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# Antimicrobial Resistance in Morocco: A Real Threat to Poultry Productivity and a Major Public Health Risk

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## ABSTRACT

The excessive use of antibiotics in poultry farms has become a significant worldwide concern, including in Morocco, where it has led to the development of multi-resistant avian pathogenic *Escherichia coli* (APEC). This retrospective study, conducted between 2019 and 2024, aimed to evaluate the evolution of antibiotic susceptibility profiles of APEC isolated from broiler chickens. A total of 93 samples were collected from various poultry farms across different regions of Morocco. The isolates were tested for their susceptibility to a range of commonly used antibiotics using standardized antimicrobial susceptibility testing methods. The results indicated alarmingly high levels of antimicrobial resistance (AMR) among the APEC strains. Resistance to Amoxicillin was observed in 94.62% of the isolates, followed by doxycycline (88.17%), tetracycline (84.95%), flumequine (82.80%), enrofloxacin (48.39%), sulfamethoxazole-trimethoprim (46.24%), and florfenicol (27.95%). However, resistance to fosfomycin (7.53%) and colistin (6.45%) was comparatively low. Furthermore, the study revealed that 93.55% of the *E. coli* strains exhibited multi-drug resistance, defined as resistance to three or more antibiotic classes. This high prevalence of multi-drug resistance poses a significant threat to animal health, food safety, and public health due to the potential transmission of resistant bacteria from animals to humans through the food chain. The findings highlight the urgent need for prudent use of antibiotics in poultry farming to mitigate the rise of AMR. It is essential to implement stringent antibiotic stewardship programs, promote alternative measures for disease control such as vaccination, and enhance biosecurity practices on farms. Additionally, continuous surveillance and monitoring of antibiotic resistance patterns are crucial to define a national strategy to combat AMR.

**Key words:** APEC, Colibacillosis, AMR, Broiler, Morocco

## INTRODUCTION

Avian colibacillosis is one of the most frequent and significant bacterial infections in avian species (Lozica et al. 2021). These infections are usually the main cause of antibiotic treatment in poultry farms, leading to the emergence of resistant strains, which pose a public health problem (Naghavi et al. 2022). This infection, primarily entering through the respiratory tract or sometimes the genital tract, mainly affects breeder and broiler flocks, causing lesions and significant economic losses (Barnes et al. 2008; Stokholm et al. 2010). Avian pathogenic *E. coli* (APEC) is one of the main causes of mortality in poultry (Apostolakos et al. 2021). These infections also lead to reduced performance and condemnations at slaughterhouses. Moreover, the means of combating these diseases are very expensive. Most colibacillosis cases are superinfections following viral, bacterial, or parasitic infections. The importance of colibacillosis is primarily medical and economic (Ferreira de Souza et al. 2019).

Over the last decade, the pathological situation of Moroccan poultry has undergone major and serious changes. Significant pathologies such as low pathogenic avian influenza (H9N2), which appeared at the beginning of 2016, and mycoplasma provide a breeding ground for colibacillary superinfections, whose prophylaxis is becoming increasingly tedious (Oubouyahia and Nassik 2021).

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Antimicrobial resistance (AMR) is now one of the greatest threats to global health, food security, and development (Dadgostar 2019; El-Hamaky et al. 2023; Desouky et al. 2023). The misuse and overuse of antimicrobials in humans, animals, and plants are accelerating the emergence and spread of antimicrobial resistance worldwide. WHO has alerted AMR and the United Nations (UN) estimates that 5 million people die each year from infections caused by multi-drug-resistant germs, with this figure potentially rising to 10 million by 2050 (Murray et al. 2022).

The issues of AMR are complex and multifaceted but not insurmountable. Fighting AMR with a "One Health" approach will help to save millions of lives, preserve antimicrobials for future generations, and guard against drug-resistant pathogens (Sevilla-Navarro et al. 2022; Rashid et al. 2024).

Control of avian colibacillosis has traditionally been achieved through the curative and preventive use of antibiotics (Panth 2019). However, increasing antimicrobial resistance and the judicious use of antibiotics now require alternative control strategies to protect both animal and human health (Zhang et al. 2022). To find alternatives to this problem of antibiotic resistance and to limit the use of antibiotics, the use of autovaccines is an innovative solution and a new strategy for preventing and controlling avian colibacillosis (Christensen et al. 2021).

