***Original Research Article***

**Phenotypic Characterization of the Resistance of *Klebsiella pneumoniae* Strains Isolated from Poultry Droppings in the Abidjan District**

**ABSTRACT**

Poultry farming, a promising sector in Côte d'Ivoire, is confronted with avian pathologies leading to heavy losses at several levels. **Objective:** The objective of this study is to determine the antibiotic resistance profile of *Klebsiella pneumoniae* strains circulating in poultry farms in the Abidjan District of Côte d'Ivoire. **Methods**: the methods used were isolation, bacteriological identification, and antibiotic susceptibility testing **Results**: the results reveal that 192 strains of *Klebsiella pneumoniae* were isolated from 414 cloacal swabs collected in three municipalities of the District of Abidjan. The determination of bacterial susceptibility showed high resistance rates of 88.33% to kanamycin, 61.7% to trimethoprim-sulfamethoxazole and 56.7% to tetracycline, 30% for quinolones and fluoroquinolones in the municipality of Cocody, 66.6% to amoxicillin/clavulanic acid, 33.3% to cefepime and cefoxitin in the municipality of Bingerville. In the municipality of Yopougon, only resistance to cefoxitin was observed. However, no resistance was observed in the presence of ceftazidime, gentamicin, amikacin. **Conclusion**: These results highlight the importance for poultry farmers to adopt a biosecurity approach in order to reduce the use of antibiotics and decrease the rate of resistance.

**Keywords**: *Klebsiella pneumoniae*, Poultry, Resistance, Antibiotics, Abidjan District

**1. INTRODUCTION**

Côte d'Ivoire is a country located in West Africa and whose economy is based on agriculture. It is characterized by galloping population growth influenced by high fertility, low mortality and marked by a large influx of migrants from the sub-region, particularly to urban areas including Abidjan. Its population is 29,389,150 inhabitants in 2021 (CICG, 2021). This rapid growth of 2.9% on average/year has resulted in the insufficiency of natural food resources to meet the essential needs of the population, particularly the needs of foodstuffs of animal and aquatic origin. In the 1980s, Côte d'Ivoire initiated a policy of food self-sufficiency focused on short-cycle livestock production such as poultry production (CICG, 2021). In 2017, Ivorian poultry production was estimated at 49,000 tons of poultry meat with 250 billion CFA francs in turnover and 250,000 direct and indirect jobs (Firca, 2017; Rokiatou et al., 2023). It ensures the coverage of animal proteins by providing 88% of meat production and 100% of table eggs (Koné & Danho, 2008). As in the entire animal sector, poultry farming is subject to a massive and permanent use of antibiotics to improve animal growth, which significantly influences the microbiological quality of the meat. Antibiotics are incorporated as feed additives in compound feeds, pellets or "mash", with the aim of improving growth and feed efficiency (Dawadi et al., 2021). The increasing use of antimicrobial substances in the animal sector leads to significant human exposure to antimicrobial-resistant bacteria (AMR), indirect gene transfer between bacterial species, and the spread of antimicrobial-resistant bacteria in the environment (Silbergeld et al., 2008). The unintentional use of antibiotics in poultry has led to the emergence of multidrug-resistant organisms that pose a potential threat to human health (Luangtongkum et al., 2006; Miranda et al., 2008). The increase in antibiotic resistance in bacteria in humans and animals is a major concern, as it may decrease the effectiveness of medical care (OMS, 2020). The bacteria frequently detected in birds that influence the health and production of animals are composed mainly of enterobacteriaceae such as *Salmonella*, *Escherichia coli*, *Proteus*, *Klebsiella*, *Enterobacter* and other non-fermentative bacteria such as *Pseudomonas*. (Gwida et al., 2014). The presence of these pathogens in poultry feces and poultry products raises concerns related to hygiene and food processing, due to the growing appreciation of poultry meat among consumers (Lutful Kabir, 2010). The objective of this study is to determine the level of resistance of *Klebsiella pneumoniae* isolated from poultry in the Abidjan district of Côte d'Ivoire.

