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*Original article*

# VETERINARY USE OF ANTIBIOTICS AND CONTAMINATION OF PORK CONSUMED IN CÔTE D'IVOIRE BY OXYTETRACYCLINE, TYLOSIN AND AMOXICILLIN RESIDUES

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**Summary**

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This study aimed to evaluate the concentrations of three veterinary antibiotics in pig meat consumed in the District of Abidjan, Côte d'Ivoire: oxytetracycline, tylosin and amoxicillin. Muscle samples taken from 90 pigs slaughtered at SIVAC (Ivorian Slaughtering and Cold Cuts Company) of Yopougon (Abidjan, Ivory Coast) were analysed by the "Premi®Test" analysis kit. The concentrations of oxytetracycline, tylosin and amoxicillin residues were measured using high performance liquid chromatography. Of the 90 samples analysed, 31.11% were contaminated with oxytetracycline, tylosin and amoxicillin. The average level of contamination of pork meat with oxytetracycline residues was 488.57 ± 285.95 µg/kg, Those of tylosin and amoxicillin residues were 353.21 ± 391.51 and 279.64 ± 104.82 µg/kg respectively. Compared to the reference values, the amonts of residues found in pork were well above the tolerated values. This study confirms the presence of oxytetracyclinw, tylosin and amoxicillin residues in pork meat sold in Abidjan. The levels of contamination with the three antibiotics did not depend on the sex and breed of the animals. It is therefore urgent to undertake correlational studies linking the consumption of pork in the District of Abidjan (Côte d'Ivoire) and the occurrence of metabolic diseases.

**Key words**: amoxicillin, antibiotics, contamination, HPLC, oxytetracycline, pig meat, tylosin

## INTRODUCTION

Pork meat is widely consumed in various forms by the population of Abidjan. This fact is linked to traditional consumption of pork (Koffi *et al.*, 2021) and a policy of covering the animal protein needs of Côte d'Ivoire oriented mainly towards four production sectors including the pork sector. However, various illnesses after con-

suming pork have been reported (Offianan *et al.*, 2015; Atobla *et al.*, 2018; Kouamé *et al.*, 2019).

The nutritional value and hygiene have been cited as causes of diseases relative to pork meat. Studies have reported that due to its composition and lack of hygiene, pork is an excellent environment for the proliferation of bacteria such as *Escherichia coli, Staphylococcus aureus, Clostridium* spp. and *Salmonella* (Bout & Girard, 1988; Assidjo *et al.*, 2013; Magqupu *et al.*, 2023). However, it should be noted that the above-mentioned causes cannot be the only factors to blame since Côte d'Ivoire still has no reliable quality control system for basic animal products, particularly pork (Koffi *et al.*, 2021).

Faced with this worrying situation, a broader global approach must be followed which must include the supply and marketing chain (Atobla *et al.*, 2018; Magqupu *et al.*, 2023) and the the pork production system (Koffi *et al.*, 2023). A scientific assessment of the pork industry production system is therefore required to guarantee the health of consumers. Indeed, animal products may contain residues from veterinary drugs that can have deleterious effects on human heath (Ture *et al.*, 2019; Arsɯne *et al.*, 2022; Bancali, 2024) and development of antimicrobial resistance (Caneschi *et al.*, 2023; Wu & Zeng, 2024). Unfortunately, these proportions of residues in animal products are exacerbated by poor practices in many developing countries. Non-compliance with prophylaxis, failure to consider time limits before slaughter and poor quality of veterinary medicines is observed in these countries (Mouiche *et al.*, 2019; Bedekelabou *et al.*, 2022; Ragassa & Berhanu, 2023). Thus, monitoring sales of veterinary antibiotics and knowing the level of veterinary antibiotic residues in animal products in general and pork in particular would be an important source of information for risk prevention and also an instrument decision. The work already carried out in this context, was focused on the situational analysis of the use of veterinary antibiotics in Côte d'Ivoire (Kaba *et al.*, 2013). These investigations performed an analysis of the final results of authorisations to import veterinary medicines for wholesalers in Côte d'Ivoire in 2013 and showed that various antibiotic molecules had been used in veterinary medicine in the country in 2013. The veterinary products came from eight countries with France, Belgium and the Netherlands being the main suppliers. Sulfonamides with tetracyclines and betalactams were the most used drug families. Antibiotics were mainly used for the treatment of animals intended for human consumption (Kaba *et al.*, 2013).

The present study was carried out as a logical continuation of that previous research. Its objective was to evaluate the concentrations of three veterinary antibiotics in pig meat consumed in the District of Abidjan.