To highlight the problem of antibiotic resistance in Morocco, a retrospective study was carried out between 2019 and 2024 with the aim of monitoring antibiotic resistance in *E. coli* strains isolated from broiler chicken farms belonging to ZALAR Holding, a vertically integrated company in Morocco with farms mainly located in the Fes-Rabat-El-Jadida and Casablanca-Beni-Mellal areas.

## MATERIALS AND METHODS

### Study design

A retrospective study was based on the results of bacterial investigation carried out between 2019 and 2024 in the farms belonging to ZALAR Holding group, the largest poultry integrator in Morocco. For each sample, the following information was recorded: the farm of sampling, the age of birds, the *E. coli* strain isolated and the profile of antibiotics susceptibility. The samples were taken from dead birds and organs (Lungs, liver, air sacs and heart) were collected on necropsy examination (Fig. 1).

### Samples collection

The present study concerned 93 samples of poultry broiler farms located in different areas of poultry production in Morocco. These farms were investigated between 2019 and 2024. The sampling criteria were the high mortality, clinical signs and the presence of colibacillosis lesions on necropsy. In addition to the isolation of *E. coli* after bacteriological analysis in laboratory.

For further examination and to perform a diagnosis, dead birds were collected in each sampled farm and oriented to the laboratory of avian pathology (Department of Veterinary Pathology and Public Health, Hassan II Agronomic and Veterinary Institute, Rabat, Morocco and an external laboratory of Dr Mouahid). At necropsy, tissues were collected for laboratory analysis (femoral bone marrow, lungs, liver, heart, and air sacs) as birds presented with lesions associated with *E. coli* bacterial complications (congestion and fibrin) (Fig. 2).

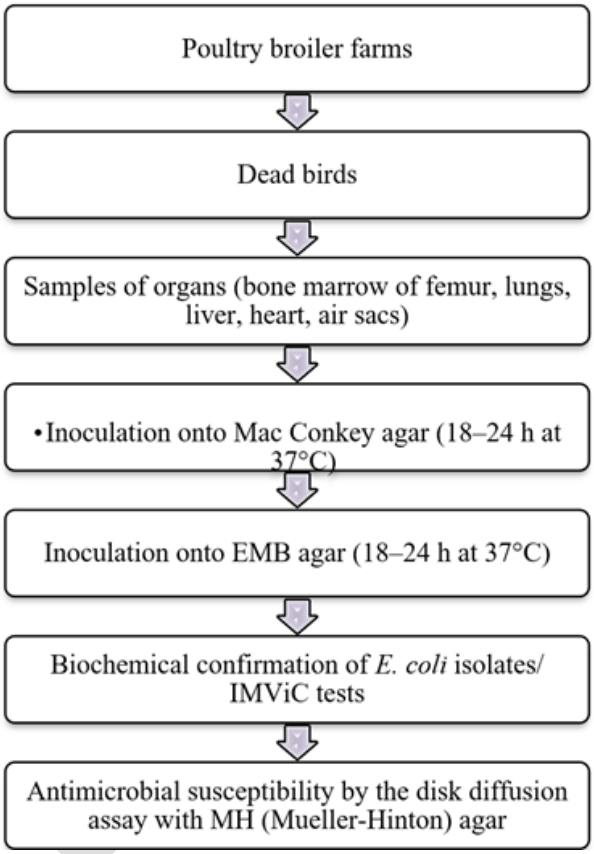


**Fig. 1:** study design: procedure of isolation, identification and determination of antimicrobial susceptibility of *E. coli.*