**2. Materials and methods**

**2.1. Study area**

The study took place in three (3) municipalities of the district of Abidjan, namely, Cocody, Yopougon and Bingerville. The district of Abidjan located at 5°20′ 00" N, 4° 01′ 00" W is composed of 13 municipalities that extend over 2119 km2; it is the most populous city in French-speaking West Africa with more than 6321 million inhabitants (UN, 2021). The Greater Abidjan area (26°C on average between 1961-2016) is humid (82% with rainfall reaching 1,675 mm). It has two rainy seasons and two dry seasons (Kouao et al., 2024) . It is characterized by abundant rainfall (1500 mm of water/year) and an average temperature of 27°C and the average annual humidity level is more than 80%.

**2.2. Type of study**

This is a prospective study that took place over a six-month period from January to June of the year 2024. Data were collected from the fact sheets developed for this purpose. This information included general information about farms and markets, the size and age of animals, sample numbers, nature of collection, origin, and the antibiotics frequently used in treatments.

**2.3. Material**

**Collection sites**

The choice of collection sites was motivated by the presence of private poultry farms or poultry markets, where live poultry can be purchased and even slaughtered directly for personal consumption. In the municipalities, farms were selected on the basis of a population of at least 500 broiler poultry or egg layers for consumption and intended for sale for the consumption of local populations. As far as markets are concerned, our choice was mainly for the sales stands where there is a slaughtering unit, also known as a "slaughterhouse". These sellers sell poultry of all types (meat, layers, guinea fowl, etc.) and have them killed immediately at the customer's request.

All poultry used in the study were purchased and harvested.

**Study population**

The population of animals selected for this study consisted mainly of broilers and layers of eggs for human consumption. The breeds of poultry are those that are generally raised in Côte d'Ivoire

**Inclusion criteria**

The study concerned adult poultry (36-40 days for broilers and at least 18 months for layers) that were apparently healthy, i.e. without observable pathological signs and which could be sold for human consumption at any time. The agreement of the owner or seller was also decisive for our choice.

**Exclusion criteria**

Farms with broiler poultry less than 36 days old, laying hens and sick poultry, as well as farms undergoing treatment, were not included in the study. Similarly, poultry sellers who refused to participate were excluded from the study.

**Biological material**

The biological material used for bacteria testing is made up of poultry droppings taken from live poultry using sterile cotton swabs.

**2.4. Methods**

**Sampling**

Sampling was carried out randomly at the different sampling sites. For the municipalities of Yopougon and Bingerville, sampling was carried out on poultry farms and for the municipality of Cocody, it was carried out on poultry markets. The sample size was calculated according to the Schwartz formula (Pourhoseingholi et al., 2013)

**Sample collection**

At each collection site visited, the objectives of this study had to be explained in order to obtain the agreement of the poultry owner or sellers to participate in the study. At each visit, 5 live poultry were bought as part of the study either from the farmers or from the market vendors and collected. With the help of a sterile swab inserted into the cloaca, a circular movement is exerted on the cloacal wall in order to collect faecal matter. The swab covered with fecal matter is then removed from the vent and placed in a tube containing approximately 2 mL of sterile buffered peptone water (BPW). The tubes were immediately placed in a cooler containing cold accumulators and then transported to the laboratory of the National Reference Center for Antimicrobial Resistance (CNR-RAM) at the Institut Pasteur de Côte d'Ivoire (Adiopodoumé site) without breaking the cold chain. Fecal samples were randomly collected from poultry farms and markets, based on the willingness of the farm owners to participate in the study and the accessibility of the farms.

**Collection form**

A collection sheet was used to collect field data on farms and markets as well as data on animals. This information concerned: general data on the farm, the number and type of animals as well as the treatments administered.

**Ethical Approval and Informed Consent**

This study did not require formal ethical approval, but consent from the poultry farm owners was obtained verbally prior to sample collection.