## MATERIALS AND METHODS

### *Ethical statement*

The study was approved by the Direction of Veterinary Services, Ministry of Animal and Fisheries Resources and Use Committee of Félix Houphouët-Boigny University. The study was conducted in accordance with the local legislation and institutional requirements.

### *Period and site of the study*

This study was realised from 1 February to 30 March 2019 at SIVAC (Ivorian Slaughtering and charcuterie Company) of

Yopougon (District of Abidjan). The district has more than five million inhabitants and covers an area of 2119 $km^2$. SIVAC is one of the leading companies in the slaughtering, processing, marketing and charcuterie of pig, and is located within the industrial zone of the commune of Yopougon, in the western part of the District of Abidjan.

*Pig muscle samplings*

The sample size was determined using the logarithmic table. For a confidence level of 95% and a prevalence of 10%, the sample size was estimated at 90 pig muscle samples. The selection of the animals slaughtered in this study was made randomly. To this end, the animals of several owners have previously been mixed in the livestock pen. The sampled animals were randomly selected among the animals entering the slaughter room at a given time. The removed muscles were kept in containers, each bearing the number that the animal had when it entered the slaughter room (Chantal & Thomas, 1976). Each muscle sample was first packaged in aluminum foil, then in a plastic bag and finally in a collection container bearing the animal number. All the samples were immediately placed in a cooler before being stored in a freezer at –20 °C. Each sample was accompanied by additional information relating to animal breed and sex.

*Sample processing*

Each meat sample was pressed using a multi-press until 250 µL of juice was obtained. Then 100 µL juice were then taken and placed in the test ampoules. These were left at room temperature for a 20 min pre-incubation. Then, the meat juice was carefully removed from the ampoule by rinsing with deionised water. The ampoules test was then covered and supplied with the kit and then incubated at 64.5°C for approximately 3 h. A negative control was carried out in parallel and allowed stopping the incubation as soon as it completely turned yellow. Positive samples were confirmed by a physicochemical method for identification and quantification.

*Detection of antibiotic residues*

Analysis kits Premi®Test (R-Biopharm AG, Germany) were used for the detection of antibiotic residues in pig muscles. It is a standardised test validated by AFNOR which allows the detection of antimicrobial substances in meat. Premi®Test is based on the inhibition of the development of *Bacillus stearothermophilus*, a bacterium very sensitive to many antibiotic and sulfonamide residues. A standardised number of spores is coated in an agar excipient containing some selected nutrients. When the meat juice added to a Premi®Test ampoule and incubated at 64 °C contains sufficient residues, the spores are inhibited and do not develop. The colour of the ampoule then remains purple and the test is considered positive. On the other hand, in the absence of residues in the added meat juice, the spores multiply at 64 °C (acidifying the zone). The ampoule then become yellow and in this case the test is considered negative.

*Quantification of antibiotic residues*

The levels of contamination of pig muscles were determined through quantification of antibiotic residues using the liquid chromatography analysis method. After cutting into small pieces and grinding the sample in a mortar, 5 g were weighed and transferred into a Erlenmeyer flask of 1000 mL. Then 50 mL of dichloromethane and 10 mL of double-distilled water were successively added. The mixture was

filtered through Whatman paper and the filtrate was placed in a separatory funnel; 1.5 g of sodium sulfate was added and the whole mixture was then shaken vigorously so as to obtain phase separation (aqueous phase above and organic phase below). The collected organic phase was filtered again on Whatman paper and the filtrate was evaporated to dryness in a rotavapor. After evaporation, 10 mL of methanol was added. The filtrate was purified using Bond Elut (C18) cartridges and the antibiotics were removed with 5 mL of methanol. The filtrate was packaged in a chromatographic tube and the analysis of antibiotic residues was carried out by High Performance Liquid Chromatography (HPLC). The residues were detected and quantified by a Shimadzu chromatograph coupled to an SPD-20A(UV) detector under the operating conditions described in Table 1.

*Data analysis*

Data analysis was carried out using R software (version 3.6.3) which required the packages (ggplot2; Publish; multcomp; car). One-way analysis of variance (ANOVA) was used to compare the concentration of antibiotics between animal breeds. Antibiotic doses were also compared according to breed using Tukey's HSD *post hoc* test at 5% threshold. The second analysis consisted of evaluating the concentrations of residues in animals compared to the reference required by the Codex Alimentarius by means of ANOVA followed by Dunnett's *post hoc* test at 5% threshold. Also, for the comparison of means, ANOVA followed by Tukey's HSD *post hoc* test at 5% threshold was carried out.