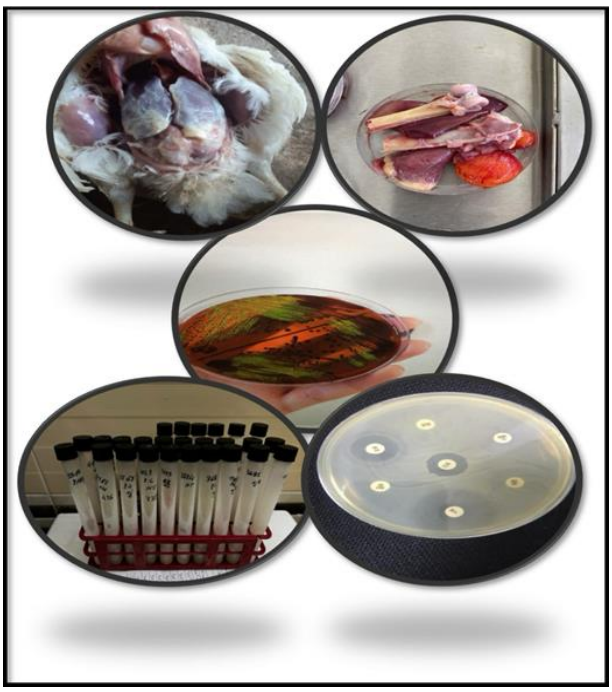

**Fig. 2:** Broiler materials, *E coli* culture and antimicrobial susceptibility.

### *E. coli* isolation and identification

For isolation of *E. coli*, sample were taken from tissue with colibacillosis lesion detected on necropsy examination

and inoculated onto Mac Conkey agar (Mac Conkey, Oxoid®), and onto Eosin Methylene Blue (EMB, Oxoid®) agar plates, then the plates were incubated for 37 °C for approximately 24 hours. The identification of characteristic colonies in both media were based on a rapid fermentation of lactose and production of strong acids.

The confirmation of *E. coli* strains was performed by using a classical biochemical identification (IMViC) and also by API 20E gallery. In fact, IMViC is an abbreviation that stands for the Indole, Methyl red, Voges-Proskauer, and Citrate utilization tests. These four tests are used to differentiate members of the family Enterobacteriaceae.

**Antimicrobial susceptibility tests**

According to the standard operational procedures, antimicrobial susceptibility of the *E. coli* isolates was established by the disk diffusion assay with MH (Mueller-Hinton) agar.

The strains were assessed for AMR. In fact, colistin (50µg); amoxicillin (25µg); tetracycline (30µg); flumequine (30µg); enrofloxacin (10µg); sulfamethoxazole-trimethoprim (23.75µg); doxycycline (30µg); fosfomycin (200µg), and florfenicol (30µg) were used to test the susceptibility of isolated *E. coli.* The results of antibiograms were interpreted as recommended by the French microbiology society (CA-SFM 2023), with cut-off values for susceptible, intermediate, and resistant phenotypes.

## RESULTS

During the period between 2019 and 2024, a total of 93 samples were analyzed for isolation of *E. coli* and antimicrobial susceptibility testing from boiler farms belonging de ZALAR Holding in Morocco, Associated recorded data regarding diseases, and mortalities rates of broiler flocks involved (Table 1). The present investigations have revealed that *E. Coli* infection could occur at different ages in poultry production and its generally a second complication of primary infection. Nevertheless, *E. coli* is usually identified in association with other pathogens, mainly, respiratory viruses such as, H9N2. Bacterial diseases were also identified, in fact, *Mycoplasma gallisepticum* (MG) were the frequent agents in the sampled poultry farms.

Antibiogram profiles of the 93 APEC strains isolated from dead broiler presenting severe colibacillosis lesions showed very high resistance frequency to most common antimicrobials prescribed in poultry medicine (Table 2). These results show very high levels of resistance to most antibiotics. Therefore, resistance to amoxicillin was 94.62%, followed by doxycycline (88.17%), tetracycline (84.95%), flumequine (82.80%), enrofloxacin (48.39%), sulfamithoxasole-trimethoprim (46.24%), and florfenicol (27.95%). However, a low level of resistant strain was identified towards Fosfomycin (7.53%) and Colistin (6.45%).

The overall rate of multiple antimicrobial resistance is 100% and none of the isolates is sensitive to all the nine antimicrobials tested (Table 3). This table shows that 93.55% of isolated *E. coli* strains are multi-resistant (resistance against 3 antibiotics and more).

## DISCUSSION

APEC strains isolated during the study period showed a high frequency of resistance to several antimicrobial families. These results align with those from a previous study on breeders in Morocco between 2018 and 2021, where 75 *E. coli* clinical strains were resistant to more than half of the tested antibiotics, except fosfomycin (6.1% resistant strains) and no resistance against colistin and florfenicol (Oubouyahia et al. 2022).