**Bacteriological analyses**

Seeding, isolation and identification techniques are the usual techniques of bacteriology commonly used by the bacteriology laboratory of the Institut Pasteur de Côte d'Ivoire. The identification of *Klebsiella pneumoniae* was based on the morphological appearance of the colonies on the Eosin Methylene Blue (EMB) agar medium giving large colonies more or less mucous, on the cultural characteristics of the bacterium, namely the Gram-negative bacilli, from 0.3 to 1.0 μm in diameter by 0.6 to 6.0 μm in length, immobile, non-spore-forming, aero-anaerobic and on its biochemical characteristics: ferments glucose with gas production, negative oxidase, positive catalase, has a nitrate reductase. *K. pneumoniae* is: Voges-proskauer+ (VP+), lysine decarboxylase + (LDC+), ornithine decarboxylase – (ODC-), (Indole) IND-, Citrate+, Urea+, Ortho-nitrophenyl-β-galactoside + (ONPG+), Hydrogen sulfide - (H2S-), tryptophan deaminase – (TDA-), reduces nitrates to nitrites (NO3+) and then confirmation with Maldi-Tof® (Biomerieux, France) was made in case of doubt.

**Determination of bacterial susceptibility: the antibiogram**

Kirby Bauer's modified WHO-recommended method based on diffusion from antibiotic-impregnated discs on Muller-Hinton agar (Kashosi et al., 2018) was used to perform the antibiotic susceptibility test. Bacterial suspensions were prepared in a salt solution to achieve turbidity equivalent to that of the 0.5 standard of the McFarland range and with the help of sterile swab sticks dipped in the suspension. Each isolate was inoculated with tight streaks over the entire surface of the agar. The inoculum was evenly distributed over the entire surface of the agar, taking care not to leave any space between the streaks. Antibiotics commonly used in the treatment of Gram-negative bacilli infections were selected: Amoxicillin-acid/clavulanic (20/10 μg), Amoxicillin (20 μg), Ticarcillin (75 μg), Cefoxitin (30μg) Cefepime (30μg); Rifampicin (5μg); Tetracycline (30μg), Gentamicin (10μg), Amikacin (30μg), Nadidixic acid (30μg), Sulfamethoxazole/trimethoprim (25μg), Ceftazidime (30μg), Enrofloxacin (5μg). The reading was made by measuring the diameters of these bright areas (inhibition zone) using the ADAGIO automatic reader® of BioRad-France and the interpretation was made according to the standards of the EUCAST-CASFM version 2023. The reference strain of *E. coli* ATCC25922 was used as quality control for antibiotic susceptibility testing.

**Detection of the ESBL phenotype according to the double synergy test**

All strains of *Klebsiella pneumoniae* isolated from poultry were detected for the production of extended-spectrum beta-lactamase by the double diffusion or double synergy assay. It was conducted under the standard conditions of the antibiogram. Ceftazidime and cefepime discs were placed around an amoxicillin/clavulanic acid disc on Müller-Hinton agar (approximately 15 mm) and then incubated at 37°C for 24 hours. The appearance of an image in the shape of a champagne cork reflects the production of an extended-spectrum beta-lactamase by the strain of*Escherichia coli* Tested (Drieux et al., 2008).

**Statistical analysis**

The data obtained were recorded with Excel software to produce descriptive statistics. The results obtained were reported on a sheet and recorded on a computer file, the Excel® software. These inhibition diameter values were interpreted (S= sensitive, I= intermediate, R=resistant) according to the EUCAST-CASFM 2023 reference (CA SFM, 2023). The data was analyzed by the Graph Pad 5 software. The Chi-2 test was used to determine a possible correlation between different variables for a significance threshold α = 0.05.

**3. RESULTS**

**3.1. Strains collected**

A total of 414 cloacal swabs obtained from broiler poultry and egg layers were sampled in three municipalities of the District of Abidjan: Cocody, Yopougon and Bingerville. The distribution of samples by collection site is recorded in **Table I**. From the various cloacal swabs, 414 bacterial strains were isolated. These isolates were composed of 50.7% *Escherichia coli* (n=210) and 46.7% *Klebsiella pneumoniae* (n=192) and 2.9% *Enteroabacter cloacae* (n=12).