## RESULTS

Out of 90 pig samples, twenty-eight (28) tested positive in the PremiTest, i.e. 31.11%. The breeds of pigs contaminated with antibiotic residues were Large white (LW), Landrace (LR) and Pietrain (P). Of examined male pigs, 32.14% were contaminated with antibiotic residues com-

**Table 1.** Operating conditions for residues dosage by using HPLC

| | | | |
|---|---|---|---|
| Column | Shim pack VP-ODS (250 L x 4,6 mm) | | |
| Mobile phase | HPLC quality acetonitrile 80%/ HPLC quality bi-distilled water 20% | | |
| Volume injected | 20 µL | | |
| Speed | 0.5 mL/min | | |
| Column temperature | 40 °C | | |
| Rinse solvent | Methanol | | |
| Analysis duration | 15 minutes | | |
| Standard concentration (mg/L) | 20 | | |
| | Oxytetracycline | Tylosine | Amoxicillin |
| Detector : SPD-20A (UV) | 280 nm | 254 nm | 254 nm |
| Maximum residue limit (MRL, mg/kg) | 0.10 | 0.20 | 0.05 |
| Detection limit (LD, µg/kg) | 0.052 | 0.041 | 0.013 |
| Limit of quantification (LQ, µg/kg) | 0.156 | 0.123 | 0.039 |
| Standard retention time | 4.32 | 2.54 | 1.32 |
| Extraction yield (%) | 98.34 | 07.30 | 97.45 |

**Table 2.** Distribution of the prevalence of antibiotic residues by sex and breed

| Variables | | Positive samples/ all samples | Prevalence | P-value (95%) |
|---|---|---|---|---|
| Breed | Landrace | 10/27 | 37.30 | 0.137 |
| | Large White | 14/36 | 39.88 | |
| | Duroc | 0/9 | 0 | |
| | Pietrain | 4/15 | 26.66 | |
| | Hampshire | 0/3 | 0 | |
| Sex | Male | 56/18 | 32.14 | 0.9809 |
| | Female | 34/10 | 29.41 | |

**Table 3.** Effect of sex and breed on antibiotic accumulation

| Active ingredients | Max | Min | Mean | Standard deviation |
|---|---|---|---|---|
| Oxytetracycline (µg/kg) | 950 | 0 | 488.57 | 285.95 |
| Tylosin (µg/kg) | 1260 | 0 | 353.21 | 391.51 |
| Amoxicillin (µg/kg) | 500 | 90 | 279.64 | 104.82 |

pared to 29.41% of females (Table 2). The statistical analysis showed that contamination levels were identical between races (P=0.13) and sexes (P=0.98).

The level of contamination of pig muscles with oxytetracycline was 488.57± 285.95 µg/kg and with tylosin was on average 353.21±391.51 µg/kg. Values oscillating between 90 and 500 µg/kg were found in samples contaminated with amoxicillin with an average of 279.64± 104.82 µg/kg. All these values are higher than the tolerated reference values (Table 3). The accumulation rate of oxytetracycline residues in tissues of LR pigs was 0.57 mg/kg (95% CI: 0.42 to 0.72 mg/kg). The values measured in LW and P pigs contaminated with oxytetracycline were 0.55 mg/kg (95% CI: 0.40 to 0.71 mg/kg) and 0.68 mg/kg, respectively (95% CI: -0.97 to 2.33 mg/kg) respectively. No significant difference was observed among the breeds of pigs (P≥0.05) (Table 3). The average accumulation rates for tylosin residues were 0.61 mg/kg (95% CI: 0.34 to 0.89 mg/kg) for LR, 0.28 mg/kg (0.03 at 0.53 mg/kg) for LW and 0.47 mg/kg (-3.15 to 4.10 mg/kg) for P. Amoxicillin residues were 0.26 mg/kg (95% CI: 0.23 to 0.36 mg/kg), 0.28 mg/kg (95% CI: 0.23 to 0.34 mg/kg) and 0.32 mg/ kg (95% CI: 0.27 to 0.38 mg/kg in the LR, LW and P pigs respectively. No significant difference (P≥0.05) was observed between the breeds (Table 3).

On average, the accumulation of oxytetracycline residues in males was 0.61 mg/kg (95% CI: 0.49 to 0.74 mg/kg) compared to 0.52 mg/kg (95% CI: 0.35 to 0.67 mg/kg) in females. Regarding tylosin residues, the amounts measured in females and males were respectively 0.50 mg/kg (95% CI: 0.20 to 0.79 mg/kg) and 0.42 mg/kg (CI 95%: 0.17 to 0.66 mg/kg). The measured amoxicillin concentrations were on average higher in males (0.31 mg/kg; 95% CI: 0.26 to 0.36 mg/kg) than in females (0.22 mg/kg; 95% CI: 0.16 to 0.28 mg/kg). The level of amoxicillin contamination in males was statistically higher than that in females (P<0.05). On the other hand, males and females presented