In broilers, a previous Moroccan study also reported an *E. coli* resistance rate above 50% for most antibiotics, except fosfomycin (16.1%) and colistin (2.94%) (Rahmatallah 2020). The most sensitive antibiotics against *E. coli* were colistin, florfenicol, and fosfomycin. These antibiotics are rarely used in coli-septicemia treatment for various reasons: colistin requires delicate administration by injection, florfenicol is associated with a drop in fertility, and fosfomycin has controversial efficacy despite high sensitivity on antibiograms.

A similar study conducted by Rahmatallah et al. (2017) on antibiotic susceptibility testing has identified a high resistance rate for oxytetracycline (100%), amoxicillin (90.9%), trimethoprim-sulphamethoxazole (82.2%), enrofloxacin (75.9%), and florfenicol (61.5%). Conversely, low resistance rates were observed for gentamicin (24.8%), fosfomycin (16.1%), and colistin (2.94%) (Rahmatallah et al. 2017).

**Table 1:** Associated recorded data regarding diseases, and mortalities rates of broiler flocks

| Age | | Diseases | | | | | | Rate of mortalities | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 18 – 28 days | 30 – 41 days | H9N2 | NDV | IBDV | MG | Enteritis | Only *E. Coli* | Less than 5% | 5 to 10% | Up to 10% |
| 29 | 64 | 71 | 3 | 8 | 5 | 9 | 3 | 41 | 46 | 6 |

**Table 2:** E. coli antibiogram profiles from broiler involved in study

| Antibiotic | Resistant | | Intermediate | | Susceptible | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % | Number | % |
| Colistin | 6 | 6.45 | 0 | 0.00 | 87 | 93.55 | 93 | 100.00 |
| Amoxicillin | 88 | 94.62 | 2 | 2.15 | 3 | 3.23 | 93 | 100.00 |
| Tetracycline | 79 | 84.95 | 4 | 4.30 | 10 | 10.75 | 93 | 100.00 |
| Flumequin | 77 | 82.80 | 2 | 2.15 | 14 | 15.05 | 93 | 100.00 |
| Enrofloxacin | 45 | 48.39 | 9 | 9.68 | 39 | 41.94 | 93 | 100.00 |
| sulfamethoxazole-trimethoprim | 43 | 46.24 | 4 | 4.30 | 46 | 49.46 | 93 | 100.00 |
| Doxycycline | 82 | 88.17 | 5 | 5.38 | 6 | 6.45 | 93 | 100.00 |
| Fosfomycin | 7 | 7.53 | 0 | 0.00 | 86 | 92.47 | 93 | 100.00 |
| Florfenicol | 26 | 27.96 | 0 | 0.00 | 67 | 72.04 | 93 | 100.00 |

**Table 3:** *E coli* multi drug resistance

| | Number | Percentage |
|---|---|---|
| R0 | 0 | 0.00 |
| R1 | 0 | 0.00 |
| R2 | 6 | 6.45 |
| R3 | 9 | 9.68 |
| R4 | 9 | 9.68 |
| R5 | 26 | 27.96 |
| R6 | 26 | 27.96 |
| R7 | 10 | 10.75 |
| R8 | 7 | 7.53 |
| R9 | 0 | 0.00 |
| Total | 93 | 100.00 |

R0: sensitive to all tested antimicrobials. R1, R2, R3, R4, R5, R6, R7, R8, R9: resistant to 1…. 9 antimicrobials respectively.

The most concerning aspect of our results is that all tested APEC strains were multi-resistant (i.e., resistant to three or more antibiotic families). Although this was not unexpected—previous studies indicated that 93% of Moroccan broilers had received at least one antibiotic treatment (Rahmatallah and El Rhaffouli 2018). These results underscore the urgent need to reduce antibiotic usage in the Moroccan poultry industry. Furthermore, the potential contamination of poultry meat by *E. coli* strains with zoonotic potential (Manges and Geum 2019; Hend and Hashad 2023) raises public health concerns. Wider use of autogenous vaccines could help reduce antibiotic usage, limit the selection of AMR on farms, and improve poultry production (Keita and Le Devendec 2022), while adding value to the downstream segment of broiler production.

*E. coli* resistance against antibiotics in Morocco has been documented since the late 1980s (Filali 1986; Amara et al. 1995). It remains a significant public health problem and continues to evolve (Baaj and Lahlou 2002). Compared with previous studies shows that antibiotic resistance is not a new phenomenon. For instance, as early as 1988, Filali et al. found an 82% resistance rate to oxytetracycline (Filali et al. 1988). Amara et al. (1995) detected a 23% resistance rate to quinolones.