**Table I: Distribution of samples by collection site**

|  |  |  |
| --- | --- | --- |
| Municipality | Number of samples (n) | Proportion (%) |
| Cocody | 348 | 84,10 |
| Yopougon | 39 | 9,40 |
| Bingerville | 27 | 6,50 |
| Total | 414 | 100 |

**3.2. Distribution of *Klebsiella pneumoniae* strains according to collection sites**

The search for bacteria in poultry droppings detected the presence of 192 strains of *Klebsiella pneumoniae*. The majority of these germs (93.75%; 180/192) came from the municipality of Cocody. Figure **1** shows the distribution of *the Klebsiella pneumoniae* strains studied according to the collection sites.

**Figure 1:** Overall distribution of *isolated Klebsiella pneumoniae* strains by study site

**3.3. Resistance profile of *Klebsiella pneumoniae* to the antibiotics tested according to the collection sites**

The highest resistance rates were observed in the municipality of Cocody in the presence of kanamycin (88.3%), trimethoprim/sulfamethoxazole (61.7%), tetracycline (56.7%) and amoxicillin/clavulanic acid (43.3%). However, excellent antibacterial activity was observed with regard to the other antibiotics tested.

No resistance was detected in Bingerville in the presence of the majority of antibiotics, however, high rates of resistance were reported for betalactams such as amoxicillin/clavulanic acid (66.6%) and (33.3%) for cefepime and cefoxitin.

In the municipality of Yopougon**,** with the exception of cefoxitin, which had a resistance rate of 100%, total bacterial susceptibility was described in the presence of the other antibiotics tested **(Table II).**

The results of this study clearly show that antibiotic resistance rates vary significantly between study sites (*p<0.05).*

**Table II:** Resistance profile of *Klebsiella pneumoniae* strains isolated from poultry manure by study site

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Tested antibiotics | Cocody  n = 180 (%) | Bingerville  n = 9 (%) | Yopougon  n = 3 (%) | *P-value* |
| Amoxicillin+clavulanic acid | 78 (43,3) | 6 (66,7) | 0 (0) | *p*<0.05 |
| Cefoxitin | 0 (0) | 3 (33,3) | 3 (100) |
| Ceftazidime | 0 (0) | 0 (0) | 0 (0) |
| Cefepime | 18 (10) | 3 (33,3) | 0 (0) |
| Meropenem | 1 (11,1) | 0 (0) | 0 (0) |
| Gentamicin | 0 (0) | 0 (0) | 0 (0) |
| Amikacin | 0 (0) | 0 (0) | 0 (0) |
| Kanamycin | 159 (88,3) | 0 (0) | 0 (0) |
| Trimethoprim+sulfamethoxazole | 111 (61,7) | 0 (0) | 0 (0) |
| Tetracycline | 102 (56,7) | 0 (0) | 0 (0) |
| Nalidixic acid | 36 (20) | 0 (0) | 0 (0) |
| Enrofloxacin | 18 (10) | 0 (0) | 0 (0) |
| Amoxicillin | 0 (0) | 0 (0) | 0 (0) |
| Ticarcillin | 33 (18,3) | 0 (0) | 0 (0) |

**III.4. Phenotypes expressed of *Klebsiella pneumoniae* strains at study sites**

The resistance phenotypes expressed by *Klebsiella pneumoniae* strains were distributed as follows:

* The "FQR" phenotype (cross-resistance to quinolones and fluoroquinolones) observed in 30% of strains from Cocody;
* The "C3GR" phenotype (resistance to 3rd generation cephalosporins) reported at low levels in the municipalities of Cocody (10%) and Bingerville (33%),
* The phenotype of Emerging Highly Resistant Bacteria (BHRe) "C3GR/CARBAR/FQR" (resistance to 3rd generation cephalosporins, carbapenems and quinolones and fluoroquinolones) observed in an isolated strain (1.6%) in the municipality of Cocody;
* The "WILD" phenotype (absence of acquired resistance) was described in proportions of 43.3% and 66.7%, respectively, in strains from the Cocody and Bingerville sites **(Figure 2).**