**Table 4.** Effect of sex and breed on antibiotic accumulation in the meat

| Active ingredients | Breed/ Sex | Sample number | Accumulation (mg/kg) | Comparison to the reference values | |
|---|---|---|---|---|---|
| | | | | CL (low) | CL (high) |
| Oxytetracycline | Breed | 23 | 0.57 | 0.478 | 0.662 |
| Ref Val: | Male | 14 | 0.61 | 0.490 | 0.737 |
| 0.2 mg/kg | Female | 9 | 0.52 | 0,354 | 0.0699 |
| Tylosine | Breed | 22 | 0.45 | 0.277 | 0.622 |
| Ref Val: | Male | 13 | 0.42 | 0.175 | 0,661 |
| 0.1 mg/kg | Female | 9 | 0.50 | 0.197 | 0.795 |
| Amoxicillin | Breed | 28 | 0.28 | 0.239 | 0.320 |
| Ref Val: | Male | 10 | 0.22 | 0.158 | 0.282 |
| 0.05 mg/kg | Female | 18 | 0.31 | 0.262 | 0.363 |

Ref Val: reference value; CL: confidence limit.

the same levels of contamination for oxytetracycline and tylosin (Table 3).

In terms of the combined incidence of sex and race on the accumulation of antibiotic residues, the results showed an average accumulation of 0.0406 mg/kg for oxytetracyline, 0.1454 mg/kg for tylosin and 0.0199 mg/kg for amoxicillin. No significant difference ($P \geq 0.05$) was observed between of sex and breed about the accumulation of antibiotic residues in pig muscles (Table 4).

The comparison of the levels of antibiotic residues measured to the reference values, has indicated that the quantities of residues found in the pig muscles were significantly above the reference values depending on sex and breed (Table 4). The multiple comparison test carried out has clearly shown a significant difference between the reference values and the amoxicillin contents contained in pig muscles depending on sex ($P=0.0404$). Also, for the same molecule of amoxicillin, a homogeneity of the combined effect of sex and race in the accumulation of residues was noted. No difference was observed in the accumulation of residues of the molecules of tylosin and oxytetracycline ($P>0.05$) depending on sex and breed (Table 4).

## DISCUSSION

The PremiTest was used in this study as qualitative method to assess the contamination of pig meat with antibiotic residues. This method is widely used in the field of food quality control because of its reliability (Shaltout, 2019). According to this analysis method, 31.11% of the pig samples analyzed were contaminated with antibiotic residues. The levels of contamination with oxytetracycline, tylosin and amoxicillin were well above the reference values tolerated in pork. This level of contamination is almost similar to those reported by Donkor *et al.* (2011) and Bor *et al.* (2023) in Ghana and Kenya respectively.

The arguments that can explain this contamination are at several levels of the production and marketing chain. The first argument relates to the fragility of the species selected for pig farming intensification programs. These are mainly geneti-

cally modified species such as Large White, Landrace and Pietrain. These species are less resistant to infections compared to local species called "African pigs". This fragility motivates excessive use of veterinary products for the prevention and treatment of pigs. The second argument is linked to the use of antibiotics by unqualified personnel. In fact, to avoid paying for the services of a veterinary doctor, breeders freely access veterinary products and administer large, inappropriate doses to animals with total ignorance of the pathology from which the animals suffer (Kantati, 2011). According to Ouattara *et al.* (2013), 73% of semi-industrial poultry farms do not consult a veterinarian and therefore resort to self-medication.

The third main reason concerns failure to respect the time between the last medical treatment administered to the animal and the moment of its slaughter (Kaba *et al.*, 2020). In fact, it is recommended to slaughter animals several weeks after the last medical treatment. This allows the animals to metabolise the injected products and thus avoid the presence of residues in the meat. Contrary to this recommendation, the majority of breeders met in the Abidjan District by Kaba *et al.* (2020), transport the animals to the slaughterhouse just a few days after the last medical treatment. At this level, the slaughter of animals could be avoided if SIVAC had a rigorous food quality control system such as PremiTest. Unfortunately, up today, no control system exists in this country. As a result, high levels of antibiotic residues in pig meat expose consumers in the District of Abidjan to numerous health problems. These problems may be linked to carcinogenic risks, changes in digestive flora and bacterial resistance to antibiotics (Reig & Toldra, 2008).

## CONCLUSION

This study confirms the contamination of pork meat with residues of oxytetracycline, tylosin and amoxicillin. Contamination levels are so high that consumers of pork in the District of Abidjan are exposed at bacterial resistance to antibiotics. It is therefore urgent to undertake studies linking the consumption of pork in the District of Abidjan (Côte d'Ivoire) to the occurrence of metabolic diseases.

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