotics. Yulistiani, et al. [54] confirmed that 25% of *Proteus* is resistant to only one and two antibiotics. While 20.83% of *Proteus* is resistant to only three antibiotics, and also that 12.5% of *Proteus* is resistant to only four antibiotics.

#### *4.5.4. Salmonella*

Arhin, et al. [36] *Salmonella enteritidis* resistant (4.7%) to different antibiotics used as prophylaxis in poultry. According to the results of the highly prevalent multidrug-resistant *Salmonella* from chicken study by Zhang, et al. [57] he showed that 33.8% of salmonella showed multi-resistance to the different antibiotics in the order of 1 to 3 antibiotics, while 43% resisted from 4 to 6 antibiotics. A percentage of 12.6% of *Salmonella* resisted from 7 to 9 antibiotics and a low percentage of 8.3 % resisted more than 10 antibiotics. Ziech, et al. [58] concluded in her study on multidrug-resistance and ESBL-producing *Salmonella* spp. demonstrated that the isolated *Salmonella* group has a multidrug-resistance of 86%. Zhang, et al. [57] also confirmed that in the whole of *Salmonella*, he notices that 97.7% resist at least one antibiotic while 2.3% is sensitive to the antibiotics tested. He noted that 81.1% of strains isolated resist more than 3 antibiotics. in a study conducted by Suresh, et al. [45], this phenomenon of multiple drug resistance was observed in 21 isolates of *Salmonella* with a percentage of 57.14%.

#### *4.5.5. Serratia*

Yulistiani, et al. [54] confirmed that 33.33% of *Serratia* is resistant to only one and four antibiotics, and also that 16.76% of *Serratia* is resistant to only two antibiotics.

### 4.6. Frequencies of bacterial group resistance to several antibiotics

All bacterial isolates were resistant to at least one of the antimicrobial agents evaluated. Overall, 84.9% of the isolates displayed microbial drug resistance (MDR) to 3 or more antimicrobials, whereas 19.2% (14 of 73) of the 73 isolates evaluated displayed MDR to 5 or more antimicrobials [51]. In a study realized by Dandachi, et al. [34] on prevalence and characterization of multi-drug-resistant Gram-negative bacilli isolated from Lebanese poultry, illustrates the current epidemiology of multidrug-resistant Gram-negative bacilli in Lebanese chicken farms.

There was a high rate of multidrug-resistance out of a total of 184 Enterobacteriaceae. A percentage of (79.9%) showed resistance to at least three unrelated antimicrobial agents. 21.2% were resistant to all eight tested antimicrobials [59].

## 5. CONCLUSION

In conclusion, the study primarily describes a group of Enterobacteriaceae isolated from various organs of sick and dead broilers. Our laboratory experimental results showed resistance to multiple antibiotics, for

more than nine antibiotics. They also showed high percentages of resistance in the same family of bacteria, as well as in all bacterial groups. These results confirmed that the problem of the resistance to the antibiotics in the breeding broilers chicken in the study zones of the west of Algeria is getting worse.

The number of isolates used in this study is humble. Nevertheless, it confirms the findings of several Algerian authors regarding the exacerbation of the problem of antibiotic resistance, with the increase in unauthorized breeding of broiler chickens especially away from veterinary control. Despite the sensibility of the subject as indicated above, we hope to expand our research on a larger scale in subsequent studies.

And finally, we have to raise the alarm concerning the urgent need to urge the competent authorities to think seriously to find a solution to the problem that threatens the lives of humans and animals.

**Authors' Contributions:** MY: contributed to the necropsy, collected the samples from chicken farms, performed the major experiments, preparing the results, and helped with the preparation and writing of the manuscript. FFF: conducted the laboratory experiments, supplied the reagents, collected and organized the result data. HK: conceived the idea of research, verified the analytical methods, coordinated between the first two, and finalized the manuscript. All authors read and approved the final manuscript.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Ethics Statement:** Verbal consent was obtained from the owners of the farms to collect chicken samples. We have also kept the identity of our collaborators.

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