**Figure 2**: Distribution of phenotypes expressed with regard to the antibiotics tested

**IV. Discussion**

The objective of this study was to determine the level of antibiotic resistance in *Klebsiella pneumoniae* isolated from poultry droppings in the District of Abidjan. Bacteriological analysis led to the identification of 192 strains of *Klebsiella pneumoniae* on all the droppings collected, with a strong presence in the municipality of Cocody, i.e. 94%. This result is in agreement with the literature data. Indeed, some authors claim that *Klebsiella pneumoniae* is a ubiquitous bacterium, present in both the digestive tract of humans and animals, in the soil and water, where it can become pathogenic under certain conditions (Bao et al., 2013; Yu et al., 2007). Several factors could explain this high number: (i) an abnormal water insufficiency condition, (ii) excessive humidity (>50%) and a temperature that is too low due to cold bedding when introducing the chicks, (iii) inadequate implementation of internal and external biosecurity protocols (Puterflam et al., 2022). The presence of *Klebsiella pneumoniae* in poultry droppings was reported in Nigeria and Mali with 4.9% and 54.54% identification respectively (Adebowale & Adeyemo, 2018; Sidibé, 2020) and 67.1% in Togo (Bedekelabou et al., 2020a)**.**

Regarding antibiotic resistance in strains of *Klebsiella pneumoniae*, all the strains tested showed no resistance to gentamicin, amikacin. A high resistance to aminopenicillins in this bacterium (43.3%) in Cocody and (66.7%) in Bingerville could be explained by the intrinsic resistance of the bacterium, by the absence of resistance markers and by the low selection pressure for the development of antibiotic resistance. The results of this study are much lower than those of Kebaili in 2019 which show (100%) resistance to amoxicillin + clavulanic acid (KebailiI & Azmani, 2019) while those in Togo show lower rates (8%) (Bedekelabou et al., 2020b).

Regarding other antibiotics, the study shows high rates of resistance to kanamycin (88.33%), trimethoprim-sulfamethoxazole (61.66%) and tetracycline (56.66%). The results of this study are significantly lower than those of Togo, which give tetracycline (96.2%), trimethoprim-sulfamethoxazole (66.7%). The intensive and exclusive use of these antibiotics as a prophylactic measure may have encouraged the emergence of resistant strains (Chauvin, 2009) in the poultry industry, which could contribute to the presence of such strains in the Abidjan district. Resistance to kanamycin and trimethoprim-sulfamethoxazole could be problematic in the event of human contamination with such strains, as these two molecules are respectively used in the treatment of human infections. It is crucial to note that Enterobacteriaceae have a demonstrated ability to acquire and exchange genes containing resistance factors, and the gut flora provides an exceptional opportunity for the transfer of genetic information between bacteria (Van Immerseel et al., 2004).

The presence of *Klebsiella pneumoniae* The Poultry Resistance Workshop in the Abidjan District highlights the importance of an integrated One Health approach, which considers the link between antimicrobial resistance (AMR) in farm animals and humans, in order to effectively combat this problem (Galán-Relaño et al., 2023). Stringent measures, including increased control of agricultural practices, good husbandry practices through the implementation of effective and efficient biosecurity measures, and control of food for human consumption, are essential to reduce the risk of transmission of these resistant strains. Finally, the implementation of a surveillance plan for pathogenic bacteria in a "One Health" context in accordance with the recommendations of the Tripartite (WHO/FAO/WOAH) is essential.

**V. Conclusion**

This study sheds light on the detection of *Klebsiella pneumoniae* in poultry droppings, with high levels of resistance to antibiotics such as tetracycline, trimethoprim-sulfamethoxazole and rifampicin that may pose a public health concern at the human level

These findings highlight the urgent need to improve the control of antibiotic use on farms and to promote a comprehensive One Health strategy to curb the transmission of antibiotic-resistant bacteria between animals and humans.

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