Our results indicate that *E. coli* resistance to certain antibiotics is increasing in Morocco. A comparison with the French antibiotic resistance surveillance network (Resapath 2014) shows worrying resistance rates in Morocco, with a 12% enrofloxacin resistance rate in France versus 76.38% in Morocco. Jaouzi et al. (2004) observed evolving resistance rates between 1998 and 2003: chloramphenicol (65% in 1999), oxytetracycline (90% in 1999), amoxicillin (65% in 1999), flumequine (43% in 2001), sulfamethoxazole-trimethoprim (68% in 2003), enrofloxacin (34% in 1999), and colistin (13% in 2003). An additional study published by El Houadfi and Zekhnini (2009) on day-old broiler chicks showed resistance rates of 96% to oxytetracycline and ampicillin, 72% to amoxicillin, and 20% to chloramphenicol, colistin, and enrofloxacin.

The comparison with Algeria shows similarly high resistance rates, except for colistin, with enrofloxacin resistance at 45% in Algeria versus 76% in Morocco (Aggad et al. 2010). Hafed et al. (2015) noted increasing resistance trends: enrofloxacin resistance rose from 23% (Amara et al. 1995) to 76%, and oxytetracycline and colistin resistance rose from 65 and 0.4% in 1995 to 97.05% and 12.58% in 2016. Their study also has showed high sensitivity to colistin (87.41%), spectinomycin (52.94%), and sulfamethoxazole-trimethoprim (67.56%), while marked resistance was observed for oxytetracycline (97.05%), amoxicillin (88.23%), enrofloxacin (76.36%), Doxycycline (73.52%), and cefalotin (60%). Moderate to low sensitivity was observed with nalidixic acid (50.20%) and flumequine (48.88%).

The increased resistance to beta-lactams and fluoroquinolones is particularly concerning. For example, resistance to ampicillin rose from 14% in 1995 to 96% in 2009. Amoxicillin, not used in poultry in 1995, saw resistance rates rise from 65% in 1999 to 90.09%. Enrofloxacin resistance increased from 23% in 1995 and 34% in 2003 to 76.38% in 2016.

Regarding multidrug resistance (resistance to more than three antibiotics), our study found a rate of 93.55%, which is consistent with a previous study in Morocco which showed that 83.3% of *E. coli* strains isolated from broiler meat were multidrug-resistant (Mounaim et al. 2020).

## Conclusion

The results of this retrospective study show respectively high antimicrobial resistance rates to, amoxicillin, doxycycline, tetracycline and flumequine. However, Fosfomycin and colistin remains the two best options for effective treatment against *E. coli* in the study area. Antibiogram profiles obtained from APEC strains isolated from broiler show a high level of AMR and multi drug resistance in the Moroccan poultry industry, which should be tackled urgently. Monitoring antimicrobial susceptibility is recommended to ensure a well-reasoned choice of antibiotic against *E. coli* and thus avoid and slow down the phenomenon of AMR toward this pathogen.

In conclusion, the problem of AMR is currently a major obstacle to poultry production and a public health risk in Morocco, and must be taken seriously by all stakeholders, in line with the "One Health" approach.

**Author's Contribution:** Lhoussaine Oubouyahia, Saadia Nassik, and Ouafaa Fassi Fihri conceived and planned the experiments; Lhoussaine Oubouyahia carried out the experiments. Amal Essalah-Bennani, Eric Badin and Hanane Balil contributed to sample preparation. Lhoussaine Oubouyahia, Eric Thibault and Hubert Gantelet contributed to the interpretation of the results. Lhoussaine Oubouyahia took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript. All authors have read and agreed to the published version of the manuscript.

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**Institutional review board statement:** All animal in the present study died, and all procedures were conducted in agreement with the recommendations of the Hassan II Agronomy and Veterinary Institute of Rabat and the Moroccan Ministry of Agriculture, which are in accordance with international ethical legislation (European Union

Directive 2010/63/EU) and complied with ARRIVE (Animal Research Reporting of *In Vivo* Experiments) guidelines (https://arriveguidelines.org/).

**Data availability statement:** The data presented in this study are the propriety of Zalar Holding poultry group and may be available from the corresponding author upon reasonable request.

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**Conflicts of interest:** The authors declare they have no conflict of interest.

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